Protocol Title: Manipulating ghrelin signaling via GOAT inhibition in alcohol use disorder

Protocol Number: 19-DA-N075

Protocol Version: 09/25/2023

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Investigational Agents:

Drug Name:	GLWL-01
IND Number:	**142,215 (cross-reference IND # 124,676 from GLWL Research Inc)**
Sponsor:	NIDA
Manufacturer:	GLWL Research

1 PRÉCIS

Background and Objective: Acyl-ghrelin is a 28-amino acid peptide that stimulates appetite and food intake. It is an endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a). Preclinical studies suggest that acyl-ghrelin increases alcohol intake and decreases in acyl-ghrelin and GHS-R1a function suppresses alcohol consumption. Furthermore, previous human studies indicate a positive correlation between endogenous ghrelin levels and alcohol craving and drinking. In clinical studies conducted by our group with individuals with AUD, intravenous (IV) acyl-ghrelin administration, versus placebo 1) increased alcohol craving during alcohol cue-exposure and 2) increased IV alcohol self-administration as well as decreased latency to first infusion of alcohol and 3) increased brain activation in the amygdala in anticipation of alcohol reward. Together, this preclinical and human data suggest that manipulating the ghrelin signal may be a novel and potentially effective pharmacological approach to treat individuals with alcohol use disorder.

After the discoveries of GHS-R1a and acyl-ghrelin, a next step was identifying ghrelin O-acyltransferase (GOAT) the enzyme that catalyzes the conversion of des-acyl-ghrelin (DAG) to acyl-ghrelin via octanoylation. GOAT is thus "the master switch for the ghrelin system", as acyl-ghrelin, not DAG, is biologically active at the GHSR-1a. GOAT's structure is highly conserved, is produced by endocrine cells in the stomach and is co-expressed with ghrelin. Therefore, GOAT is a promising target for manipulating the ghrelin system by altering the peripheral acyl-to-total ghrelin ratio (where total ghrelin = acyl-ghrelin + DAG). Recently, the ghrelin system has been investigated as a potential treatment target for AUDs. As such, an oral bioavailable GOAT inhibitor offers encouraging potential as a treatment for alcohol use disorder. GLWL-01 is an existing GOAT inhibitor for which GLWL Research Inc. has recently and successfully completed a first-in-human safety clinical trial. The goal of this protocol is to conduct a proof-of-concept human laboratory study to assess a potential early signal of efficacy of GLWL-01 in relation to alcohol-related outcomes.

Study population: Males and females (*N* = 43) with alcohol use disorder.

Study Design: A within-subject, counterbalanced, double-blind, placebo-controlled study. Participants will take GLWL-01 450 mg b.i.d. or matched placebo for a minimum of 4 days (Stage I). After a minimum 2 day wash-out window, Stage II will take place during which the counterbalanced study drug will be administered for a minimum of 4 days.

Primary outcome measure: The co-primary aims will be to determine whether: 1) the number of adverse events (AEs) experienced differ in the GLWL-01 condition, compared to placebo; and 2) GLWL-01, compared to placebo, reduces alcohol cue-elicited craving using a validated alcohol cue-reactivity procedure.

Secondary outcome measures: The main secondary aim will be the effects of GLWL-01 on food choices using a "virtual buffet" experimental procedure. We will also monitor a wide range of

behavioral measures including e.g., pain, anxiety, depression, alcohol craving and withdrawal, and smoking.

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3 BACKGROUND

3.1 Statement of the problem: searching for new neuropharmacological targets in alcohol use disorder

Alcohol use disorder (AUD) is a chronic relapsing brain disease that results in severe consequences including serious morbidity and mortality (NIAAA,2018). According to recent data, approximately 30% of the US adult population meets criteria for lifetime diagnosis of AUD (Grant et al., 2015; Miller & Cohen, 2001). Treatment of AUD consists of psychological, social and pharmacologic interventions (Garbutt, West, Carey, Lohr, & Crews, 1999). When combined with psychosocial treatments, medications can improve outcomes for some individuals; however, these treatments are not effective for many others (Edwards, Kenna, Swift, & Leggio, 2011; Heilig & Egli, 2006). The Food and Drug Administration (FDA) has only approved disulfiram, naltrexone and acamprosate for AUD, and all these medications have limited efficacy (Edwards et al., 2011; Heilig & Egli, 2006). Developing new drugs for AUD is therefore a high scientific and public health priority. There is a crucial need to identify novel drug targets that may lead to the discovery of new effective medications for AUD. One source of new drug targets comes from research on the neurobiology of obesity, as alcohol- and food-seeking behaviors share some overlapping features and neural circuits (Tomasi & Volkow, 2013). While alcoholism, obesity and binge eating are complex conditions with likely diverse genetic and environmental contributions to their etiologies (Tomasi & Volkow, 2013), it is notable that medications used to treat AUD often result in weight loss. For example, naltrexone, topiramate and ondansetron are used in the treatment of AUD and have also been utilized to treat obesity and/or eating disorders (Leggio et al., 2011). Therefore, research focused on identifying feeding-related pathways may be of utility in understanding the neurobiology of alcoholism, and may yield possible neuropharmacological targets for AUD. As outlined below, there is an increasing body of evidence supporting the notion that the ghrelin system may be a novel neuropharmacological target for AUD.

3.2 What is ghrelin and what does it do?

Ghrelin was first isolated from the stomach (Kojima et al., 1999). It is a 28-amino acid peptide acting as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a), a G-protein coupled receptor that induces growth hormone (GH) release from the pituitary (Kojima et al., 1999). The n-octanoyl bearing ghrelin is known as active ghrelin (acylated), although the des-acylated ghrelin is not totally inactive (Stengel & Taché, 2009). The acylation process is facilitated by ghrelin O-acyltransferase (GOAT) the enzyme that catalyzes the conversion of des-acyl-ghrelin (DAG) to acyl-ghrelin via octanoylation. Ghrelin activates hypothalamic orexigenic neurons and inhibits anorectic neurons to induce hunger (Solomon, De Fanti, & Martínez, 2005; Toshinai et al., 2003); accordingly, intracranial ghrelin administration stimulates feeding in mammals (Cowley et al., 2003; Tschöp, Smiley, & Heiman, 2000) and non-mammals (Furuse et al., 2001; Unniappan et al., 2002). In humans, intravenous (IV) ghrelin administration increases appetite and food intake (Akamizu et al., 2008; Druce et al., 2005; Neary et al., 2004). Ghrelin stimulates appetite and food intake by acting on the arcuate nucleus (ARC), an area known for its role in alcohol reinforcement

via opioidergic neurons (Loose, Ronnekleiv, & Kelly, 1991). The highest expression of GHS-R1a's is in the central nervous system (CNS). GHS-R1a is also expressed in several peripheral tissues such as the stomach, intestine, pancreas, thyroid, gonads, adrenal, kidney, heart and vasculature. It is unclear how circulating ghrelin reaches CNS targets. It may do so by crossing the blood-brain barrier (BBB) or by direct diffusion (or passage) as the BBB is incomplete at the ARC (Chollet, Meyer, & Beck-Sickinger, 2009). Local CNS regulation may occur as small quantities of ghrelin are produced in the hypothalamus and thus, may also regulate neurons expressing GHS-R1a's (Carpino et al., 2002).

3.3 Rationale for studying the ghrelin system in alcohol use disorder

In addition to the ARC, GHS-R1a's are also highly co-expressed with dopamine (DA) receptors in other brain regions (e.g., dentate gyrus of the hippocampus, midbrain, substantia nigra, raphe nuclei and ventral tegmental area [VTA]) (Guan et al., 1997; Jiang, Betancourt, & Smith, 2006; Katayama, Nogami, Nishiyama, Kawase, & Kawamura, 2000; Zigman, Jones, Lee, Saper, & Elmquist, 2006). Feeding behavior, DA release and locomotor activity are triggered by ghrelin after intracerebral ventricular (ICV) infusion as well as peripheral ghrelin administration (Jerlhag, Grøtli, Luthman, Svensson, & Engel, 2006; Kawahara et al., 2009; Naleid et al., 2005; Quarta et al., 2009). 6-Hydroxydopamine lesions of the VTA suppress ghrelin's ability to elicit food-reinforced behavior (Weinberg, Nicholson, & Currie, 2011). Alcohol- and food-seeking behaviors share common neurobiological mechanisms, and both alcohol and food exert their reinforcing effects, in part, by increasing DA in limbic regions (reviewed in: ((Leggio et al., 2011); (Tomasi & Volkow, 2013)). Cortico-mesolimbic DA pathways may mediate alcohol's rewarding effects (including craving) associated with its abuse liability (reviewed in: (Koob, 1992; Tupala & Tiihonen, 2004)) Thus, involvement of ghrelin in the DA reward system and the role of the DA reward system in AUD suggest a significant role of ghrelin in the pathophysiology of AUD, as detailed in the animal and human studies summarized below.

3.4 Animal studies:

<u>Ghrelin modulates cholinergic and dopaminergic reward pathways</u>

Ghrelin administration into the VTA increases extracellular concentrations of accumbal DA in mice (Jerlhag et al., 2007). Peripheral administration of ghrelin or direct administration into the intra-laterodorsal tegmental area (LDTg) concomitantly increases release of ventral tegmental acetylcholine and accumbal DA in rats (Jerlhag, Janson, Waters, & Engel, 2012). The non-selective nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine attenuates the stimulatory and DA-enhancing effects of ghrelin infused into the third ventricle (Jerlhag et al., 2006), indicating that nAChRs mediates these neurochemical properties of ghrelin; nAChRs also mediate the locomotor stimulatory and DA-enhancing properties of ghrelin administered into the VTA (Jerlhag, 2008). Only alpha3-beta2, beta3, and/or alpha6 nAChR subtypes are implicated in the central alcohol rewarding actions of ghrelin (Jerlhag, 2008). The same nAchRs (alpha3-beta2, beta3, and/or alpha6) in the VTA mediate the rewarding properties of alcohol, modulating voluntary-ethanol-consumption induced increases in both VTA acetylcholine and accumbal DA

levels (Larsson, Edström, Svensson, Söderpalm, & Engel, 2005; Larsson, Jerlhag, Svensson, Söderpalm, & Engel, 2004). Peripherally injected ghrelin also activates reward-related measures, such as locomotor activity, accumbal-DA release and conditioned place preference (CPP), an established measure of alcohol reward where mice are conditioned to associate an environment with previous alcohol exposure in the absence of alcohol itself (Jerlhag, 2008). In summary, these studies demonstrate that both ghrelin and ethanol share a common substrate, the cholinergic-dopaminergic reward system, for their reward processing properties.

Ghrelin is required for alcohol reward

Another set of experiments demonstrated that ICV ghrelin administration to mice significantly increased alcohol intake compared to vehicle treatment in a 2-bottle (alcohol/water) free choice limited access paradigm, and this increase was even more robust when ghrelin was administered bilaterally into either the VTA or the LDTg (Jerlhag et al., 2009). The effects of ghrelin on the VTA and LDTg were specific for alcohol intake (i.e., food intake was increased by ghrelin ICV in comparison to vehicle but was not affected by bilateral ghrelin administration into either the VTA or the LDTg). Notably, ghrelin administration into the lateral hypothalamus or paraventricular nucleus had no effects on ethanol intake (Schneider, Rada, Darby, Leibowitz, & Hoebel, 2007), confirming that ghrelin works in specific brain reward nodes (i.e., VTA). Furthermore, the effects of ICV ghrelin on alcohol intake were absent in GHSR knockout mice (Jerlhag et al., 2009). Additional experiments reported that the rewarding properties of alcohol (i.e., alcohol-induced accumbal DA release and locomotor stimulation) are also attenuated in ghrelin receptor knockout mice compared to wild type (Jerlhag, Landgren, Egecioglu, Dickson, & Engel, 2011), suggesting that the relationship between ghrelin and alcohol reward is primarily mediated through the GHSR-1a's. In summary, these studies demonstrate that central ghrelin signaling is required for alcohol reward.

