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HPA AXIS REACTIVITY: PHYSIOLOGICAL UNDERPINNINGS OF NEGATIVE URGENCY?

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ABSTRACT

VanderVeen, John Davis. M.S., Purdue University, December 2015. HPA Axis Reactivity: Physiological Underpinning of Negative Urgency. Major Professor: Melissa A. Cyders.

Hypothalamic-pituitary-adrenal (HPA) axis dysfunction is found in heavy alcohol users. Negative urgency is a personality trait reflecting the tendency to act rashly in response to negative emotional states, and is associated with problematic alcohol consumption. The current study examined the relationship between negative urgency and HPA axis functioning following (1) negative mood induction and (2) intravenous alcohol administration among heavy social drinkers (proposed $n = 40$). I hypothesized the following: (1) Negative mood induction would result in an increase of cortisol release as compared to neutral mood induction; (1a) Negative urgency would be related to increased cortisol release in response to negative mood induction; (1b) Negative urgency would partially mediate the relationship between mood induction and cortisol release; (2) Acute IV alcohol administration would result in increased cortisol levels in the neutral mood condition, but decreased cortisol levels in the negative mood condition; and (2a) Negative urgency would be related to the suppression of cortisol release in the negative mood condition in response to acute IV alcohol administration. Repeated measures analyses of variance, the PROCESS macro, and paired samples t-tests were used to examine study

hypotheses. Hypotheses were largely unsupported. Writing mood induction procedures reduced salivary cortisol levels in negative mood ($t(35)= 2.49$, $p= 0.02$) and there was a trend decrease in neutral mood ($t(35)= 1.87$, $p= 0.07$). Alcohol administration also reduced salivary cortisol levels in both negative mood ($t(35)= 3.99$, $p< 0.01$) and neutral mood ($t(35)= 2.60$, $p= 0.01$). However, salivary cortisol changes were no different than typical circadian patterns in response to mood induction ($t(231)= 0.37$, $p=0.71$) or in response to acute alcohol administration ($t(231)= 0.44$, $p= 0.64$). Negative urgency had a trend main effect on salivary cortisol level in response to acute IV alcohol administration, such that those higher in negative urgency were more similar to typical circadian patterns ($F(19,28)= 1.59$, $p=0.13$). This could serve as preliminary support for a psychological mechanism for the alcohol sensitivity hypothesis. Overall these findings suggest the current study failed to sufficiently manipulate salivary cortisol levels. Future studies should consider methodological techniques when exploring these relationships, including IV compared to oral alcohol administration, mood compared to stress manipulations, and cortisol compared to other HPA axis biomarkers.

INTRODUCTION

Impulsivity is associated with several harmful behaviors, including alcohol use and abuse (Sher & Trull, 1994; Congdon & Canli, 2005; Verdejo-Garcia, Lawrence & Clark, 2008). Recently, a multidimensional conceptualization of impulsivity has allowed for a more comprehensive understanding of this trait (e.g., Whiteside & Lynam, 2001; Smith et al., 2007; Dick et al., 2010). One dimension of impulsivity that has been most highly related to problematic alcohol behaviors is negative urgency (Coskunpinar, Dir, & Cyders, 2013), which is defined as the tendency to act rashly in the presence of a negative emotion (Whiteside & Lynam, 2001). Though negative urgency has been related to such behaviors, its mechanism of action is unknown. One possible underlying factor explaining emotion-based rash action is through activation of the hypothalamic-pituitary-adrenal (HPA) axis, which responds to negative affectivity (Jacobs et al., 2007) and alcohol use (Shuckit, Gold, & Risch, 1987; King et al., 2002; Stalder et al., 2010; Thayer et al., 2006). The current study examined negative urgency's relationship to problematic alcohol use behaviors and hyperactive HPA axis response to negative stimuli.

