Protocol #: 15-0933
Project Title: Effects of acetate and alcohol on brain function
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Overall goal: This is a pilot study to determine the feasibility of measuring effects of oral alcohol and intravenous acetate on cerebral blood flow and brain function in moderate drinkers and individuals with alcohol use disorder (AUD). Data from this pilot will be used to calculate the power needed for a larger, R21 application.

I. Hypotheses and Specific Aims:

Acute alcohol alters behavior and cognition through effects on brain metabolism and function. Although the risks of abuse are known, *there is a gap in our understanding of the effects of acute alcohol and its metabolite, acetate, on brain function*. Alcohol is first metabolized to acetaldehyde and then to acetate, primarily in liver but also in the gut. Alcohol consumption increases plasma acetate, which then accumulates in the brain (Volkow et al., 2013). Normally the brain utilizes glucose as its energy substrate. After acute alcohol there is a switch in brain energy substrate utilization from glucose to acetate. This switch may explain why moderate alcohol can decrease brain glucose metabolism by 25%-30% with relatively little effect on cognitive processing (Volkow et al., 2006). It has been hypothesized that brain acetate is a form of caloric "reward" that perpetuates drinking and that falling acetate levels associated with sobriety precipitate withdrawal (Jiang et al., 2013).

Alcohol increases endotoxin produced by the gut, stimulating inflammatory cytokine release from the liver which induces neuro-inflammation. The neurotoxicity of excessive alcohol is partially mediated through prolonged activation of microglia, the resident immune cells of the brain. The liver is critical in regulating the immune system's response to alcohol since liver stimulates both pro-inflammatory and anti-inflammatory cytokines. Given the role of inflammation in alcohol neurotoxicity, it is intriguing that acetate has been shown to decrease microglial activation (Brissette et al., 2012) and inflammatory cytokine production (Soliman et al., 2012a), suggesting a neuroprotective role.

Acute alcohol is associated with increased cerebral blood flow (CBF). It is unknown if changes in CBF are due to alcohol, acetate, or both. Determining the differential effects of alcohol and acetate on brain function is significant as it could lead to new treatments targeting alcohol metabolites. The goal of the proposed study is to determine feasibility of measuring effects of oral alcohol and intravenous acetate on cerebral blood flow and function in moderate drinkers with the objective to use the data collected for an R01or R21application. Although currently there is no data in the literature on this, finding such changes would be important and could direct future prevention and treatment plans for alcohol use disorders. For this feasibility pilot study we propose to investigate the effects of acetate and alcohol on CBF and brain function with two specific aims.

Aim 1. Investigate the effects of <u>acetate</u> on cerebral blood flow and function in controls and AUD individuals
Preliminary data will be collected for the hypothesis that compared to placebo given IV acetate given IV will: a) increase CBF as measured by arterial spin labeling (ASL); and b) decrease blood oxygen level dependent (BOLD) activity during functional magnetic resonance imaging (fMRI); and c) these changes will be greater in AUD individuals than controls

Aim 2. Investigate the effects of <u>alcohol</u> on cerebral blood flow and function in controls and AUD individuals
Preliminary data will be collected for the hypothesis that compared to non-alcoholic jello shots, orally ingested alcohol jello shots will: a) increase CBF, and b) decrease BOLD activity; and c) these changes will be greater in AUD individuals than controls.

II. Background and Significance:

<u>What is the problem and the critical barrier to progress in the field?</u> Many studies have looked at the effects of acute alcohol on CBF (Sano et al., 1993; Strang et al., 2014; Volkow et al., 1988), activity (Bjork and Gilman, 2014; Van Horn et al., 2006) and metabolites (Biller et al., 2009). Although work is beginning to emerge on the brain effects from acetate (Jiang et al., 2013), there have been no studies that fully evaluate the effects of acetate and compare them to that of alcohol. We review the literature to describe the gaps that exist and then describe how the proposed project will fill the gaps. *Answering these questions will increase scientific knowledge and guide clinicians toward better strategies for the treatment and prevention of alcohol disorders.*

Alcohol and brain metabolism: In the United States, alcohol is the most widely abused addictive drug and the third leading cause of preventable deaths (CDC, 2014). Alcohol impairs cognition, likely through direct and indirect effects on brain metabolism and altered neurotransmitter function. The mechanisms for cognitive and behavior effects of an

acute alcohol challenge are partially understood. Moderate alcohol significantly reduces brain glucose metabolism up to 25%, surprisingly high, given that these doses produce only mild intoxication (Volkow et al., 2008, 2006). The liver rapidly metabolizes alcohol to acetaldehyde, and then to acetate. Acetate then enters the circulation and can be used as an energy source. Volkow et al., demonstrated that the decrease in glucose metabolism resulting from alcohol ingestion is associated with an increase in brain acetate, suggesting a switch in brain substrate utilization (Volkow et al., 2013). Wang et al. (2013) found that rats exposed to alcohol had higher levels of brain acetate than control rats.