*Specificity of Ghrelin receptor (*GHS-R1a*) antagonism for reduction of alcohol reward and consumption*.

Apart from energy homeostasis, gut peptides communicate with pathways involved in the rewarding effects of food (Perello & Dickson, 2014). Ghrelin administration increases the activity of dopamine (DA) neurons in the ventral tegmental area (VTA) and DA release in the nucleus accumbens (NAc) (Abizaid et al., 2006) , suggesting that ghrelin affects motivation and reward processing (Perello & Dickson, 2014). 6-Hydroxydopamine lesions of the VTA suppress ghrelin's ability to elicit food-reinforced behavior(Weinberg et al., 2011). Consistent with the commonality of pathways regulating motivation for food and alcohol (Volkow, Wang, Tomasi, & Baler, 2013), independent labs using different animal models have provided evidence for a role of ghrelin in alcohol-seeking behaviors. Briefly, central or peripheral ghrelin administration increases NAc DA overflow and alcohol reward measured by conditioned place preference (CPP) (Jerlhag, 2008; Jerlhag et al., 2011; Jerlhag et al., 2009). Ventricular, VTA, or dorsolateral tegmental area injections of ghrelin increase alcohol intake (Jerlhag et al., 2009). Alcohol intake, NAc DA release, and alcohol-induced CPP are abolished in ghrelin peptide or receptor knock-out mice (Bahi et al., 2013; Jerlhag et al., 2011; Jerlhag et al., 2009) Furthermore, in mice, rats or prairie voles, GHS-R1a

antagonism reduces NAc DA release, alcohol CPP, as well as alcohol intake, preference and operant self-administration (Cepko et al., 2014; Davis et al., 2012; Gomez & Ryabinin, 2014; Jerlhag, Egecioglu, Dickson, Svensson, & Engel, 2008; Kaur & Ryabinin, 2010; Landgren et al., 2012; Stevenson et al., 2015; Suchankova et al., 2013; Suchankova, Engel, & Jerlhag, 2016; Szulc et al., 2013). These effects do not appear to be due to the caloric content of alcohol as GHS-R1a antagonism also reduces: 1) intake of saccharin (a non-caloric sweetener with high reward value) in mice and rats (Landgren et al., 2011); 2) CPP, where reward is measured alcohol-free (Jerlhag, 2008; Jerlhag et al., 2011; Jerlhag et al., 2009); and 3) the rewarding effects of other addictive drugs which have no caloric content (e.g., cocaine and nicotine)(Engel & Jerlhag, 2014). Finally, we have recently shown that GHS knock-out rats self-administer less alcohol in a short-term binge-like drinking paradigm (Zallar et al., Under Review)

GHS-R1a antagonism inhibits both evoked and spontaneous GABAergic activity in the central nucleus of the amygdala (CeA)

Cruz and colleagues (2013) used quantitative reverse transcription polymerase chain reaction to demonstrate the presence of GHS-R mRNA in the CeA (an area with a key role in regulating ethanol consumption whose GABAergic transmission is enhanced by acute and chronic ethanol administration) and used electrophysiological methods to demonstrate tonic ghrelin signaling in the CeA (Cruz, Herman, Cote, Ryabinin, & Roberto, 2013). In naïve animals, superfusion of ghrelin increased the amplitude of evoked inhibitory postsynaptic potentials (IPSPs) and the frequency of miniature inhibitory postsynaptic currents (mIPSCs); co-application of ethanol further increased the ghrelin-induced enhancement of IPSP amplitude. In chronic ethanol-treated animals, superfusion of the GHS-R1a antagonists D-Lys3-GHRP-6 and JMV 3002 decreased evoked IPSP and mIPSC frequency, revealing tonic ghrelin activity in the CeA. Blockade of GHS-R1a receptors with D-Lys3-GHRP-6 and JMV 3002 had a significant inhibitory effect on both evoked and spontaneous GABAergic activity, suggesting constitutive activation of GHS-R1a's or tonic activity of endogenous ghrelin signaling in rat CeA. Pretreatment of CeA neurons with the GHS-R1a antagonists completely blocked the ghrelin-induced facilitation of IPSP amplitudes, indicating that ghrelin exerts its effect through GHS-R1a's. These results suggest that the ghrelin system may constitute part of a brain pathway modulating reinforcement properties of alcohol consumption, and provide additional evidence for the potential role of ghrelin receptor antagonism as a novel pharmacological approach to treat AUD.

3.5 Human studies:

Blood ghrelin levels, alcohol intake and craving

In healthy volunteers, blood ghrelin levels are significantly reduced after acute alcohol consumption (Calissendorff, Danielsson, Brismar, & Röjdmark, 2005, 2006; Calissendorff, Gustafsson, Holst, Brismar, & Röjdmark, 2012; Zimmermann, Buchmann, Steffin, Dieterle, & Uhr, 2007). Additional studies reported that non-abstinent alcoholics had lower blood ghrelin concentration compared to controls (Addolorato et al., 2006; Badaoui et al., 2008; de Timary et al., 2012), and abstinent alcoholics had increased levels (D. J. Kim et al., 2005; J. H. Kim et al.,

2013; Kraus et al., 2005). Together, these studies suggest that ghrelin levels are suppressed by acute alcohol intake and are increased during abstinence. The PI, Dr. Leggio, conducted a study that provided the first preliminary evidence of a significant positive correlation between blood ghrelin levels and the Obsessive-Compulsive Drinking Scale (OCDS) craving scores in active drinking alcoholic individuals (Addolorato et al., 2006), a finding also confirmed by another more recent study (Koopmann & von der Goltz et al., 2012) . Other studies partially confirmed the relationship between blood ghrelin levels and alcohol craving in alcoholic individuals, albeit only in those with positive family history of alcoholism (Hillemacher et al., 2007) or in females (Wurst et al., 2007). Dr. Leggio led the first longitudinal study of serum ghrelin levels in alcoholic patients over the course of 12 weeks treatment. Ghrelin concentrations were assessed at baseline (T0; after 72 hours abstinence), then repeatedly at 2 weeks (T1), 6 weeks (T2), and 12 weeks (T3); levels in abstinent patients were compared to those who did not remain abstinent during the 12-week treatment period. At baseline, blood ghrelin levels were significantly higher in the non-abstinent group ($p=0.035$) and there was a significant *group x time* interaction effect on ghrelin levels ($F=4.193$, $df=3$, $p=0.012$), [**Figure 1**]. These findings suggest that ghrelin level in early abstinence predicts later relapse. We also found a significant positive correlation between baseline ghrelin levels and craving during the 12-week period (Leggio et al., 2012). In summary, consistent with the preclinical data, human studies show that higher ghrelin levels may be associated with higher craving and alcohol consumption, suggesting that ghrelin is a potential new pharmacological target for AUD. Moreover, a recent PET study (although in a different population of obese subjects) showing that peripheral ghrelin levels were inversely associated with the availability of DA type 2 receptor (D2R) in key reward processing areas such as the caudate, putamen, ventral striatum, amygdala, and temporal lobes, i.e., higher ghrelin levels were associated with lower D2R availability, presumably from increased DA release and receptor occupancy (Dunn et al., 2012). Notably, although only peripheral ghrelin levels were tested, circulating ghrelin may reach CNS targets directly by crossing the BBB (Chollet et al., 2009). Taken together, these results support the hypothesis that ghrelin may influence alcohol craving and consumption via the DA-related rewarding processing.

Figure 1

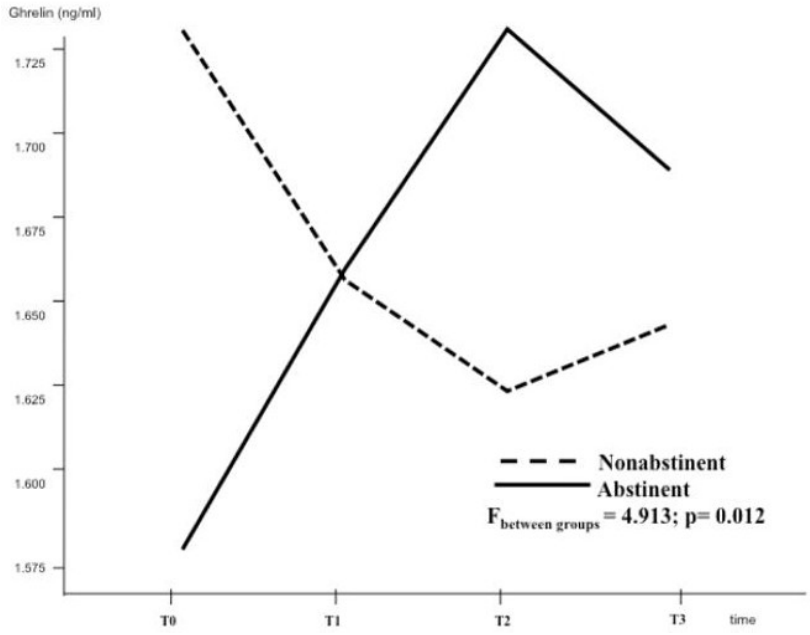


Figure 1 Differences in blood ghrelin levels between abstinent and non-abstinent alcoholic patients.

Human acyl-ghrelin administered intravenously acutely increases alcohol craving in alcohol-dependent heavy-drinking individuals

We conducted the first study administering IV ghrelin to alcoholic individuals (PI:Leggio). In this double-blind, placebo-controlled, between-subject, randomized study, 45 non-treatment seeking heavy drinking alcoholic individuals received a bolus of IV ghrelin 1 μg/kg, IV ghrelin 3 μg/kg or saline solution (placebo) and then immediately participated in a cue-reactivity (CR) experiment in a controlled laboratory setting. During the CR procedure, participants were exposed to alcohol cues (e.g., the sight and smell of one's preferred alcoholic beverage), compared to control cues (while water is usually used in CR studies, juice cues were used in this study to specifically control for non-alcoholic appetitive behaviors). Both subjective (i.e., urge to drink and attention to the cues) and physiological (i.e., heart rate, blood pressure, salivation) responses to cues were collected. The main results of this study (Leggio et al., 2014) showed a main ghrelin effect in increasing the urge to drink alcohol [$F(2,40) = 3.36$, $p = .045$] using a repeated measures ANCOVA (**Figure 2**). Post-hoc comparisons revealed that alcohol urge was significantly greater for ghrelin in 3 mcg/kg than placebo ($p = .046$) (**Figure 2**), with a large effect size ($d = 0.94$). No statistically significant differences were found neither in the ghrelin 1 mcg/kg vs. placebo condition nor between ghrelin 1 mcg/kg and ghrelin 3 mcg/kg. In contrast with urge to drink alcohol, IV ghrelin was not significantly more effective than placebo in increasing urge to drink juice.