Negative Urgency

Impulsivity's relationship with alcohol use outcomes has varied across studies (*see* Coskunpinar, Dir, & Cyders, 2013). One primary reason for this inconsistency in

findings is the way in which impulsivity is defined and assessed (*see* Smith, Fischer, & Fister, 2003). Because of this, recent research has shifted towards a multidimensional approach to understanding impulsivity (e.g., Dick et al., 2010). One multidimensional model, the UPPS-P Model of Impulsive Behavior (*see* Lynam et al., 2006) proposes that impulsivity is comprised of multiple separate, though related, traits; including *sensation seeking* (the tendency to seek sensory pleasure and excitement), *lack of planning* (the tendency to delay action in favor of careful thinking and planning), *lack of perseverance* (the ability to remain with a task until completion and avoid boredom), *positive urgency* (the tendency to act rashly in positive emotional states), and *negative urgency* (the tendency to act rashly in negative emotional states) (Lynam et al., 2006) [1]. Research has found that use of these separable traits increases the predictive and clinical utility of impulsivity for alcohol use outcomes (e.g., Coskunpinar et al., 2013; Smith et al., 2007). Importantly, negative urgency is the distinct trait that has been studied most in depth and has the most robust relationship to problematic levels of alcohol use, including alcohol dependence (Coskunpinar et al., 2013).

Though negative urgency has been suggested as the most clinically relevant of the impulsivity traits for problematic alcohol use and dependence, how and why it imparts this risk is still unknown. It has been theorized that negative urgency is related to emotional lability or reactivity. Attempts to support this theory have been largely unsuccessful (Cyders & Coskunpinar, 2010; Cyders et al., 2009), and studies have been

[1] Impulsivity can also be measured using behavioral lab tasks. However, these tend to be conceptualized as state measures of impulsivity and have little overlap with trait measures (Coskunpinar and Cyders, 2011). Therefore, the current study only employed personality trait measures of impulsivity.

limited by examining only self-reported mood changes to emotional stimuli, which are fraught with biases that compromise their validity (Podsakoff et al., 2003). More recent attempts have examined how negative urgency relates to underlying physiological mechanisms related to emotional experiences, and these avenues have been more successful. Negative urgency is related to blood-oxygen-level dependent (BOLD) responses to negative emotional images in the right lateral orbitofrontal cortex and the left amygdala (Cyders et al., 2014), two regions related to assigning affective value to reinforcers, decision making, and emotional processing (Kringelbach, 2005; Amunts et al., 2005). In fact, negative urgency mediates the relationship between activation in these regions and general risk-taking tendencies, suggesting that hyperactivity in these regions in response to negative stimuli might underlie tendencies toward rash action in response to negative emotional states (Cyders et al., 2014). These findings suggest that even though negative urgency is unrelated to self-reported affect changes, it likely mediates physiological responses to negative emotional stimuli leading to increased rates of risk-taking behaviors. The current study aimed to examine how hypothalamic-pituitary-adrenal (HPA) axis responses to emotional experiences relate to negative urgency and its influence on alcohol use behaviors.

Hypothalamic-Pituitary-Adrenal (HPA) Axis

HPA axis functioning is most commonly measured using cortisol levels found in blood plasma and saliva samples. Cortisol levels tend to reach their peak approximately 30 minutes after waking, sharply decline for the next two hours, and gradually decline for the remainder of the wake period in most people, although individual differences exist

(Smyth, 1997). Cortisol levels have been consistently related to the body's stress response (McQuade and Young, 2000**;** Harrison, 2002**;** Young, 2004), but likely vary based on individual difference factors. For example, women tend to display more consistent cortisol elevations in response to distressing psychosocial situations compared to men (Kirschbaum, Wüst, & Hellhammer, 1992; Kudielka & Kirschbaum, 2005). Furthermore, negative mood induction results in increased cortisol release (Gadea, Góez, González-Bono, Espert, & Salvador, 2005), and the relationship between laboratory stress tasks and cortisol release is mediated by state negative affect (Al'Absi et al., 1997). This suggests that negative urgency, which is a facet of negative affectivity (Cyders & Smith, 2008), is a prime candidate to examine as mediating the relationship between mood induction and cortisol release.