Alcohol, acetate and knowledge gaps about acetate's functional significance: While acetaldehyde toxicity is firmly established as a mechanism for tissue injury due to alcohol, less is known about the effects of acute and chronic acetate. The current proposal will systematically study the effects of acetate and alcohol on CBF and brain activity. Acetate is a short chain fatty acid normally produced by gut bacteria. Basal acetate levels are low and modulated by diet. In the brain, acetate is nearly exclusively metabolized by glia, being preferentially taken up by glia 18 times faster than by synaptosomes (Waniewski and Martin, 1998). In fact, a major interest in acetate arises from its potential as a specific marker of astrocyte activity (Wyss et al., 2011). After it is absorbed from the gut, alcohol is metabolized to acetate in the liver resulting in a 3-5 fold increase in plasma acetate (Sarkola et al., 2002). It enters the TCA cycle and can eventually be used in the glutamate-glutamine cycle (Håberg et al., 1998) (Figure 1). Acetate is not an inert substance. Its biological actions on the CNS can potentiate and antagonize the effects of alcohol in animals (Carmichael et al., 1991; Correa et al., 2003; McLaughlin et al., 2008). Large amounts of acetate have adverse effects on neural function (Israel et al., 1994). In alcoholics, one hypothesis is that chronically high brain acetate may be a form of caloric "reward" that may perpetuate drinking and that falling acetate levels from acute sobriety may precipitate withdrawal (Jiang et al., 2013). Acetate also exhibits anti-inflammatory properties. Specifically, acetate reduces microglial activation, pro-inflammatory cytokine release (Brissette et al., 2012) and endotoxin production (Soliman et al., 2012b). In addition, acetate decreases alcohol and thiamine deficiency-related thalamic neurodegeneration (Qin and Crews, 2014). Outside the CNS, acetate and other short chain fatty acids have been shown to suppress inflammation (Ishiguro et al., 2014; Vinolo et al., 2011). These findings suggest that acetate may have protective effects against alcohol-induced neuro-inflammation.

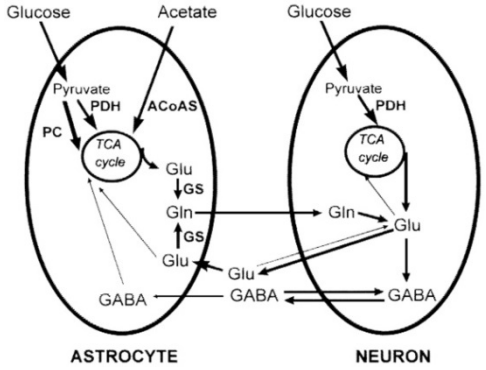

Figure 1. Glucose is taken up by neurons and astrocytes. Astrocytes have a unique ability to take up acetate as substrate for the TCA cycle (from Haberg et al. (1998)).

Acetate is also implicated in neurovascular "decoupling" observed after acute alcohol. Normally, lowering glucose metabolism results in a decrease in CBF due to tight neurovascular coupling. Instead, most studies have shown that alcohol increases CBF (Mathew and Wilson, 1986; Newlin et al., 1982; Strang et al., 2014). One explanation involves differences in the time scale of PET studies of glucose metabolism compared to blood flow assays. Another explanation involves the vasodilatory actions of acetate-derived adenosine (Schwartz et al., 1993). Studying the alcohol-induced changes in CBF will help disentangle the effects of flow and metabolism and clarify alcohol- induced changes in neural circuitry (Gilman et al., 2012; Marxen et al., 2014).

Acute alcohol and brain MRI studies: Structural and functional MRI and MRS brain studies of alcohol users has given us valuable insight into cumulative toxic effects of alcohol on the CNS (Meyerhoff, 2014; Sullivan et al., 2013). There is less information on brain effects of acute alcohol. Acute alcohol decreases fMRI BOLD activity in cortical regions implicated in cognitive control (Calhoun et al., 2004; Schuckit et al., 2012; Van Horn et al., 2006). One fMRI study showed that acute alcohol increased activity in the striatum during decision-making (Gilman et al., 2012).

How will the project improve scientific knowledge or practice? This is a pilot study to determine the feasibility of measuring effects of oral alcohol and intravenous acetate on CBF and brain function in moderate drinkers and individuals with alcohol use disorder (AUD). Data from this study will be used to apply for funds to conduct a larger study. The proposed experiments will allow us, in the larger study, to parse out the effects of alcohol and acetate on brain function. We will test if acetate increases CBF, as was suggested in a prior alcohol administration study (Schwartz et al., 1993).

Theoretical Model

The working model is shown in Figure 2. Possible pathways through which brain function is altered following alcohol intake include through hepatic metabolism of alcohol to acetate (Aim 1) and through the direct effect of alcohol on the brain, including the effect from acetate (Aim 2).

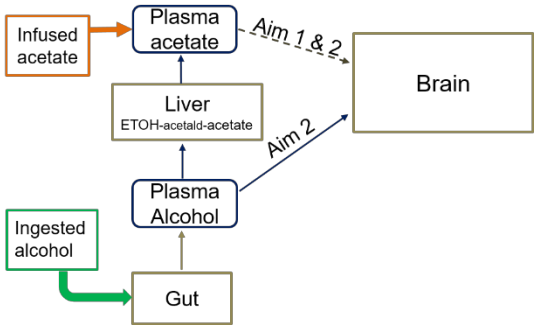

Figure 2. Working Model

III. Preliminary Studies

Practice effects: It is possible that differences in brain activity could reflect learning effects. To test for learning effects we administered two versions of the decision-making task to a healthy control, 2 hours apart. The two versions differed in the color and position of the decks and the scale of the magnitude of outcomes, while keeping the value of the decks balanced. The frequency of wins and losses was identical. There was no difference in Play response on good cards (93 vs. 95), bad cards (54 vs. 55), or on net score (good minus bad cards played) (39 vs. 40). *This lack of significant practice effect is consistent with a prior study showing stable performance on the Iowa Gambling Task, on which our task is based, given multiple times to controls* (Xiao et al., 2013) *and patients* (Waters-Wood et al., 2012) *and, together, suggest that there will not be a significant learning effect.*

IV. Research Methods

A. Outcome Measure(s):

Our goal in this pilot study is feasibility.