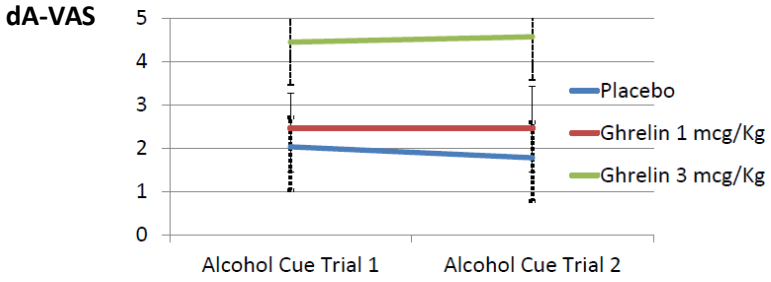


Figure 2. Intravenous ghrelin 3 mcg/kg administration, compared to placebo, resulted in increased urge to drink alcohol, measured as change in an Alcohol-Visual Analogue Scale (dA-VAS) during a human laboratory study with alcoholic individuals. There was a main ghrelin effect in increasing the urge to drink alcohol [F(2,40) = 3.36, p = .045] using a repeated measures ANCOVA. Post-hoc comparisons revealed that alcohol urge was significantly greater for ghrelin 3 mcg/kg than placebo (p = .046), with a large effect size (d = 0.94).

Post-infusion serum total ghrelin level was correlated with the increase in alcohol urge during both the first and the second alcohol trials. Similarly, the maximum serum ghrelin peak level across the six measurements correlated with the increase in alcohol urge during both the first and second alcohol trials. By contrast, neither urge to drink juice nor food craving questionnaire scores were significantly correlated with serum ghrelin levels.

Albeit preliminary, these data have clinical implications given that craving is a predictor of alcohol use. Craving has been proposed as a clinically relevant endophenotype, as higher craving is associated with an increased rate of relapse (Bottlender & Soyka, 2004; Marlatt, 1978; Rohsenow et al., 2001). Furthermore, the CR procedure is well-validated (Leggio, Schwandt, Oot, Dias, & Ramchandani, 2013; Monti, Rohsenow, & Hutchison, 2000) Exposure to alcohol cues can simulate a high-risk situation for relapse and urge to drink, as assessed in the CR, and predict drinking after treatment (Monti et al., 2000). Notably, medications (e.g., naltrexone) that reduce alcohol consumption also reduce alcohol craving during the CR procedure (Monti et al., 2000; Swift, 1999).

Human acyl-ghrelin administered intravenously acutely increases alcohol self-administration and modulates brain activity in alcohol-dependent heavy drinkers

In this second human laboratory study (13-AA-0043; PI Leggio), we hypothesized that IV ghrelin increases intravenous alcohol self-administration (IV-ASA). We further hypothesized that it modulates neural activity in central pathways related to alcohol- and food-seeking behaviors. We conducted two experiments: 1) IV-ASA and 2) functional magnetic resonance imaging (fMRI). Each experiment consisted of two identical visits with crossover, counterbalanced, randomized, double-blind administration of IV placebo or acyl-ghrelin.

IV-ASA: The primary outcome was the number of self-administered alcohol infusions. Participants self-administered a significantly higher number of alcohol infusions under IV ghrelin compared to placebo

[percent change: M (SEM) = 24.97 (10.65), one-sample t-test: t = 2.34, df = 9, p = 0.04; mixed-effects model: F1,80 = 4.86, p = 0.05] (**Figure 3**). The effect size of IV ghrelin in increasing ASA was robust (Cohen's dz = 0.74). We also found that, participants initiated button pressing sooner (F1,8 = 9.71, p = 0.01), receiving their first infusion earlier (F1,8 = 6.18, p = 0.03) under ghrelin compared to placebo (Farokhnia E Grodin MR Lee et al ....and L Leggio). Consistent with preclinical experiments, ghrelin administration increased alcohol intake in AUD heavy drinkers, providing the first evidence in humans for a role of ghrelin in ASA. Notably, we used a progressive ratio procedure, which is commonly used in animal models and provides translational evidence on the role of ghrelin in humans in increasing motivation for alcohol reward.

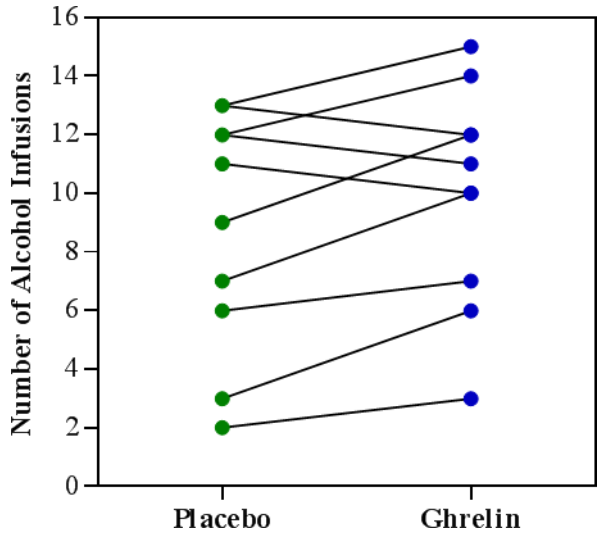


Figure 3 IV ghrelin versus placebo significantly increases the total number of alcohol infusions self-administered during the IV-ASA (p ≤ 0.05). Each line represents a participant and the circles indicate the number of alcohol infusions (some circles overlap because multiple participants administered the same number of infusions during the session; there were missing data for one participant in the placebo condition).

fMRI: We used the Alcohol-Food Incentive Delay (AFID) task, which was developed at NIAAA (Vatsalya et al., 2015) as a modified version of the monetary incentive delay (MID) task. The MID task, also developed at NIAAA (Knutson, Westdorp, Kaiser, & Hommer, 2000), is a widely used validated task to investigate anticipatory reward 60. During the task, participants respond for alcohol, food, or neutral cues to earn points for alcohol (at the end, while still in the scanner, an IV alcohol clamp is administered to rise BrAC to 0.08 mg/dL), food (at the end of the session, participants receive snacks), or no reward. Using a counterbalanced, within-subject, Drug (Ghrelin, Placebo) x Cue type (Alcohol, Food, Neutral) design, we tested whether IV ghrelin affects BOLD activation in response to viewing an alcohol or food cue symbol signaling the receipt of a reward, i.e., anticipation of food and alcohol reward. To control for between-subject differences, participants performed a baseline (pre-administration of ghrelin/placebo) AFID task.

Then, the task was run after IV ghrelin or placebo administration (AFID-1). The task was repeated under IV alcohol administration while still in the scan receiving IV ghrelin or placebo (AFID-2). There were significant Drug×AFID-run×Cue-type interactions in the left amygdala ($F_{4,26.8} = 3.54$, $p = 0.01$) and right medial orbito-frontal cortex (mOFC) ($F_{4,27.0} = 3.66$, $p = 0.01$), and an interaction at a trend level in the left NAc ($F_{4,26.6} = 2.35$, $p = 0.08$) (Farokhnia E Grodin MR Lee et al. ...and L Leggio). Pairwise comparisons indicated that, during the AFID-1, IV ghrelin compared to placebo increases food-related signal in the left NAc ($p = 0.03$). During the AFID-2, IV ghrelin compared to placebo increases alcohol-related signal in the left amygdala ($p = 0.03$) and decreases food-related signal in the right mOFC ($p = 0.04$). We had anticipated overlapping effects of ghrelin on brain activity in response to alcohol and food cues. Rather, our results suggest that distinct brain regions involved in motivation, reward and stress processing are engaged by IV ghrelin in anticipation of alcohol (amygdala) versus food (mOFC, NAc).

GHS-R1a blockade is safe and may reduce craving in heavy drinkers: preliminary findings

We are currently testing PF-5190457, a GHS-R1a inverse agonist. This drug was originally developed by Pfizer for diabetes. Common side-effects were sedation and sleepiness. In a preliminary human laboratory study (14-AA-0042; PI: Leggio), we reported the safety of PF-5190457 in heavy drinkers and we also found that this compound reduces alcohol- and food-induced craving in the bar-lab (Lee et al., 2018). A follow-up Phase 2a human laboratory study is ongoing (16-DA-0080; PI: Leggio).

3.6 RATIONALE FOR STUDYING GOAT AS A NOVEL TARGET TO TREAT AUD

After the discoveries of GHS-R1a (Howard et al., 1996) and then acyl-ghrelin (Kojima et al., 1999), identifying the system in charge of ghrelin's octanoylation was key, as acyl-ghrelin but not DAG binds with high affinity to GHS-R1a. Independent efforts from the Goldstein & Brown (Gutierrez et al., 2008) and Eli Lilly (Lim, Kola, Grossman, & Korbonits, 2011) labs led to the discovery of GOAT (**Figure 1**). GOAT's structure is highly conserved among species (Lim et al., 2011) and its mRNA expression pattern in the body resembles that of ghrelin (Romero et al., 2010). GOAT activity is required for octanylation of ghrelin and is specific for ghrelin only (Damdindorj et al., 2012). The attention to GOAT has recently increased in the ghrelin field. In fact, in addition to its central actions (Banks, Tschop, Robinson, & Heiman, 2002), there is now robust evidence that acyl-ghrelin acts on appetite and food intake via peripheral pathways. For example, GHS-R1a's are located on vagal afferent soma (Date et al., 2002; Grabauskas et al., 2015) and ghrelin-induced food intake is blocked by vagotomy in rodents and humans (Inui et al., 2004; le Roux et al., 2005). Systemic ghrelin administration suppresses vagal afferent firing, and selective blockade of the gastric vagus nerve, either by capsaicin (afferent only) or surgical differentiation (afferent and efferent pathways), blocks c-Fos expression within the arcuate nucleus in response to ghrelin-induced feeding (Asakawa et al., 2001; Benso DH St-Pierre et al., 2012; le Roux et al., 2005). These observations support that, by preventing acyl-ghrelin formation, GOAT inhibition may be a promising approach to block acyl-ghrelin effects mediated by indirect vagal and direct central mechanisms. Furthermore, GOAT inhibition increases DAG and decreases the acyl-to-total ratio. DAG was thought to be non-active, as it binds to GHS-R1a with 1000-fold lower affinity than acyl-ghrelin, and its putative receptor is unknown. It has now been shown that DAG may counteract some of the effects of acyl-ghrelin, e.g., DAG infusion lowers appetite and blood glucose, and enhances insulin secretion (Ozcan et al., 2014). Also, DAG infusion

lowers acyl-ghrelin concentrations, which may be responsible for reduced appetite and improved glucose and insulin secretion (Monteiro, 2014).

Consistent with the physiology and pharmacology of GOAT and the role of the ghrelin system in AUD discussed above, we hypothesize that GOAT inhibition may represent a novel pharmacological approach to treat AUD. Our hypothesis is further corroborated by additional human and rodent data: 1) in our pilot human laboratory study testing PF-5190457 in AUD individuals (14-AA-0042; PI: Leggio), GHS-R1a blockade reduced alcohol cue-induced craving and, in parallel, reduced the acyl-to-total ghrelin ratio (Lee et al., 2018), thus indirectly providing additional support for the hypothesis that the balance between acyl-ghrelin and DAG plays an important role in alcohol craving in humans; and 2) recent independent work conducted in the Kunos lab at NIAAA shows that GOAT inhibition reduces alcohol drinking in mice using a 2-bottle free choice procedure (*Dr. Kunos, personal confidential communication, June 2017*).

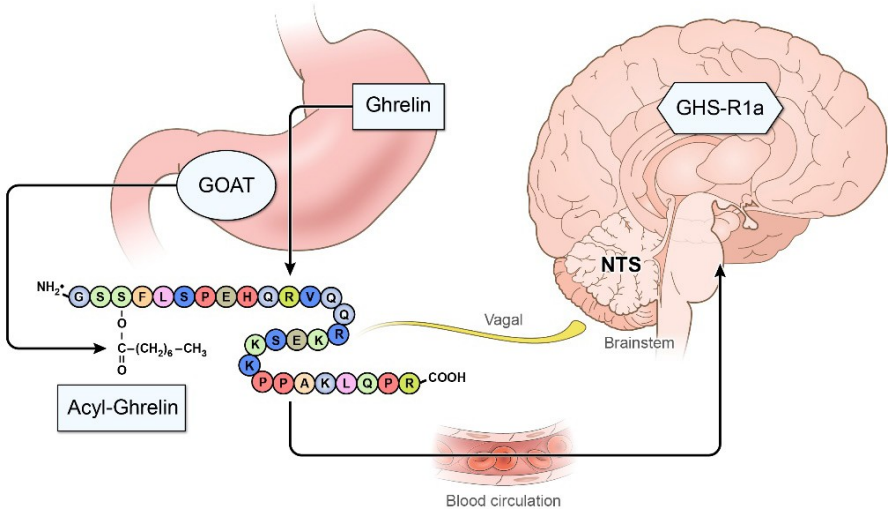


Figure 4. **The Ghrelin System**

3.7 GLWL-01: PHARMACOLOGICAL PROPERTIES AND SAFETY PROFILE IN PHASE 1 STUDIES

GLWL-01: We will test GLWL-01, a highly selective, potent reversible uncompetitive GOAT inhibitor. GLWL Research Inc. has conducted *in vitro* and *in vivo* rodent work and, notably, a first-in-human clinical study in healthy individuals. To our knowledge, GLWL-01 is the first GOAT inhibitor tested in humans. GLWL-01 inhibits human GOAT with an IC_{50} of 192 nM in a primary enzyme assay and 8.20 nM in a cell-based assay. Toxicology and pharmacology studies support GLWL-01 safety. It is readily absorbed with oral bioavailability across distinct species ranging from 69% to > 100%; time to reach maximum plasma concentration is reached consistently within 0.5 hours after oral dosing in rats, dogs, and monkeys (*GLWL Research Inc., unpublished/confidential*). Based on PK data in healthy subjects, both renal clearance and hepatic metabolism are involved in the clearance of GLWL-01. Mean plasma elimination half-life values following oral dose administration ranged from 2 to 5.5 hours across different species.

For additional details, please see the GLWL-01 Investigational Brochure (IB Version Date 17OCT2017).

Previous work with GLWL-01 in humans: The first-in-human study conducted by GLWL Research Inc. established the safety and tolerability of GLWL-01 up to 450 mg b.i.d. in humans; the same highest allowed dose will be used in our clinical study. This is also consistent with an ongoing clinical trial that GLWL Research Inc. is conducting in patients with Prader-Willi Syndrome (ClinicalTrials.gov NCT03274856). GOAT engagement was studied *in vitro* and *in vivo* in mice and humans. *In vitro* and *in vivo* mouse studies show that GLWL-01 dose-dependently reduces acyl-ghrelin and increases DAG concentrations. The first-in-human study also indicates that GLWL-01 up to 450 mg b.i.d. significantly and dose-dependently reduces acyl-ghrelin and increases DAG concentrations (*GLWL Research Inc., unpublished/confidential*). Specifically, administration of single GLWL-01 doses of up to 600 mg QD in healthy subjects, and of multiple (up to 28 days) GLWL-01 doses of up to 600 mg BID in obese patients with type 2 diabetes was safe and generally well tolerated (*GLWL Research Inc., unpublished/confidential*). Doses of GLWL-01 equal to or higher than 150 mg BID resulted in numerical decreases of plasma AG levels in obese patients with type 2 diabetes over 28 days, with the maximum decreases observed for the 450-mg BID dose group. Therefore, a 450-mg BID dosing regimen was chosen for this study as it has been demonstrated to be safe and to achieve target engagement. For additional details, please see the GLWL-01 Investigational Brochure (IB Version Date 17OCT2017).

3.8 INNOVATION

Since 2006, the preclinical and clinical literature on the role of ghrelin and its receptor in AUD has been exponentially increasing. As such, targeting the ghrelin receptor system to treat AUD is a highly innovative approach. Recent translational work conducted by the PI and his team indicate a role of ghrelin in alcohol-seeking behaviors (Farokhnia et al., 2018; Leggio et al., 2014). The first translational step taken by the PI and his team toward medication development has been to block GHS-R1a as a novel target to treat AUD. Safety and preliminary behavioral work have been conducted (Lee et al., 2018) and additional human work is ongoing under our current protocol, 16-DA-0080. The increasing knowledge of the ghrelin system has highlighted the potential role of the GOAT enzyme as a novel approach to target the ghrelin system. Therefore, this protocol is highly innovative as it represents the first attempt to test in humans the potential role of GOAT inhibition in AUD.

4 STUDY OBJECTIVES:

We propose to test the GOAT inhibitor GLWL-01 as a novel treatment for AUD. This will be a Phase 1b / 2a, proof-of-concept (early-signal) human laboratory study with GLWL-01 (450 mg b.i.d.).

GLWL-01 and matched placebo are provided by GLWL Research Inc. under a fully-executed NIH CRADA.

4.1 Primary outcome measures:

The co-primary aims will be to determine:

1) Whether the number of adverse events (AEs) experienced differ in the GLWL-01 condition, compared to placebo

2) Whether GLWL-01, compared to placebo, reduces alcohol cue-elicited craving using a validated alcohol cue-reactivity procedure.

4.2 Secondary outcome measures:

The secondary aims will be to determine:

a. Whether GLWL-01, compared to placebo, reduces food choices in a "virtual buffet" conducted in a virtual reality context.

Additional outcomes measures:

b. We will also monitor behavioral assessments of pain, anxiety, depression, alcohol craving and withdrawal, and smoking.
c. We will also monitor weight as well as food intake via a food intake

5 STUDY DESIGN AND METHODS

5.1 Overview

This study is aimed to test GLWL-01 450 mg b.i.d. for safety and an early-signal of efficacy in treating AUD.

5.2 Design

A within-subject, counterbalanced, double-blind, placebo-controlled study using GLWL-01, in individuals with AUD. Subjects will be randomized to the order of the study drug condition (GLWL-01 and then placebo; or placebo and then GLWL-01) using a block randomization based on the following two variables: sex and smoking status. Associate Investigator Melanie Schwandt, Ph.D., will develop the block randomization grid which will be sent to the NIDA IRP Pharmacy staff. No other members of the study team will have access to the block randomization grid.

5.3 Screening

The initial screening process for this protocol is conducted under the 06-DA-N415 ,"Evaluation of potential research subjects – screening protocol for clinical studies" (hereto referred to as the screening protocol). This is a protocol led by the Office of the Clinical Director (OCD) at the National Institute on Drug Abuse Intramural Research Program (NIDA IRP) to assess potential research participants' eligibility for entering all protocols at the NIDA/IRP. Additional details can be found in the screening protocol documents. As routinely done at the NIDA IRP, the screening procedures and data collected under the screening protocol will capture information above and beyond that which is necessary to determine eligibility for this protocol but allows the Investigators to assess the eligibility criteria for this protocol.

If suitable, subjects will be offered to participate in this protocol. If subjects agree and sign the consent form, they will be enrolled in the study and start the study procedures.

Once enrolled participants may undergo an additional screening process if they leave the inpatient unit between Stages 1 and 2 after being granted temporary leave due to personal emergencies or extenuating circumstances (see Overview). Upon returning to the inpatient unit after the temporary leave, they will

undergo a standard search procedure required by the inpatient unit and an alcohol and drug screening to determine if there are any changes to their eligibility status. A positive BAC or urine during screen that is positive for any of the following substances: benzodiazepines, barbiturates, cocaine metabolites, morphine, oxycodone, methadone, amphetamine, and / or buprenorphine may result in an extension of the wash-out window until they are able to provide a negative urine drug screen and BAC or termination of study participation and not move forward to the Stage 2 based on the clinical judgement of the MAI and PI. The urine drug screening also includes testing for THC/cannabis however this is not an exclusion criterion for this study nor will it result in termination study. The reason why information on cannabis use is collected is because, consistent with the initial drug screening, if cannabis use is detected it will be assessed and entered a covariate in the data analysis.
The inpatient unit is operated and managed by Johns Hopkins Institute for Clinical and Translation Research (ICTR) and has standardized search procedures for any person staying on the unit. This search procedure is to prevent any items that are prohibited or illegal from entering the unit. If any prohibited items are found during the search procedure, the study team will be informed, and the items will be confiscated per ICTR policy. This will not result in the termination of study participation.

5.4 Overview:

Eligible participants will undergo Stages 1 and 2, where study drug (GLWL-01 or placebo) administration will be counterbalanced. A study drug wash-out window of at least 2 days will take place between Stage 1 and Stage 2. During each Stage, subjects will take the study drug twice a day (b.i.d.) for a minimum of 4 days to allow flexibility, maximize feasibility and minimize scheduling problems. At the end of each Stage, the last evening dose of the study medication will not be administered. As part of this study, subjects will be admitted to an inpatient facility (Clinical Research Unit (CRU)) on the Johns Hopkins Bayview Medical Center (referred to as either Bayview or JHBMC) campus and will remain as inpatients for the duration of the two Stages and the wash-out window for an inpatient stay of approximately 14 days. We allow some flexibility to accommodate patient and CRU scheduling needs with a maximum possible 21-day length of total number of inpatient stays allowed. As for many other NIDA clinical protocols, study procedures take place either in the Bayview CRU or the NIDA IRP Biomedical Research Center (BRC) building which are both on the Bayview campus in Baltimore, MD. Participants may only leave the CRU during the wash-out window for personal emergencies and extenuating circumstances pending the approval of the PI, MAI or other covering health care provider who will evaluate the pass request and determine whether the pass may be granted. Participants on approved leave during washout do not receive remuneration while on leave and the total number of days on leave do not count toward the 21-day ceiling of stay in our clinical facilities during their participation in this study.

When the participant returns to the CRU after the temporary leave, they will undergo the standard CRU participant search procedure and alcohol and drug screening. A positive BAC or urine drug screen that is positive for any of the following substances: benzodiazepines, barbiturates, cocaine metabolites, morphine, oxycodone, methadone, amphetamine, & buprenorphine may result in an extension of the wash-out window until they are able to provide a negative urine drug screen and or negative BAC or may

result in termination of study participation and not moving on to the Stage 2 based on the clinical judgement of the PI and MAI. The urine drug screening is identical to the initial drug screen used in the screening procedure and it also includes testing for THC/cannabis however this is not an exclusion criterion nor will it result in termination study. Consistent with the initial drug screening, if cannabis use is detected it will be assessed and entered a covariate in the data analysis. After the inpatient stay, one outpatient follow-up visit in the clinic will be scheduled approximately a week after the discharge (the exact date may vary a little, based on the patient's schedule) to repeat the blood liver panel.

The following research procedures (1-3 listed below) will take place once in Stage 1 and once in Stage 2. These procedures will be the same for Stage 1 and Stage 2. Furthermore, beginning of study procedures may be delayed or participant may be withdrawn from the study for any clinical concerns (for example, clinically relevant severe alcohol withdrawal and/or any other clinical condition requirement immediate medical care).

These study procedures are similar to our ongoing protocol 16-DA-0080, therefore we will be able to compare the potential effects of GLWL-01 versus PF-5190457.