Preliminary outcome measures:
1. CBF before and after acute alcohol or acetate
2. Brain activity during decision-making before and after acute alcohol or acetate

B. Description of Population to be Enrolled:

This is a single blinded placebo controlled test of the effects of acetate and alcohol on brain function.

Inclusions-all: (1) age 21-55, (2) English proficient, (3) Participants must understand the nature of the study and must sign an informed consent; (4) be currently non-smoking.

Inclusions-moderate drinkers: (1) participants must be moderate drinkers (male: 1-14 drinks per week: female: 1-7 drinks per week); and (2) have drunk at least 2 drinks in one hour in the past; and have no history of dependence on drugs or alcohol.

Inclusions-AUD: (1) must meet DSM-V criteria for lifetime history of Alcohol Use Disorder (AUD)

Exclusions-all: Participants will be excluded if they: (1) have a "facial flushing" response to alcohol; (2) are pregnant or nursing; (3) have major medical problems including diabetes, uncontrolled high blood pressure (> 150/90), prior neurological or psychiatric history (schizophrenia, bipolar, or current major depressive disorder, epilepsy (alcohol withdrawal seizures is not an exclusion)); (4) have ever been told they have or been diagnosed with liver or kidney disease; (5) have a diagnosis of chronic gastrointestinal disease; (6) are obese (BMI>30 kg/m2); (7) have prior head trauma resulting in loss of consciousness>15 min.; (8) have MR exclusions which include claustrophobia, intracranial, orbital, or spinal metal, pacemakers, cochlear implants, cardiac stents or other non-MR-compatible implants or devices.

C. Study Design and Research Methods

Study Design

This is a pilot study to determine the feasibility of measuring effects of acetate alone and acetate obtained as a byproduct of alcohol metabolism on CBF and brain activity. Our recruitment goal will be 50 subjects: 40 healthy controls who are social drinkers (1-14 drinks per week) to achieve a target of n=20 scanned for Aim 1 and n=20 scanned for Aim 2, with a final usable healthy control target of n=15 for Aim1 and n=15 for Aim 2 (a few subjects may not qualify for data analysis due to excessive head motion in scanner); and 10 AUD individuals, to achieve a target of n=7 scanned for Aim 1. AUD participants seeking treatment will be excluded from Aim 2 (alcohol arm). Moderate drinkers will be recruited through advertisements and flyers. AUD subjects will be recruited from the Center for Dependency, Addiction and Rehabilitation (CeDAR) at the University of Colorado Anschutz Medical Campus.

Order effects: Before the study commences, test-retest reliability will be conducted on two healthy controls on MR measures by scanning them twice in a single day, with no intervention. The null hypothesis of no difference in brain activity and CBF will be tested, between the two sessions. Notably, the literature shows high reliability for test-retest of CBF using ASL at various time intervals (Floyd et al, 2001, Chen et al., 2011).

Session 1: Consent and diagnostic assessment (total time ~1 hour)

Potential participants will take part in a short interview at the Anschutz Medical Campus consisting of explanation of the study, screening for MRI compatibility, signing informed consent, and answering questions on drug and alcohol use.. Control participants will be randomized to the acetate or alcohol branch of the study but will not know which branch they are assigned to until Session 2. AUD participants will be assigned only to the acetate branch of the study. Participants will be asked to not drink alcohol for 48 hours prior to the study and to eat a light low-fat breakfast before coming to the University for Session 2. Session 2 (scan day) will be scheduled as soon as possible, about 1-2 weeks after Session 1.

Questionnaires:

(1) Composite International Diagnostic Interview Substance Abuse Module (CIDI-SAM). Dependence on alcohol, cannabis and nicotine will be measured using the computerized CIDI-SAM, a structured interview designed for trained, lay interviewers (Cottler et al. 1995). (30 min)

(2) Alcohol Use Disorders Identification Test (AUDIT). This test screens for alcohol abuse and average alcohol use (5 minutes)

(3) Timeline Followback assessment. This assessment measures frequency and quantity of alcohol consumption for the most recent 1-3 months of alcohol use.

Session 2: Acetate or Alcohol and MRI scanning (~7 hours)

Research participants will arrive at the Clinical and Translational Research Clinic (CTRC) in the Leprino Building on the University of Colorado Anschutz Medical Campus (AMC) at 7:30 am, turn in their stool samples and 2 day food log, and provide urine samples to screen for alcohol, drugs and pregnancy.

If the subject is randomized to acetate: Control participants will need to arrange for a driver to and from the second session or we will provide a voucher for up to $50 for them to take a taxi to Session 2 and up to $50 for them to take a taxi home from Session 2. AUD participants will be accompanied to and from CeDAR for the second session. Two IVs will be placed by a CTRC nurse or a physician on the study. One IV will be for infusion and one will allow blood draws without repeat sticks. The infusion IV and blood draw IV will be placed in separate arms to prevent infusate from confounding blood sample metabolite measures. The Session 2 timeline is illustrated in Figure 2. The first blood draw will be immediately after IVs are placed. Participant will then be escorted over to the Brain Imaging Center at the University of Colorado AMC for MRI scans. Participant will be blinded as to when they are receiving placebo and when they are receiving acetate. A placebo infusion will be started and continued through the first scan. The participant will be given the Subjective High Assessment Scale 7 (SHAS7), an analogue scale evaluating 7 subjective feelings of intoxication, before the first scan and after each scan (Schuckit et al., 2000). After each scan, participants will perform several motor tasks (psychomotor vigilance task, simple and choice reaction time task, and grooved pegboard task) to measure attention and arousal after placebo and drug. The participant will have the first MR scan of the brain. Immediately after the scan, the second blood sample will be obtained. One hour later, at 11:00 am, the placebo infusion will be switched to an acetate infusion and the participant will be placed in the MR scanner. The acetate will be prepared by the University of Colorado Hospital Research Pharmacy and will be based on methods of Jiang et al.(Jiang et al., 2013) (See below). The participant will have the second MR scan using the same protocol. Immediately following the second MRI scan, the third blood sample will be obtained. Then both IVs will be removed, participants will be given