5.4.1 Cue-Reactivity (CR) procedure

This procedure will be similar to that used in our previous studies. The CR procedure is performed in a bar-like laboratory at NIDA IRP. During the CR procedure, participants are exposed to visual, tactile, olfactory, and proprioceptive stimuli associated with the beverage. Participants first will undergo a 3-minute relaxation period (''please sit quietly and do nothing'') to collect pre-CR levels of urge and physiological arousal (this first relaxation period may take place outside in a clinical testing room or inside the bar-like laboratory). There will be a tray containing a glass half full of water and a commercially labeled bottle of water located in the room. An audiotape will instruct the participant to sniff the glass of water when s/he will hear high tones and stop sniffing when s/he will hear low tones. This procedure will include thirteen 5-second olfactory exposures during each 3-minute trial, with variable intervals between each exposure. The water trial provides a controlled baseline that controls for all aspects of stimuli and movement except for the nature of the beverage. After that, another 3-minute relaxation period will follow. Next, participants will undergo a similar procedure with food cues. Food cues will be actual food (e.g. snack foods, etc.) and will be personalized based on participants preferences (participants will be asked about their preferred snacks or other food and the study team will try to match what they prefer to what it is available). Time of last meal will be standardized within- and between-subject. Participants will have breakfast and the CR procedure will take place at approximately noon. Lunch will be served after the CR procedure and food craving will be assessed using the State Food-Cravings Questionnaire (GFCQ). Finally, two 3-minute alcohol cue exposure trials that will be identical to the water trial except the glass of water will be replaced with their preferred alcohol beverage and the bottle of water will be replaced with the appropriate commercially-labeled alcohol bottle. Two alcohol trials will be conducted to gain a stable estimate of participants' reactions to alcohol cues and because two exposures have proven most sensitive to differential effects in previous studies (trials will be presented in the same order for all participants because of known carryover effects). After every 3 minutes of beverage exposure, participants will rate their urge to drink alcohol by completing the Alcohol

Urge Questionnaire (AUQ) (Bohn, Krahn, & Staehler, 1995). The term urge to drink is explained to the subject as "want, desire, craving, thirst for or wish to drink". The AUQ will be complemented by the Alcohol Attention Scale (AAS), which consists of two 10-point Likert-type scales assessing attention to the sight and smell of water and alcohol cues.

5.4.2 Food choices in a virtual reality environment (VR)

As reviewed by Volkow et al. (2013), there is increasing evidence that disruption of energy homeostasis can affect the reward circuitry and that overconsumption of rewarding food can lead to changes in the reward circuitry resulting in compulsive food intake akin to the phenotype seen with addiction. Consistent with this concept, several lines of experimental research demonstrate significant commonalities between the neural substrates underlying addiction and at least some forms of obesity (Volkow et al., 2013). Consistent with this body of literature, it is conceivable that a medication effective in reducing alcohol craving and drinking, may also have effects in reducing appetite and food intake. This is the case, for example, of the FDA-approved naltrexone and of topiramate (for review, see: (Leggio et al., 2011). We will explore the potential role of GLWL-01 administration on food-related behaviors. To achieve this goal, we will use a "virtual buffet" procedure, which is consistent with the same procedure used by AI Dr. Persky at the National Human Genome Research Institute (NHGRI) Immersive Virtual Environment Test Unit (IVETU). The "virtual buffet" has already been tested by Dr. Persky's group in other IRB-approved clinical protocols at NHGRI (Bouhlal, McBride, Ward, & Persky, 2015). This procedure permits us to investigate, in a well-controlled setting, direct observations of patients' food selection behaviors. We used this procedure in protocol 16-DA-0080. Participants will be given verbal instructions and will have a training session on how to use the "virtual buffet". Participants will be instructed to choose foods and a beverage from a hypothetical buffet. In order to perform the virtual reality task, participants will wear a head mounted display connected to computer equipment and will use a pointing device to select the desired types and amounts of food and beverage. Included on the "Virtual Buffet" will be foods and beverages representative of those typically found at buffet restaurants and comprising a range of nutrient profiles and calorie densities. Consistent with the procedures already described in Bouhlal et al. (2015), participants will be instructed to choose as many and as much of the virtual food and beverages during one trip to the buffet as they would normally choose. Once the virtual plate is full, participants will have an opportunity to go back to the buffet and select additional virtual food. The buffet will contain at least two options for each food category that would typically be present at lunch (main dish, vegetable, fruit, starch, dessert, and beverages). Foods will be categorized as "Go" (the healthiest options), "Slow" (less healthy options) and "Whoa" (relatively unhealthy options), as described before (Bouhlal et al., 2015). Participants will indicate their food choice by using a pointing device and then will select the amount of food that will go on the plate (e.g., one spoonful, one piece). Following food selection, participants will be able to choose from several possible beverages. The virtual scenario will end when participants indicate they are finished selecting food and drink. During all phases of the scenario, we will digitally record participants' movement and location in the room, the direction and pattern of participants' gaze, duration of the session, and all details regarding food selection. The patient's food choice behavior will be assessed by calculating the total calories selected including foods and the beverage. Calorie content will be assessed by using the volume of the virtual food chosen by participants to calculate the appropriate number of calories based on information contained in food nutrient databases. Food-related outcomes

will be analyzed, including calorie content of food and drink chosen, patterns of engagement with the food, order of food selection, time spent in the buffet, portion sizes selected, distribution of different food types on the plate, total calorie count of selected food with and without beverages, proportion of high calorie foods, sweetened beverage selection, spatial measurements, and food choice process. At the end of the procedure, participants will also fill out questionnaires to assess participant's experiences in the virtual environment.

5.4.3 Additional data that will be collected:

During the study, the following data will also be collected:

- Alcohol drinking patterns using the Alcohol Timeline Follow Back.
- Smoking patterns using the Smoking Timeline Follow Back.
- The E-cigarette Fagerström Test of Nicotine Dependence (e-FTND) [Only for tobacco vapers/e-cigarette users]
- The urge to drink alcohol will be assessed by the Alcohol Urge Questionnaire (AUQ).
- Food craving using the General Food-Cravings Questionnaire (GFCQ) Trait (at baseline of Stage 1) and State.
- Affective mood states using the Profile of Mood States (POMS-2).
- Measures of sleepiness and possible sedation will be monitored by Stanford Sleepiness Scale (SSS).
- Signs and symptom of alcohol withdrawal will be monitored by Clinical Institute Withdrawal Assessment for Alcohol-revised (CIWA-Ar).
- History of familial alcohol use problems will be assessed by The Family Tree Questionnaire (FTQ).
- Craving for alcohol will also be assessed by Penn Alcohol Craving Score (PACs).
- Anhedonia will be measured by The Snaith-Hamilton Pleasure Scale (SHAPS).
- Personal Preference Log to gather information on food and alcohol preferences for CR procedures.
- Dietary Conditions Log will be used to establish a menu for standardized meals during the study and to assess dietary restrictions and preferences that may affect the food choices in the virtual reality environment. Measurements of pain using the Symptom Checklist
- Blood samples for will be drawn for pharmacokinetic (PK), pharmacodynamic (PD), and research use will be taken during STAGE 1 and STAGE 2 on each dosing day and the last Study Day. Additional blood samples will be taken on experimental days (Virtual Reality Study Procedure Day and Cue Reactivity Study Procedure Day). For all blood collection time points there are 2 x 3mL and 1 x 4mL samples collected for research bloods and 2 x 3mL samples collected for pharmacokinetics and pharmacodynamics. On Dosing Day 1, a 3mL blood sample will be taken for genetics. The genetic sample is meant to be collected for potential future exploratory analysis aimed at investigating pharmacogenetic aspects of GLWL-01. This is approximately 131mL per stage and the maximum PK/PD/research blood collected for the entire study period will not exceed 455mL for any given participant**.** (see "Use of Samples"

section G below for details).

- Waist circumference will be assessed twice during each Stage (first and last day of each Stage) to calculate the body shape index (BSI), which is, compared to BMI, a better indicator of health risks related to weight fluctuations.

Suicidality Assessment will be performed using the Columbia Suicide Severity Rating Scale (C-SSRS) at baseline (day of consent) as well as the (Stage 2), last study day **or** day of discharge if the participant completes study participation prior to (Stage 2), last study day, whichever occurs first.

Upon determination that screening reveals signs of suicidal ideation or concerning behavior, a mental health evaluation will be performed. This evaluation will determine the level of risk and take appropriate steps to ensure the patient's safety.

Subject characterizations:

The following are characterization measures of various behaviors and preferences administered either in questionnaire or task form. With the exception of smoking all of these will be done once at the beginning of the study before study drug is administered. (please see– Study Outline for timing of these procedures).

a) Sweet Preference Test. There is preclinical and clinical evidence linking the consumption of sweets to alcohol intake in both animals and humans (Leggio et al., 2011). We will test the possible effects of individuals' sweet preference characteristic on our study outcomes. Prior to administration, a visual examination of the sucrose solution for any particulate matter will be conducted by a clinician, proceeding only if no particulate matter is identified. Each subject will be instructed to sip, swish around his/her mouth and then spit out five different concentrations of a sweet solution (0.05, 0.10, 0.21, 0.42 and 0.83 M) each of which will be presented five times in a pseudorandom order for a total of 25 tastings. After each tasting, participants will rinse their mouths with distilled water before proceeding to the next solution. Then, participants will rate the intensity and pleasurableness of each tasting using a 200 mm analog scale. Subjects will be characterized as sweet-liker or sweet-disliker based upon their hedonic response to the various sucrose concentrations. To be categorized as a sweet-liker, a subject must rate the highest concentration of sucrose (0.83 M) as the most pleasurable. Sweet-dislikers could prefer any of the other four concentrations. As part of this test, subject will also fill out the Sweet Preference Questionnaire(Kampov-Polevoy, Garbutt, & Janowsky, 1997; Looy, Callaghan, & Weingarten, 1992).

b) Brief Addictive Behavior Social Density Assessment (BASDA). The BASDA is a self-reported assessment where participants self-report on their closest associates' drinking behaviors (Fortune et al., 2013; MacKillop et al., 2013). Participants are not asked to provide the names of their closest biological and non-biological associates. The goal is to explore the social milieu that usually surrounds the participant and to see if this information might help to predict craving behavior.

c) Behavioral approach system (BAS)/behavioral inhibition system (BIS) scale. The BAS/BIS scale was developed to measure individual differences in the sensitivity of the BIS and BAS (Carver & L. White,

1994). The scale is divided into 4 subscales: BAS Drive, BAS Fun Seeking, BAS Reward Responsiveness, and BIS. Research suggests these systems play an important role in vulnerability to addiction. The individual differences in reward sensitivity measured by these scales are especially relevant to addiction when examining the role of the DA reward system. The goal of this assessment in the current study is to further explore how BAS and BIS personality traits relate to alcohol craving and the activation of reward circuitry.

d) Virtual Buffet Questionnaires: The following questionnaires are assessments of individuals' attitudes and cognitions relating to eating and weight that have been linked with behavior change. These characterization measures may help to explain variations in subject behavior in aspects of the trial (e.g., the virtual buffet).

1. Perceived Family History: weight and alcohol problems (Eisenberg, Street, & Persky, 2017)
2. Implicit Theories of Weight, Alcohol Use and Eating (Burnette, 2010)
3. Causal Beliefs Scale for Alcohol Dependence and Casual Beliefs Scale for Body Weight (Link, Phelan, Bresnahan, Stueve, & Pescosolido, 1999; Moss-Morris et al., 2002)
4. Perceptions of Personal Control and Treatment Control (Broadbent, Petrie, Main, & Weinman, 2006)
5. Self-Stigma and Alcohol Dependence (Schomerus et al., 2011)
6. Perceptions of passing down propensity for disease (Persky, McBride, Faith, Wagner, & Ward, 2015)

f) Smoking. The high co-morbidity between alcohol and nicotine dependence reflects a strong connection between alcoholism and smoking (Littleton, Barron, Prendergast, & Nixon, 2007). We will assess the severity of nicotine dependence with the Fagerström Test for Nicotine Dependence (FTND; (Heatherton, Kozlowski, Frecker, & Fagerström, 1991)), cigarettes smoked before and then during the duration of the study (via the TLFB) and Breath CO levels, for possible exploratory analyses, which might guide future alcohol/smoking studies. We will be able to collect the precise number of cigarettes smoked during the inpatient stay via the smoking Timeline Follow Back. Furthermore, when experimental procedures will take place, smoking breaks will be standardized across and between subjects.

5.4.4 Nutrition

Participants will receive standardized meals to control and monitor caloric intake within and across participants. Participants will not be able to eat snacks or meal leftovers ad libitum while on the standardized meals. Food-related data, including % of meal eaten, will be recorded. Participants may receive a regular diet during the washout period.

5.4.5 Study Medication Check

There is a possibility that subjects and/or Investigators might identify or assume they received the GLWL- 01 compound or placebo by recognizing different physiological and/or interoceptive responses to the study medication. Therefore, at the end of each Stage, we will ask subjects and study physician which they believed they received. This information will be recorded and assessed to determine whether

awareness or perceived awareness of study medication type effected the primary and secondary outcomes of the study and to assess whether the double-blind process was preserved.

5.4.6 Measurements for safety monitoring

The following safety measures will be performed repeatedly as per below (and as needed, as the clinical situation dictates):

- **Vital signs (every day during each Stage)**
- **Check for potential adverse events (every day during each Stage)**

5.4.7 Additional Considerations

Medication Dose Justification. The dose of 450 mg b.i.d. is consistent with the previous clinical work conducted by the manufacturer GLWL Research Inc.

Missing data. While missing data will be minimized, we do expect that we will not always be able to collect all planned data (an example of a scenario is missing blood samples due to problems with the cannula, hemolysis, and other technical problems; other examples include schedule conflicts including

e.g. week-ends, holidays, etc.). Nonetheless, every effort is made to minimize these unforeseen problems with equipment or study procedures. If one should occur, we may ask the participant to repeat a specific experimental procedure (if allowed by the overall study schedule, if it is feasible and if the participant agrees) in order to avoid having to discard other usable data from a participant. Subjects may receive additional compensation if experimental sessions are repeated, consistent with the NIDA Remuneration Policy.

Data sharing with other protocols. Data obtained under this protocol and the NIDA screening protocol may be shared and combined for analysis. This will also allow us to avoid repeating assessments that are scheduled in both protocols during the same period of time, therefore avoiding duplication and minimizing participant fatigue. Participants may also consent for other NIH protocols and data collected under those protocols may be combined with data from this protocol for exploratory purposes.

This protocol does not meet criteria for genomic data sharing. NIH Human Data Sharing (HDS) policy is not applied in this protocol because it is limited by the agreement (CRADA) with GLWL Research Inc.

5.5 Inclusion and Exclusion Criteria

5.5.1 **Description of study population**

This study will enroll individuals with AUD.

5.5.2 Inclusion/Exclusion criteria

5.5.2.1 Inclusion criteria

___ ____ Alcohol Use Disorder (Minimum 2 symptoms on a validated diagnostic tool e.g., the Mini-International Neuropsychiatric Interview (MINI) or the Structured Clinical Interview for DSM Disorders (SCID)

____ ____ Male or female individuals 18-70 years old (inclusive)

____ ____ Able to speak, read, write and understand English

____ ____ Most recent urine drug test for benzodiazepines, barbiturates, cocaine metabolites, morphine, oxycodone, methadone, amphetamine, & buprenorphine is negative.

____ ____ Most recent Clinical Institute Withdrawal Assessment for Alcohol – revised (CIWA-Ar) score is ≤ 8

Males only:

____ ____ Males agrees agree to sexual abstinence or to use a reliable method of birth control during the study and 3 months following the last dose of the study drug. Acceptable methods of birth control may include: 1) condom with spermicide; 2) diaphragm with spermicide; or 3) female condom with spermicide.

Females only:

____ ____ Women of child-bearing potential may participate in the study:

1. if they test negative for pregnancy (based on a urine pregnancy test) prior to initiation of treatment

2. they must also agree to use either 1 highly effective method of contraception or a combination of 2 effective methods of contraception during the study.

Highly effective method may include hormonal contraceptives (e.g., combined oral contraceptives, patch, vaginal ring, injectables, and implants); intrauterine device or /intrauterine system; vasectomy and tubal ligation.

Effective methods may include barrier methods of contraception (e.g., male condom, female condom, cervical cap, diaphragm, contraceptive sponge)

Women may choose to use a double-barrier method of contraception. Barrier methods without concomitant use of a spermicide are not reliable or an acceptable method. Thus, each barrier method must include use of a spermicide. It should be noted that the use of male and female condoms as a double-barrier method is not considered acceptable due to the high failure rate when these methods are combined.

OR

____ ____ Women not of child-bearing potential may participate in the study and include those who have

- spontaneous amenorrhea for at least 12 months, not induced by a medical condition such as anorexia nervosa and not taking medications that induced amenorrhea e.g., oral contraceptives, hormones, gonadotropin-releasing hormone, anti-estrogens, selective estrogen receptor modulators, or chemotherapy; or

- spontaneous amenorrhea for 6 to 12 months and a follicle-stimulating hormone (FSH) level greater than 40 mIU/mL; or

- women with a history of hysterectomy or bilateral oophorectomy must be at least 40 years of age and FSH >40 mIU/mL.

5.5.2.2 Exclusion criteria

___ ___ Lifetime clinical diagnosis of schizophrenia or bipolar disorder

___ ___ BMI < 18.5 kg/m^2 and weight less than 60 Kg (both must be met)

___ ___ BMI ≥ 40 kg/m^2

___ ___ History of epilepsy and/or seizures

NOTE: individuals who have a history of alcohol withdrawal seizures may be in the study as long as they have been abstinent from alcohol for at least 2 weeks prior to consent and during that period of abstinence, there were no seizure episodes (otherwise, participant remains not eligible).

___ ___ Creatinine ≥ 2 mg/dL, AST or ALT > 1.5x the upper normal limit, hemoglobin <10.5 g/dl

___ ___ Diagnosis of liver cirrhosis

___ ___ Clinically significant history or current eating, pituitary or adrenal gland disorders or disorder of gastric

motility as judged by a study clinician as determined from medical history and/or current clinical screening

information

___ ___ Current thyroid disorders that are not stable on dose of FDA-approved medications for that disease, as judged by a study clinician as determined from medical history and/or current clinical screening information. A pre-existing stable medical condition is defined as a disease not requiring significant change in therapy or hospitalization for worsening disease during the past 3 months.

___ ___ Clinically significant abnormal 12-Lead ECG

___ ___ QTcF > 450 msec for men and > 470 msec for women

___ ___ Family history of Long QT Syndrome.

___ ___ Patients on weight loss medications within 30 days of dosing

____ ____ Patients with a history of bariatric surgery

____ ____ Diagnosis of diabetes and currently on medication

____ ____ Unable to refrain from or anticipates the use of:

- Any drugs known to be significant inhibitors of cytochrome P450 (CYP)3A enzymes and/or P-glycoprotein (P-gp) including regular consumption of grapefruit or grapefruit juice for 14 days prior to the first dose of study medication.
  [medications like acetaminophen (up to 2 g per 24-hour period) and ibuprofen may be permitted during the study]
- Any drugs known to be significant inducers of CYP3A enzymes and/or P-gp, including St. John's Wort, for 28 days prior to the first dose of study medication.
- Any medications that prolong the QTcF, unless the patient has been stable on the medication for at least 3 months and has a QTcF equal to or < 450 msec
- Benzodiazepines. If a participant received a benzodiazepine as part of their treatment during the alcohol detoxification, then s/he can still be enrolled. However, 5 half-lives (for that benzodiazepine) will be required to elapse before the anticipated date of first study drug administration

____ ____Currently taking simvastatin >10 mg per day, atorvastatin >20 mg per day, or lovastatin >20 mg per day.
The doses of these statins in combination products should not exceed these defined dose levels.

____ ____ Patients with a history of statin-induced myopathy/rhabdomyolysis.

____ ____Vision is unable to be corrected to (Snellen) 20/100

____ ____ Clinically-significant history of motion or car sickness, or history of vestibular disorders

____ ____ FIB-4 Score > 1.30

____ ____ Any other reason or clinical condition for which the PI or the MAI or other study clinician will consider unsafe for a
possible participant to participate in this study

These criteria are based on the target population for this study. Other criteria are consistent with an ongoing study that GLWL Research Inc. is conducting with GLWL-01 in patients with Prader-Willi Syndrome (ClinicalTrials.gov NCT03274856).

6 COLLECTION AND STORAGE OF HUMAN SPECIMENS OR DATA

6.1 Use of samples

Blood samples will be used for:

Research use: samples may be used for future analyses performed either at NIH laboratories or other labs outside NIH (the latter will be pending IRB approval and execution of appropriate agreement). Future research use of these samples includes blood ghrelin levels, GLWL-01 concentrations, biomarkers like feeding-related peptides (e.g., leptin, GLP-1, amylin, GIP, pancreatic polypeptide, PYY, growth hormone (GH), thyroid hormones, insulin, C-peptide, etc.), stress-related hormones (e.g., ACTH, cortisol, etc.) and/or inflammatory markers (e.g., cytokines, etc.), and other biomarkers which may be related to the outcomes of the study. Furthermore, as part of the fully executed CRADA between GLWL Research Inc. and NIH, GLWL Research Inc. may receive de-identified stored blood samples collected in Dr. Leggio's NIH clinical study in order to run biomarkers e.g. blood concentrations of hormones like acyl-ghrelin, UAG and other biomarkers well as blood concentrations of GLWL-01. This latter aspect of the CRADA is planned to generate consistent pharmacokinetic / pharmacogenetic / pharmacodynamic data between Dr. Leggio's clinical study and clinical trials that GLWL Research Inc. is conducting in parallel. The use of the same assays will allow for side-by-side comparison and analysis, therefore providing the best possible information on the role of GLWL-01 on ghrelin signaling across populations affected by different medical disorders.

Clinical use: samples (e.g., glucose, AST, ALT, GGT, bilirubin, creatinine, thyroid and electrolytes) will be analyzed at the Johns Hopkins Bayview Medical Center clinical laboratories or other clinical laboratories approved by the NIDA IRP Clinical Director. In addition to what outlined in the Study Outline, clinical blood labs can be repeated and/or additional clinical labs (blood/urine) may be performed if deemed clinically necessary by the clinicians involved in the study.

6.2 Sample Storage

Electronic data will be stored on the NIDA IRP secure, password-protected electronic medical records system (Clinical Data Warehouse; CDW; Human Research Information System (HuRIS)). Paper records are stored under double lock in an area where individuals who are not part of the study staff do not have access. The existence and types of information contained in the data management system have been publicly reported as required by the FOIA.

Samples not used immediately for clinical reasons will be processed and stored in -80 °C freezers at the NIDA IRP, Baltimore, MD. All biological specimens obtained under this protocol will be stored in coded form (protocol plus subject number) in freezers located an access-controlled area of NIDA.

7 STATISTICAL ANALYSIS

7.1 Analysis of data/ study outcomes

Outcome data will be examined for homogeneity of variance, and if necessary transformed to meet this criterion. As this is a within-subjects design, repeated measures analysis of covariance (ANCOVA) will be used to analyze the primary outcome, with drug condition as the within-subjects factor. Similarly, for secondary outcome measures such as food choices, repeated measured ANCOVA will also be used.

Biomarkers and hormonal measurements, such as feeding-related peptides stress-related hormones and/or inflammatory markers (see Section G,1), will also be correlated with changes in primary and secondary outcomes to explore the relationship between hormonal responses of interest and behavioral changes. A probability level of 0.05 or lower will be considered significant.