lunch and observed in the Brain Imaging Center for 1 hour. Participants will be given a stool sample kit and a prepaid return envelope for mailing the sample back to us. Participants will be reimbursed and allowed to go home with a driver or by taxi (details at top of paragraph). AUD participants will be accompanied back to CeDAR. All participants will receive a follow up call that evening. They will collect a stool sample the day after the scans and mail it back to us that same day.

If the subject is randomized to alcohol: The procedure is identical to that described for acetate with the following differences: (a) only one IV will be placed, for blood draws, (b) participant will receive a jello shot rather than an IV infusion. The participant is blinded as to when they will receive a placebo jello shot and when they receive an alcohol jello shot. The participant will be given a placebo jello shot 15 min before the first scan, and an alcohol jello shot 15 min before the second scan (Van Horn et al., 2006). Following the MRI, breath alcohol (BrAC) will be taken every 30 min until it reaches a level ≤0.02%, at which time participant will be allowed to go home with a driver or will be given a voucher for up to $50 for a taxi (as described in first sentence of acetate section, above). We expect BrAC levels to be ≤0.02% by 3 pm. All participants will receive a follow up call that evening.

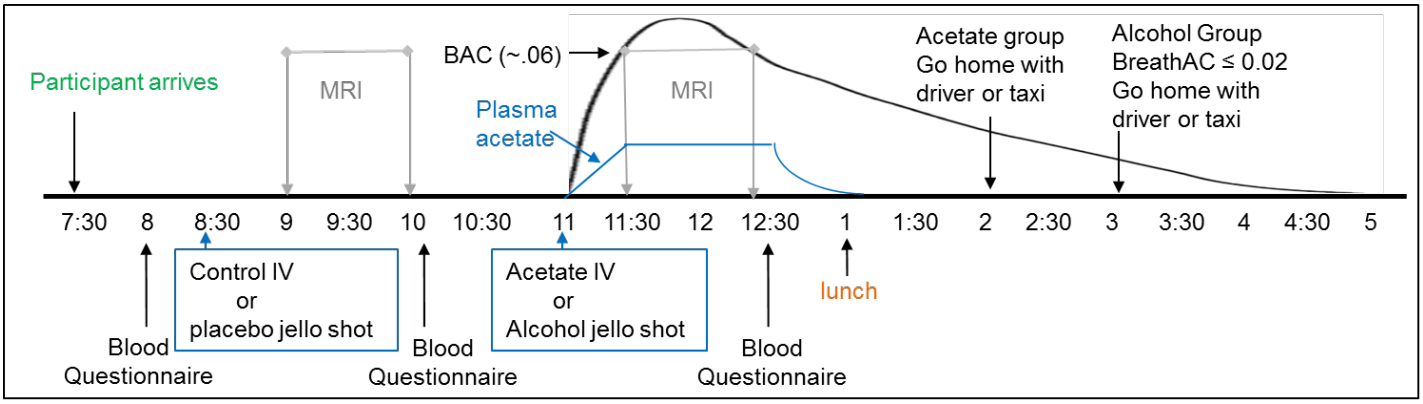

Figure 2. Procedure for acetate and alcohol administration

Research Methods

IV acetate and placebo: Infusions will be prepared by the University of Colorado Hospital Research Pharmacy at the University Hospital the morning of the study. To maintain the single blind, the placebo bag will be labeled "Morning" and the acetate bag labeled "Afternoon." A study team member will bring the infusion bags to the CTRC immediately before the study. Based on Jiang et al., acetate infusion will be 5 mg/kg/min for the first 7 minutes, followed by continuous infusion of 2.5 mg/kg/min for 68 minutes (Jiang et al., 2013). This rate results in safe plasma acetate levels of ~1.2 mM, comparable to acetate levels after moderate drinking (Jiang et al., 2013; Mason et al., 2006; Smith et al., 2007).

Alcohol and control jello shots: Methods will be as described by Van Horn et al (Van Horn et al., 2006). For the alcohol condition, participants will consume more or less of the jello shot based on the Widmark formula which takes into account their weight and sex, so as to bring the breath alcohol concentration (BAC) for each subject to the same level of 0.07%. Jello shots will be prepared with 100 proof Vodka, according to calculations based on Widmark's equation: Number of fl.oz. EtOH = [[BAC g%/ml + (hours since last drink) × Widmark b] × Weight in lbs. × Widmark r] / [0.0514 lb/fl.oz. EtOH × 100% × 1.055 g%/h]. The Widmark b factor will be set to 0.017. The Widmark r factor will be set to 0.68 for male and 0.55 for female participants, respectively. Two mL (< ½ tsp) of alcohol will be spread on top of the nonalcoholic jello shot to give the smell and taste of alcohol, but keep BAC at 0%.

Blood samples: Figure 2 shows the timing of blood draws. Five cc of blood will be drawn three times during the MR study. Samples will be kept on ice until the end of Session 2. They will then be processed and assayed for acetate and alcohol levels.

Behavioral response to alcohol (SHAS7): After each blood draw, participants will be given the Subjective High Assessment Scale 7 (SHAS7) (Eng et al., 2005; Schuckit et al., 2000) which is an analogue scale evaluating 7 subjective feelings of intoxication. The SHAS7 includes three general items (Effects of Alcohol, Drunk, and High) and four specific aspects of the effects of alcohol (Clumsy, Confused, Dizzy, and Difficulty Concentrating). Items are rated on a

36-point Likert scale. The SHAS has been shown to have high reliability and validity and may be predictive of possible alcohol use disorders (Schuckit et al., 2000; Schuckit and Smith, 2000).

Motor tasks to measure arousal and attention: After each scan, participants will perform three motor tasks, (psychomotor vigilance task, simple and choice reaction time task, and grooved pegboard task) to measure arousal and attention after placebo and drug. These tasks will measure how alcohol and acetate affect arousal and attention.

MR scan: Studies will be performed on the research-dedicated 3T MR system (Skyra, Siemens Healthcare, Erlangen, Germany) and 32-channel head coil at the Brain Imaging Center (Bldg. 400)**.** An anatomic 3D T1-weighted image will be acquired for image registration and normalization. Pulsed arterial spin labeling (ASL) will be used to measure brain CBF. Blood oxygen level dependent (BOLD) functional MRI will be acquired to measure brain activity during the decision-making task.

MR acquisition (Total MR time = 60 minutes): Linear and second order shimming will be performed to reduce static field inhomogeneities at the skull base prior to scans.
1. *Anatomic:* Structural high-resolution isotropic 3D T1-weighted image will be acquired for image registration and normalization with TR/TE/FA 45 ms/20ms/45°, 256x256 matrix, 240x240 mm^2 field-of-view (0.9 x 0.9mm^2 in-plane resolution). Scan time = 5 minutes
2. *Pulsed arterial spin labeling (ASL) CBF images:* 2D Pulsed arterial spin labeling (ASL) using: TR/TE/TI 4500/10/1800 ms, 240 mm FOV, 128 x 128 matrix (1.8 x1.8 mm^2 in-plane), 4 mm thick slices, sensitivity encoding (SENSE) factor= 2, and 60 pairs of interleaved control and tag images, axial plane. Scan time=~6 minutes
3. *Blood oxygen level dependent (BOLD) fMRI:* T2* weighted echo-planar imaging BOLD fMR images will be acquired with 3.4 x 3.4 x 3 mm3 voxel size, TR/TE/FA 2100/30/70. Images will be acquired to measure brain activity during the decision-making with which we have extensive experience (see below for details). Scan time=24 minutes

Decision-making task (Fig 2): Participants will play a modified version of the Iowa Gambling Task (Bechara et al., 1994) during fMRI scanning. The modified IGT assesses risk avoidance and sensitivity to negative outcomes (Tanabe et al., 2013; Thompson et al., 2012). We have many years of experience giving the task to substance users and controls. Briefly, participants are presented four decks of cards and instructed to earn as much hypothetical money as possible by choosing to either Play or Pass on a given deck. "Play" results in a single positive or negative monetary value, along with the running total. "Pass" results in no change. To perform well, participants must learn to Pass on the two bad decks that result in a net loss and Play on the two good decks that result in net gain over time. The payout of the decks is controlled on the frequency and magnitude of wins and losses. The decision period is 2 seconds, and the outcome period is 4 seconds, separated by a variable jitter of 0 to 4 seconds. There are 50 trials of each deck, 200 trials total, plus 40 baseline trials to control for motor effects, and 10 six-second fixation crosses presented in pseudorandom order. Total task scan time is 24 minutes consisting of two 12 minute runs. The task will be given twice, once in the morning and once in the afternoon. To reduce practice effects for the afternoon study, the color, win/loss amounts (but not the ratio of wins:losses), and the order of the decks will be changed.

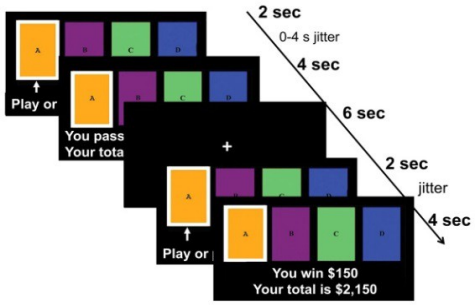

Figure 3. Decision-making task and timing parameters.

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

Session 1

1. Consent Procedures: Participants will have time to ask questions and have the study explained by PI. They will fill out an MRI screening form to ensure safety prior to entrance to the scanner.
2. The session will be conducted in a private room to facilitate confidentiality. Data collected during the initial screening and between and after the MR sessions will be associated with the participant number only and will assess alcohol use (frequency, amount, and abuse/dependence symptoms).
3. Participants will be paid $35 for completing Session 1.