Potential covariates will be evaluated such that covariates that significantly predict the outcome measure will be retained in the model. Negative affect, as measured by the POMS-2 and Symptom Checklist, and cannabis dependence, if present, will be entered as covariates of particular interest in analysis for primary and secondary outcome responses. Additional covariates include baseline characteristics, like age, race, years of education, alcohol drinking levels, ADS score, family history of alcoholism density and number of days of alcohol abstinence prior to enrollment.

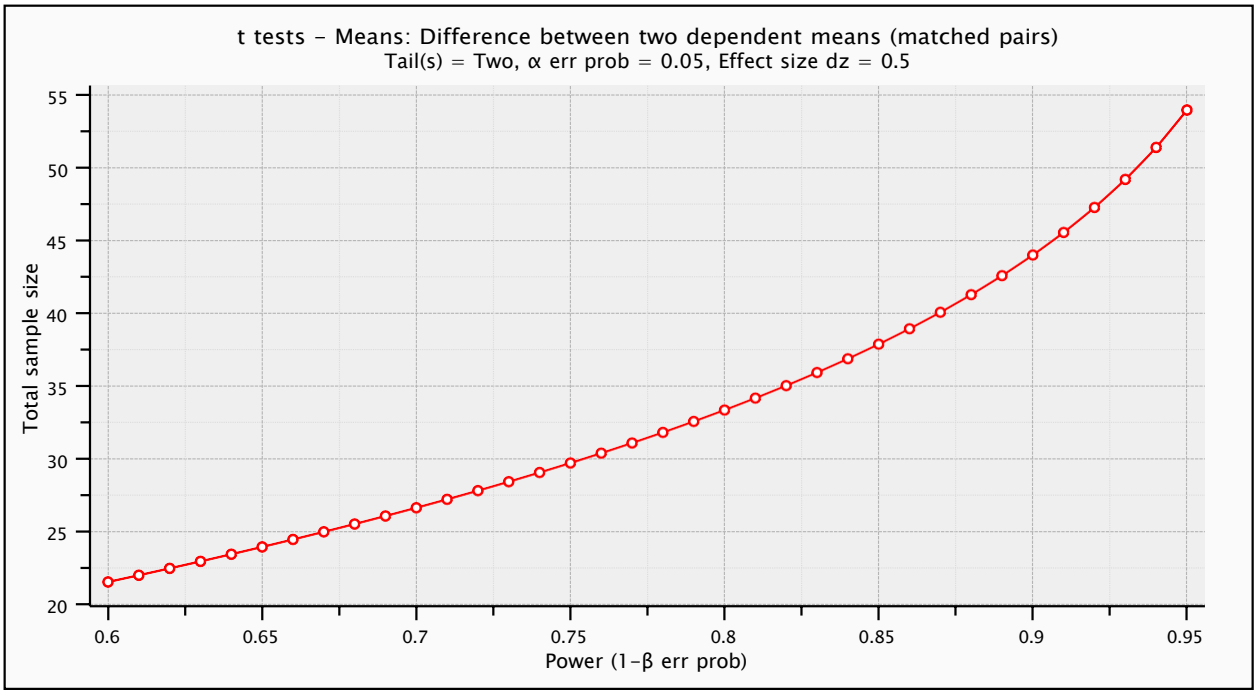
7.2 Power analysis

We performed a power analysis for the primary outcome and it is consistent with: 1) our previous Phase 1b study (N = 12 completers) with PF-5190457, where the primary outcome was the number of AEs experienced differ in the drug condition, compared to placebo; and 2) effect sizes (dz = 0.5) of alcohol cue-reactivity studies for other medications like the FDA-approved naltrexone and other experimental compounds (George et al., 2008; Miranda et al., 2014; Monti et al., 1999, 2000). The goal will be to reach up to 34 completers. A sample size of 43 subjects will be needed: a) considering the following parameters: dz = 0.5, α err prob = .05, and power (1-β err prob) = .80 (see Table 1 for additional details); and b) considering an attrition rate of approximately 20%.

Table 1: Power Analysis

t tests - Means: Difference between two dependent means (matched pairs)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	Two
	Effect size dz	=	0.5
	α err prob	=	0.05
	Power (1-β err prob)	=	0.8
Output:	Noncentrality parameter δ	=	2.915476
	Critical t	=	2.034515
	Df	=	33
	Total sample size	=	34
	Actual power	=	0.807778



7.3 Accrual number

Consistent with the within-subject design and other human laboratory studies we and others have performed, we anticipate that approximately 20% of the enrolled participants may not complete the study. Therefore, we anticipate enrolling up to 43 participants in order to reach up to 34 completers.

8 HUMAN SUBJECTS' PROTECTION PLAN

8.1 Consent documents and process

Procedures conducted before obtaining consent for this protocol
Screening: subjects are prescreened during a phone screening which is part of the NIDA screening protocol. Then, potential candidates are screened in-person under the NIDA screening protocol, which will provide the necessary data to determine eligibility to this protocol.

Clearance for eligibility: inclusion/exclusion criteria will be reviewed by a team of three individuals and signatures will be required in order to establish participants' eligibility to this protocol. Once cleared for eligibility participants, will be contacted and scheduled for consent procedures.

8.1.1 Consent procedure

On the day of consent eligible participants will undergo an alcohol and drug screening. A positive BAC or urine drug screen (benzodiazepines, barbiturates, cocaine metabolites, morphine, oxycodone, methadone, amphetamine, & buprenorphine and/or any other clinical condition requiring further assessment and evaluation may result in consenting procedures being either rescheduled or the participant being classified as ineligible. Eligible subjects are enrolled in this protocol after the study-specific consent form for this protocol is obtained. Authorized study investigators will obtain informed consents for this protocol. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants as detailed in the consent form. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing as a copy of the consent will be handed to the participant during their screening visit and again on the day of consent. Participants will be asked to sign and date the informed consent. A copy of the signed and dated consent will be given to each participant. As part of the consent procedure, participants' ability to understand the consent will be evaluated using the 'NIDA/NIAAA Evaluation of Potential Research Participants' Ability to Consent'. Subjects must answer all questions correctly to proceed with consent signing.

Consent Review Process (in person or remote)

After the potential participant has had the opportunity to review the consent form, the PI or co-investigator may conduct the verbal review of the consent form a. in a face-to-face discussion in a private study room or b. via encrypted video chat program or c. via a remote-onsite video conference between on-site private study rooms, all approved by NIDA's ISSO. The remote platforms allow a virtual face-to-face interaction which we believe enhances the consent process while also reducing person-to-person contact. The format to be used will be decided by the PI or co-investigator in consultation with the prospective participant. Regardless of the platform selected for the review of the consent, e.g., face to face or remote from outside of NIDA or within NIDA's physical space, the PI/co-investigator will obtain the signature of the participant in person only after answering all questions and confirming participant

understanding of the protocol including completion of the NIDA/NIAAA Evaluation of Potential Research Participants Ability to Consent.

Consent Signing Process (paper or electronic)

Signatures will be obtained on the consent in-person only, there will be no remote consent signing process for this study. Signatures will not be obtained until a. the PI/co-investigator has verified the identity of the participant including confirmation of the individual's name and birthdate b. the PI/co- investigator has confirmed all questions raised by the prospective participant have been adequately answered and c. the PI/co-investigator have completed the NIDA/NIAAA Evaluation of Potential Research Participants Ability to Consent. Documentation will be provided confirming all three assessments have been completed in CDW on the day of consent.

The PI or co-investigator along with the participant have two options for applying their signature and date to the consent (PDF) document prior to uploading in the electronic medical record in CDW. a. manual pen/paper signing may take place. Once the participant and PI/co-investigator have both manually signed and dated the paper consent, a member from the research team will scan and upload the signed consent into CDW or b. electronic signing may take place on the PDF consent document. Adobe Acrobat endorsed by the NIH and is the approved program installed by NIDA BIS to allow for the safe and secure electronic signing of PDF documents in CDW and is 21 CFE part 11 compliant. The following steps will be followed to obtain electronic signatures.

1. The PI/co-investigator log into CDW using their unique identification code/password. This ensures the authenticity, integrity and confidentiality of electronic records by authorized individuals.
2. Open the PDF document to be signed on the designated government approved device (laptop/tablet)
3. Click the Sign icon (alternatively click 'Fill & Sign') in the toolbar.
4. Hover the mouse over the field on the PDF where you want to add your signature and date
5. Use the stylus to import a signature image in the selected field.
6. By default, the signature color is black
7. Click apply and then click at the place in the PDF where you want to place the signature.
8. Click save, when you save the document, the signature, and date become part of the PDF and a permanent record in the participant electronic medical record in CDW.

Whether the consent is signed via pen/paper or electronic, the signed/dated copy of the consent will be printed out and delivered to the participant on the day of consent. Note, only CDW (EISnet) network compatible printers can be used to print out signed consent documents on site at NIDA further ensuring restricted access to documents containing PII to authorized personnel only and on an as needed basis.

8.2 Subject Selection

Adults who fulfill the qualification criteria will be included, regardless of race, ethnicity, sex, or religious

affiliation. Individuals must be able to speak, read, write, and understand English as this study employs scales and experimental procedures that are validated only in English. This includes the assessments conducted to test the primary and secondary outcomes and is therefore required to maintain the research integrity of the study.

8.3 Recruitment

Consistent with the other clinical protocols in the NIDA IRP, recruitment efforts will take place as part of the NIDA screening protocol. Participants will be recruited through referrals from the NIDA Recruitment Contractor, NIH Volunteer Office, NIH clinicians and from the NIH Office of Patient Recruitment as well as through ResearchMatch.org (facilitated through OPR). ResearchMatch is a voluntary service that matches people interested in being research participants with researchers conducting a wide range of studies.

ResearchMatch is currently used for other protocols in our Section, as well as for many other NIH protocols. ResearchMatch is a Clinical and Translational Science Awards (CTSA) initiative funded by the National Center for Advancing Translational Sciences (NCATS), part of the National Institutes of Health. Furthermore, participants will also be recruited by word of mouth and through local pre-approved advertisements created by the recruitment contractor. Advertisement language will be used as flyers with tear off tabs, posted on the NIDA and Johns Hopkins campuses and universities, colleges, local business establishments and medical institutions in the greater Baltimore Maryland metro area and in the DC area. Additionally, advertisements will be placed on billboards of public places and public transportation services in the greater Baltimore metro area. Advertisements will also be posted on the radio, in electronic (including social media) and printed local media, including newsletters, websites, and local newspapers in the greater Baltimore area (for example, the 'Baltimore Examiner'). Listserv ad will be used on craigslist.org, as well as in NIH and other local email distribution lists, that are moderated, and following approval from the managers of these lists. 'ClinicalTrials.gov' may also represent a source of recruitment. Finally, participants will also be recruited via the NIDA Screening Protocol), as these protocols serve as the screening protocols not only for this study but also for other protocols at the NIDA Intramural Clinical Program.

8.4 Children

Children < 18 years of age will not be studied.

8.5 Vulnerable populations

Vulnerable populations will not be studied.

8.6 Evaluation of Risks/Discomforts and Benefits ratio

Anticipated Benefit

This study does not offer direct benefit to participants.

9 PROTECTION OF PARTICIPANTS' PRIVACY AND CONFIDENTIALITY

This protocol will be covered by a Certificate of Confidentiality (CoC). Strict subject confidentiality will be maintained throughout the study. Confidentiality and information technology standards are in place at the NIDA intramural program to protect electronic repositories of patient data as well as other clinical patient related material. It is reasonably expected that these safeguards will protect participants' medical and personal health information, ensuring their privacy.

Information obtained in the course of participating in this protocol will become part of the patient's NIH medical record. Third parties may request access to this information. However, access will not be granted without the explicit, written consent of the subjects. Samples and data will be stored using codes (protocol number plus participant study number) that we assign. Only the PI, MAI, AIs and authorized staff can link the codes back to the subject's identity. Data will be kept in password protected computers. Samples will be kept in locked storage. Only study personnel will have access to the samples and data.