Session 2

1. Participants will be asked to not drink alcohol for 48 hr prior to Session 2 and to eat a light low-fat breakfast on the day of Session 2 to avoid interference with alcohol absorption.
2. Participants will need to arrange for a driver to and from the second session or we will provide a voucher for up to $50 for them to take a taxi to Session 2 and up to $50 for them to take a taxi home from Session 2.
3. CTRC nurse or a physician co-investigator will start IV, monitor infusions, and perform blood draws.
4. Participants will change into scrubs and provide a urine sample. Participants will be excluded from the MRI if positive for drugs, alcohol, or pregnancy.
5. Participant will enter MR scanner. There will be two scans, each ~1 hour. The first scan will be from 9-10am, the second scan will be from 11:30am-12:30pm.
6. Participants will either be in the acetate arm or in the alcohol arm of the study.
7. Participants receiving alcohol will have BrAC samples measured at 30 min intervals until BAC is ≤0.02%, at which time they will be allowed to go home with a driver or will be given a voucher for up to $50 for a taxi (details in 2., above).
8. Participants receiving acetate will be observed for 1 hr to give acetate time to metabolize and then will be allowed to go home with a driver or will be given a voucher for up to $50 for a taxi (details in 2., above).
9. The participant will change back into clothes and will be paid $95 per scan. Thus they will be paid $190 for completing all scans

Follow-up

1. Participant will receive a follow-up email or call from the PI on the evening of Session 2.

This study involves answering questionnaires, performing several motor tasks, having oral alcohol or an acetate infusion, and having a functional MRI of the brain. Breaches of confidentiality are a risk. It is necessary for participants to answer questions about private things like drug use, to rule out or diagnose substance dependence and other psychiatric conditions. Participants' names will be associated with a participant number to insure confidentiality. Behavioral data and imaging data will be stored using the participant number. All documents indicating the identity of participants will be kept in a locked room inside a locked file cabinet. Computer files include no identifiers except a code number, and will be password-protected. If the results of the study are published, data that might reveal the identity of any particular subject will be disguised. An MRI is a non-invasive routinely used procedure that poses minimal risk to participants.

Potential Risks

Risks of alcohol. Ethyl alcohol in the amount used in the current study presents minimal risk to the participants. The amount of alcohol they receive will be less than the legal limit and is expected to produce mild intoxication. There is a chance of gastrointestinal irritation, nausea, vomiting, light-headedness, diarrhea with alcohol. These symptoms are self-limited and are expected to dissipate with time. Any subject who has never had alcohol in the past will be excluded. The PI will be with the participants for the length of the study until they leave.

Risks of acetate. Sodium acetate presents minimal risk to the participants. It can cause eye and skin irritation in the crystal form and ingesting large amounts may cause gastrointestinal irritation. The acetate will be in solution, and thus not expected to be an irritant. There is a rare possibility that a reaction may occur during or immediately after the infusion of acetate characterized by flushing or redness of the skin which may resemble a rash. No subject will receive both acetate and alcohol as they will be randomized to one arm. An established protocol will be used for the acetate infusions (Jiang et al., 2013; Mason et al., 2006).The infusion rate for this protocol results in plasma acetate levels of ~1.2mM, lower than another previous study (Akanji and Hockaday, 1990) that resulted in plasma acetate levels of 1.38 mM for healthy participants and 1.65 for diabetics. One study reported a mild metabolic alkalosis which was well tolerated by healthy participants (Smith et al., 2007). Participants will be observed until about 2:00 pm, 1.5 hours after infusion, for any adverse effects. The PI will be with the participants for the length of the study until they leave.

Venous Cannulation. Venous cannulation is a routine clinical procedure that carries minimal risks when performed by trained personnel. The major risk is pain and discomfort. It is possible bruising could occur in some participants. The risk of phlebitis or infection is very remote.

Blood draws. Blood will be drawn through the venous access catheter. Fifteen cc of blood per person will be taken, which is a minimal amount that does not pose significant risk to the participants.

Brain Imaging. The University of Colorado Anschutz Medical Campus research dedicated Brain Imaging Center (BIC) is run by full time trained technicians with extensive experience and training to conduct the proposed studies safely. In addition, all research personnel involved in this study are/will be trained in MR safety by the imaging center staff. The primary physical risk is the risk of injury from metal objects being drawn into the magnet. Additionally, some

participants may experience transient peripheral nerve stimulation or nausea in the bore of the magnet. Nausea can be minimized by stabilizing the head. The participant may experience discomfort due to noise or confined environment of the scanner (claustrophobia), or discomfort and frustration carrying out the tasks.

Exposure to a high magnetic field. The only known hazard associated with exposure to a static high magnetic field is that the magnet exerts a strong force on ferromagnetic objects. For this reason, ferromagnetic objects are excluded from the vicinity of the magnet so that they will not become projectiles. At our Center for Functional MRI, the research systems for human use have field strengths of 3T. Imaging at these field strengths is not considered a significant risk according to FDA guidelines. The scanning sequences applied are within the FDA guidelines for human MR scanning. In addition, every subject undergoes extensive safety screenings to determine whether he/she has any implanted materials or braces and dental retainers that may pose a risk. This screening will be done once the subject is consented, and another time on each imaging study day. If there is any doubt about the nature of any implanted material, or any other contraindication to MR scanning, the subject will not be scanned.

Heating from radiofrequency (RF) pulses. The RF pulses that are used for creating the MR signal deposit some energy in the body in the form of heat, but no ionizing radiation is used with MRI. For the same pulse sequence, the RF power deposited is higher at higher magnetic field strengths. However, the pulse sequences that will be used at 3T have relatively low power depositions. In the future, pulse sequences with higher RF power depositions may be developed, but it will be insured that the power deposited is always below the FDA guidelines.