10 STUDY AGENTS/INTERVENTIONS

10.1 Study Drug

Based on GLWL-01 nonclinical and preliminary clinical data (for details, please see Investigational Brochure, IB Version Date 17OCT2017), no clinically significant safety or tolerability concerns have been identified in patients or subjects to date for GLWL-01 up to the highest single dose given 600 mg in healthy subjects and multiple doses given 600 mg BID for up to 28 days in obese patients with type 2 diabetes. Of note, this protocol will test a lower dose compared to the maximum dose previously tested and the total days of drug exposure will also be lower. However, it is also important to keep in mind that GLWL-01 is investigational, and all of its possible side effects may not be known. There may be rare or unknown side effects and some of these may be life-threatening. Drug administration may be temporarily held or stopped due to side-effects or other safety concerns.

As of June 2017, GLWL-01 has been given to 24 healthy overweight/obese humans and 38 obese patients with type 2 diabetes mellitus in a clinical study. The most commonly reported side effects in this study were headache, diarrhea, decreased appetite and stomachache. From preclinical and clinical studies (hERG and single/multiple dosing human studies) conducted by GLWL Research Inc., the proarrhymic potential of GLWL-01 is low (GLWL-01 Investigational Brochure, IB Version Date 17OCT2017). Specifically, single 12-lead ECG's were collected in 28-day MAD dosing persons with diabetes up to 600 mg bid (patients were excluded for ECG's that demonstrated findings that might obscure safety evaluation, including conduction abnormalities). The following were noted (GLWL Research Inc., unpublished data and personal communications): a) one subject treated with GLWL-01 600mg BID dose experienced the following changes from a baseline QTcB of 396 ms: on Day 3, QTcB was 477 msec, then again on Day 3 two hours later it was 474 msec, and finally on Day 5 it was 408 ms. Three subjects dosed with GLWL-01 at 450 mg BID had QTcB as follows after dosing: 445 msec on Day 10 in one subject; 459 msec on Day 5 in one subject; and 457 msec on Day 21 in one subject. In summary, three cases of QTc prolongation (> 450 msec) were observed, but there were no arrhythmias and no cardiovascular symptoms. There were no QT intervals measured that were >500 msec (a cut-off that, if persistent, is a risk factor for the development torsade's de pointes).

The ongoing clinical study conducted by GLWL Research Inc. in Prader-Willi Syndrome patients is dosing GLWL-01 at the 450 mg BID level; thus far, there has not been a signal for QTc prolongation. In this Prader-Willi Syndrome study, the investigators are using the Fridericia equation for QT correction which should minimize false positives that may arise from concurrent increases in heart rate (GLWL Research Inc., personal communications on September and October 2018).

Update (May 6, 2022): during the execution of this clinical study, a moderate adverse event of transient asymptomatic transaminitis was reported in one participant (possibly related to the study drug) and the consent was updated accordingly. Of note, this potential risk was already described in the IB from rodent studies conducted by the manufacturer.

Study drug and matched placebo will be provided by the company (GLWL Research Inc.) and its storage, dispensing and waste processes and procedures will be managed by the NIDA Pharmacy. Study drug is administered by NIDA IRP and CRU nurses and study physicians following instructions provided in CDW order entered by the PI, MAI or other authorized NIH health care provider.

10.2 Blood draw

The total amount of research blood to be drawn for this protocol will be up to 461 cc. Risks of blood draws include discomfort or possible bruising (hematoma) at the site of needle entry. For blood draws, there is also a small risk of fainting or infection at the site of the needle stick. Risks from blood draws are minimized by experienced medical personnel who will perform these procedures using sterile technique and following universal precautions.

10.3 ECG recording

Subjects may experience some discomfort when electrodes will be removed from the skin, which should resolve quickly.

10.4 Rating Scales, Questionnaires and other Behavioral Assessments

Participants are asked to complete rating scales and questionnaires (paper-and-pencil or computerized) as part of the study. Some individuals may feel emotional discomfort answering some of the items on rating scales. Such stress will be managed by the study team. If needed, participants have immediate access to licensed health care professionals.

10.5 Cue-Reactivity

Alcohol cue-reactivity experiments are performed on a regular basis by Dr. Leggio's team. Increased craving and distress may appear during the CR procedure. Staff members will monitor the participant via 1 way mirror in the 'bar-like' room and the participant will always be able to communicate with staff members. Should clinically significant symptoms appear, participants will have immediate access to licensed health care professionals. Additionally, these risks are minimized by the inpatient design of this protocol. Of note, alcohol cue reactivity experiments are performed only during the cue reactivity procedure, and the study does not include any alcohol administration at any point.

10.6 "Virtual Buffet"

Previous studies conducted at the NHGRI Immersive Virtual Environment Test Unit (IVETU) located at the NIH Clinical Center show the safety of this experimental procedure. In the virtual environment encounters previously conducted at NHGRI, there were no incidences of serious cybersickness. Some individuals may experience mild symptoms of motion sickness (also called cybersickness in this context). These symptoms may include mild dizziness, headache, eyestrain, blurred vision, light sensitivity, and/or nausea. Such symptoms are rarely strong, and we will discontinue the virtual session should participants report onset of these symptoms. We will continue the virtual session only if the participant desires, all symptoms have subsided, and a clinician approved the continuation of the experiment. Participants will be screened so as to exclude individuals with conditions or history that would make them particularly susceptible to cybersickness. Furthermore, they will be instructed and reminded to report any symptoms during the encounter. Dr. Leggio's team is experienced in executing this procedure in one of his ongoing clinical protocols.

Counseling: At the end of the study, consistent with previous studies from our team (e.g. 13-AA-0040; 13-AA-0043; 14-AA-0042, 16-DA-0080 and 17-AA-0093) participants will be debriefed on their drinking and receive counseling guidance and recommendations aimed at enhancing their readiness for behavioral change and treatment as recommended by the NIAAA guidelines (NIAAA, 2005). This counseling session will follow the NIAAA pamphlet "*Helping patients who drink too much: a clinician's guide*" (US Department of Health and Human Services, 2005). Their alcohol consumption pattern and quantity will be reviewed with them and linked to consequences they may have experienced; clear recommendations will be made for them to reduce or stop drinking.

10.7 Risk of compensation for tasks

Although we expect that this will be rare, it is possible that being compensated will cause a participant with alcohol dependence to desire to purchase alcohol to such an extent that they will leave the CRU against medical advice. In order to decrease the risk of this, participants will not be paid in cash on the same day of testing.

11 PLAN FOR REPORTING UNANTICIPATED PROBLEMS AND ADVERSE EVENTS

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), serious adverse events are defined as described in NIH HRPP Policy 801-Reporting Research Events. The NIH Principal Investigators (PIs)/designee and, as applicable, non-NIH Investigators must report events to the IRB via the Reportable Event Submission Form (REF) in NIH iRIS. All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review in accordance with this policy.

Consistent with the Common Terminology Criteria for Adverse Events (CTCAE), an adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease

temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure.

12 DATA SAFETY AND MONITORING PLAN

The DSMP is consistent with the NIH policy 503 Specifically:

1. *Monitoring mechanism*: this study is monitored by the NIDA/NIAAA Addictions DSMB

2. *Frequency of the monitoring*: Initial DSMB review takes place before the study begins. Subsequent DSMB reviews take place after each 10 patients who have been exposed to study drug, unless otherwise recommended by the DSMB during the interim monitoring reviews
3. *Stop or change rules*: The following rules are followed:
   3a. Criteria for individual subject withdrawal:
   - A Serious Adverse Event (SAE) that is judged by the Investigators as being related to the study drug;
   - At the discretion of the PI and/or MAI and/or other health care provider involved in the study based on tolerability as well as adverse event (AE) severity, or self-reported symptoms and/or observed changes in patient's well-being as reported by health care providers including clinically significant changes in vital signs, weight/BMI, blood/urine laboratory values, etc.
   - ECG's (See Study Outline) will be monitored during each Stage as follows:
     ECG 12 lead with QTcF measurement (Fridericia equation) collected during STAGE 1 at Baseline (prior to dosing), Dosing Day 3, on Last Study Day, and on Washout Day -2 (or last Washout day if washout period extends beyond two days). ECG 12 lead with QTcF measurement (Fridericia equation) collected during STAGE 2 at Dosing Day 3 and Last Study Day. The ECG will be timed to coincide approximately with peak drug level. Specifically, according to the manufacturer data, Tmax ranged 1-6 hours at steady state in humans. Therefore, ECG will be done within 6 hours post study drug administration. If there is prolongation of the QTcF interval, either to >500 ms, or to > 60 ms over that on Day 1 ECG AND the absolute QTcF > 450 ms for males or >470 ms for females (**both criteria must be met**), ECG will be repeated and evaluated;

   - Significant non-compliance with protocol procedures or Investigator request;
   - Patient request.

4. *Advanced plans for any interim analyses and/or futility analyses*. There are no current plans for interim analyses and/or futility analyses

5. *Information to be monitored*. Progress of the study, including assessment of participant recruitment and accrual and adverse events are reviewed to determine whether there is any change to the risk: benefit ratio of the study. Specific parameters that are monitored include vital signs and potential adverse events.

6. *Communication*. The information to be monitored referenced above is reported to the DSMB

at the time of the DSMB, to the Clinical Director (via iRIS) and IRB at the time of continuing review, and to the FDA at the time of annual report. Adverse events are recorded and reported to the Clinical Director, the NIH IRB, the Addictions DSMB, and the FDA in accordance with all NIH requirements for adverse event reporting

13 CLINICAL MONITORING PLAN

Quality assurance (QA) will be performed by the Investigators, as well as by a QA monitor, which according to established practice is considered sufficient for small, single-site trials, like the present project. The QA monitor for this study will have considerable expertise in clinical trials and will monitor the study binders on a regular basis and consistent with recruitment pace. Additionally, quality assurance will be monitored independently by the NIAAA/NIDA Combined Monitoring Plan, Intramural Research Program Auditing Committee (IRPAC) Coordinated by the Offices of the Clinical Director at NIAAA and NIDA. The NIAAA/NIDA Combined Monitoring Plan, Intramural Research Program Auditing Committee (IRPAC) monitors intramural research studies to ensure compliance with GCP, organizational policies and applicable federal, state and local laws and the reliability of study data.

14 COMPENSATION

Participants will be compensated consistent with NIDA remuneration policy for time and research-related inconveniences, to the extent to which they complete them, as follows:

Procedure	**Compensation**
Stage 1 and Washout	
Virtual Buffet…….	$150.00
Cue-Reactivity…….	$200.00
Performance Incentive for Completion of all assessments Stage I…….	$100.00
Stage 2	
Virtual Buffet…….	$150.00
Cue-Reactivity…….	$200.00
Performance Incentive for Completion of all assessments Stage II……	$100.00
Maximum Residential Overnight Stay $50/per night	$1000.00
Follow Up Outpatient Clinic Visit	$100.00
Incentive for Completion all study procedures	$200

POSSIBLE TOTAL COMPENSATION	**$2200.00**

Compensation will be prorated if participants do not complete the study. Cost of cigarettes purchased for use at the CRU through the study team will be tracked and deducted from final compensation. Payments to participants may be in-kind, cash, or check, in accordance with NIDA participant remuneration policy.

Transportation will be provided to and from NIDA or remuneration $15.00 for travel time per study visit.

Participants on approved leave during washout do not receive remuneration while on leave and the total number of days on leave do not count toward the 21-day ceiling for participation.

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