Peripheral nerve stimulation from rapidly switched magnetic fields (dB/dt). Magnetic field gradients are switched on and off during imaging to encode the spatial distribution of the MR signal. Gradient switching rate depends on the gradient coil used, but does not depend on field strength. For this reason, the gradient switching rates will be similar to those for the 1.5 T scanners we have used in the past, and these rates will not exceed FDA recommendations. The FDA guideline states that a significant risk is involved only when "dB/dt sufficient to produce severe discomfort or painful stimulation" is used. All of the fMRI studies performed in the past on our 3T system are well below this threshold.

Acoustic noise. Acoustic noise is an unwanted side effect of MR imaging. As currents are pulsed through the gradient coils within the magnetic field, the system acts like a loudspeaker, making a repetitive tapping sound. In all of our studies, participants will wear ear plugs to reduce the noise to a comfortable, safe level. Additionally, head phones will be placed over the ears. The FDA guideline for a significant risk due to acoustic noise is "peak acoustic noise over 140 dB", and we will ensure that the acoustic levels remain well below this value.

Potential risk of loss of confidentiality. Since this study includes drug and alcohol screening, and pregnancy test assessment, there is the potential that this information may not be kept confidential (for instance by theft of study material). The investigator team will make every effort to keep all information confidential. All study material will be stored in locked cabinets in the Research-2 Building, University of Colorado Anschutz Medical Campus sponsored facilities. Furthermore, a unique study number will be used for each person in data sets and spreadsheets that do not readily identify a name. The identifying name information containing material will be locked.

Psychological risk: These studies do not pose any significant psychological risk to the patients. If patients are anxious they can be trained in the mock scanner located adjacent to the MR scanner to mitigate anxiety and help the subject adjust to the scanning environment.

Social Risk. There is no social risk associated with participation in this study; all results will be kept strictly confidential and will not be used to influence clinical decision making. There are no other known risks associated with participation in this study.

E. Potential Scientific Problems:

1. Sex effects: Alcohol metabolism differs in men and women and several studies find an interaction between sex and alcohol's effect on terms of brain morphometry (Hommer et al., 2001), function, and CBF (Marxen et al., 2014; Rickenbacher et al., 2011). It should be noted, however, that not all studies have confirmed sex differences in brain morphometry (Demirakca et al., 2011). There will not be power to detect sex effects. Our goal is to demonstrate feasibility.

2. Vasomotor effect: The BOLD signal reflects complex interactions between CBF, cerebral blood volume, and oxygenation. We plan to study differences in global and regional vascular effects in acetate vs. alcohol treatment. The fMRI BOLD measurements are insensitive to time lags or shifts in the hemodynamic response of the brain during decision-making. Therefore in exploratory studies a finite impulse response (FIR) model will be used, according to prior work (Yamamoto et al., 2014).

3. Diurnal variations: We cannot exclude the possibility of diurnal fluctuations confounding our findings. The alternative to conduct blinded placebo and treatment studies on two separate days in the same participant would be a hardship on participants.
4. Dose-response: A dose-response for acetate or alcohol will not be obtained. Fortunately, the pharmacokinetics of alcohol are well established and the timing of the MR scans is planned according to Figure 2. The pharmacokinetics of acetate are not as well established. The IV infusion protocol will give us better control of plasma levels, and serum acetate and alcohol will be assayed.

F. Data Analysis Plan:

Blood alcohol and acetate: Levels of blood alcohol and acetate will be analyzed using colorimetric enzyme-linked immunosorbent assays (ELISA).

MR measures: MR CBF and fMRI BOLD activity data will be processed in SPM12 (The FIL Methods Group, 2014). First, each participants' T1-weighted anatomic image will be skull-stripped then coregistered to the Montreal Neurological Institute (MNI) MNI152 template. The transformation matrix generated from this step will be applied to CBF and BOLD contrast maps of interest.
MR CBF: Motion-corrected ASL CBF images (mL/100g/min) will be generated by the scanner software. CBF maps will be skull-stripped and co-registered to each participant's T1-weighted anatomic image in native space followed by application of the transformation matrix to MNI space.
BOLD fMRI pre-processing: After discarding the first 4 volumes for saturation effects, images will be corrected for movement with a 3-dimensional rigid body transformation. Participants with scan-to-scan head movement >2 mm will be excluded. Motion parameters will be modeled as nuisance variables. Images will be normalized to MNI space using the two step transformation procedure described for MR CBF. Images will be spatially smoothed with a 6 mm full-width-half-maximum isotropic Gaussian kernel, filtered to remove low-frequency fluctuations (128 s high pass filter), and corrected for temporal autocorrelation.
BOLD fMRI: Model specification and contrast of interest: The time series will be predicted by nine regressors, consisting of decision and outcome for each of the four decks, plus motor control. Motion parameters will be modeled as nuisance regressors. The stimulus function will be convolved with the canonical hemodynamic response function (HRF). We are primarily interested in effects of acetate or alcohol on brain activity during the decision phase of the task. The primary outcomes will be the contrast of Decision>motor control baseline and Outcome>motor control baseline. For each participant these contrast maps will be brought to the second level for subsequent ROI and whole-brain analyses.

Regions-of-interest (ROI): ROI and whole brain analyses will be conducted for outcome measures of CBF and fMRI BOLD activity. Six anatomically- defined ROIs will be based on the neural circuitry found to be involved during decision-making on the modified IGT and which overlap with brain regions showing altered signal after acute alcohol (Anderson et al., 2011; Bjork and Gilman, 2014). ROIs will be bilateral ventral striatum (VST), dorsal striatum, insula, and anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and dorsolateral prefrontal cortex (DLPFC). VST and DST will be manually drawn, based on published landmarks (Mawlawi et al., 2001). For the OFC, insula, ACC, and DLPFC, the automated anatomical labeling atlas (AAL) (Tzourio-Mazoyer et al., 2002) will be used. Figure 3 shows examples of VST=green, DST=orange, OFC=blue, insula=red.

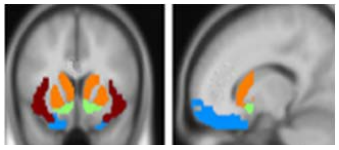
Fig 3. Regions of interest

Statistical Analysis: We have three outcomes of interest: 1) CBF, 2) fMRI BOLD signal during decision-making, 3) behavioral measures. A bio-statistician employed part-time by the Department of Radiology will provide statistical support. The aim of this pilot study is simply to determine the feasibility of performing the experiment and to acquire the necessary data to calculate power needed for a larger study.

CBF: ROI analyses: For this feasibility pilot study, a paired t-test will be used to investigate the effect of acetate and the effect of alcohol separately. In an exploratory manner, drug x time interactions will be examined through descriptive and graphical analyses, though we acknowledge insufficient power to reject the null hypothesis of no interaction. Nevertheless, a noticeable difference in outcomes based on factors of drug or time could provide data to compute power for a larger, more powered study.

To provide context for this pilot, the statistical plan for the later, larger study is included: We will fit a general linear multivariate model with repeated measurements of CBF as the outcomes. Each participant will have 12 repeated measurements (6 ROIs x 2 treatments (acetate and placebo or alcohol and placebo)). Age and number of drinks per week will be included as covariates. MCMC imputation (Li, 1988) will be used to address missing data. A Chi-Muller test will be conducted (Chi and Muller, 2013) for the main effect of treatment (acetate versus placebo or alcohol versus placebo) at any ROI. Residual analysis will be used, and Box-Cox transformations will be considered to account for deviations from multivariate normality. An alpha spending approach will be used to handle multiple comparisons problems, by testing the overall hypothesis of no effect at any region at the alpha = 0.04 level, and then, if we attain significance, testing the treatment effects at each ROI at the alpha =0.01/6 level. This yields a total Type I error for this aim of 0.05 =0.04 + 6*(0.01/6).

CBF: Whole brain analyses: The main effects of acetate on CBF will be analyzed in SPM12 using a repeated-measures ANCOVA with drug (alcohol, or acetate vs. placebo) as within-subject factor and adjusting for age and number of drinks per month. As this is a feasibility study, a less stringent threshold of $p<0.05$, uncorrected will be used to explore for possible main effects and interaction effects as well as nonparametric approaches. In the later, larger study, significance levels will be set at $p<0.05$, corrected for multiple comparisons with family-wise error (FWE) using AlphaSim Monte Carlo simulations, 10,000 iterations, to determine cluster size criterion. The voxel-level significance will be set at $p<0.005$.

fMRI BOLD activity: *ROI analyses:* For each ROI and each subject, the percent signal change will be extracted using Marsbar toolbox. The feasibility analyses are the same as CBF: ROI approach. To provide context for this pilot, the statistical plan for the later, larger study is included: To examine the main effect of alcohol or acetate on BOLD response, a general linear multivariate model with 12 repeated BOLD response measurements as the outcomes will be employed. Covariates will include education level and number of drinks per week. From prior experience, BOLD activity during decision-making correlates with education level. Analyses are otherwise the same as CBF: ROI approach.

fMRI BOLD activity whole brain analyses: The feasibility analyses are the same as CBF: whole brain analyses. In the later, larger study, the main effects of alcohol or acetate on fMRI BOLD signal during decision- making will be analyzed in SPM12 using a repeated-measures ANCOVA with drug (alcohol or acetate vs. placebo) as within-subject factor and adjusting for education and number of drinks per month. Analyses are otherwise the same as for the CBF: whole brain analyses.

Behavioral: Decision-making performance will be analyzed with repeated measures ANOVA on net score, adjusted for education level. Net score is the number of plays on good decks minus bad decks. The behavioral effects of treatment will be analyzed using repeated measures ANOVA on SHAS7, on the psychomotor vigilance task, simple and choice reaction time task, and grooved pegboard task.

Power and Confidence Interval Width: This study is a pilot study, designed to estimate the size of the effect, and provide accurate parameter estimates for a later, more definitive study. The power analysis here is based on the work of Schwartz et al. (Schwartz et al., 1993), who studied CBF before and after drinking and observed a mean difference in flow measured as a ratio of cortex/cerebellum (after – before) of 0.054 with standard deviation of the difference of 0.085. The smallest difference in CBF that a study with 10 people can detect using repeated measures ANCOVA with 90% power and a Type 1 error of 0.05 is a difference of 0.09 (ratio of cortex/cerebellum). Therefore we should have adequate power to detect a difference with alcohol. There is no prior CBF data on acetate in the literature to perform power analysis for acetate. This study is designed to provide preliminary data for a later study and an estimation of the N necessary for that study to provide sufficient power for analysis.

G. Summarize Knowledge to be Gained:

This proposal is relevant to public health because of the considerable impact alcohol has on the health of individuals and in terms of legal, medical, and social costs to the country. Again, this is a pilot study to determine the feasibility of measuring effects of oral alcohol and intravenous acetate on CBF and function in moderate drinkers and AUD individuals. Data from this study will be used to apply for funds to conduct a larger study. The results from this the later,

larger project will add to our knowledge about mechanisms of acute alcohol's effects on brain function and the role of acetate in these effects.

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