Cortisol also responds to alcohol consumption; however, the pattern of cortisol release to alcohol varies based on the course of use and the participant's alcohol use history. Generally, in healthy populations, with no family history of alcohol abuse, acute alcohol consumption results in increased cortisol levels (Smyth et al., 1998; King et al., 2002). The HPA axis response to alcohol differs in people with alcohol dependence. In these populations, basal cortisol levels are up to four times higher than in healthy populations (Stalder et al., 2010), likely due to alcohol withdrawal-related stress. Alcohol intake then acts to *reduce* cortisol levels (Schuckit, Gold, & Risch, 1987). It appears that although alcohol generally *increases* cortisol release in the absence of another stressor (King et al., 2002), alcohol consumption *suppresses* cortisol levels in the presence of a stressor (such as a stress induction or withdrawal stress), as the individual already has elevated cortisol levels (Schuckit, Gold, & Risch, 1987; Stalder et al., 2010). If negative

urgency is related to increased physiological responsivity to stressors (as in Cyders et al., 2014), negative urgency could be related to (1) increased cortisol response to negative mood induction and (2) a more marked decrease in cortisol in response to alcohol consumption during negative mood. This would support the self-medication model of alcohol consumption (Colder, 2011; Kuntsche et al., 2005) and implicate negative urgency as a mediator in this model.

The Current Study

The purpose of the present study was to examine how HPA axis functioning, as assessed by salivary cortisol levels, is related to negative urgency in response to negative mood induction and intravenous (IV) alcohol administration. Based on the findings discussed above, I hypothesized the following:

Hypothesis 1. There will be a main effect of mood induction on cortisol release, such that negative mood induction will result in an increase of cortisol release as compared to neutral mood induction (Al'Absi et al., 1997; Gadea et al., 2005).

Hypothesis 1a. Negative urgency will be related to increased cortisol release in response to negative mood induction (Cyders et al., 2014).

Hypothesis 1b. Negative urgency will partially mediate the relationship between mood induction and cortisol release (Cyders et al., 2014).

Hypothesis 2. There will be an interaction between acute IV alcohol administration (1 drink prime) and mood condition (negative/neutral) on cortisol level, such that acute IV alcohol administration will result in increased cortisol levels in the neutral mood

condition, but decreased cortisol levels in the negative mood condition (Smyth et al., 1998, King et al., 2002; Schuckit, Gold, & Risch, 1987).

Hypothesis 2a. Negative urgency will be related to the suppression of cortisol release in the negative mood condition in response to acute IV alcohol administration (Cyders et al., 2014; Schukit, Gold, & Risch, 1987).

METHOD

Participants

Participants were recruited from the Indianapolis, IN area for a larger parent study (Title: *Analysis of emotion-based alcohol consumption using fMRI and experimental paradigms: A career development proposal*; PI: M. Cyders, Supported by NIAAA K01AA020102). There were 358 potential participants that contacted the lab about study participation. Of these, 86 met criteria based on our phone screen and 67 potential participants came in for our in depth screening session. Participants were excluded if they had current or past alcohol dependence or alcohol use treatment, current Axis I psychiatric illness (DSM-IV-TR, 2000), or (women only) were pregnant, breastfeeding, or had the intention of becoming pregnant.

46 potential participants qualified to participate in the two self-administration sessions. Of these, 8 withdrew from the study, leaving a total of 38 participants that completed all study components. Common reasons for withdrawal were a lack of ability to dedicate two full-day sessions and a strong aversion towards needles. Also, 2 participants were excluded because they did not have a sufficient quantity of saliva for cortisol analysis, leaving a final sample of 36 participants. There were no differences in study variables between participants that were excluded and participants that completed the study. However, there were several differences between participants that dropped out

of the study and participants that completed the study. Participants that dropped out had trends for fewer drinking days ($t(40)= -1.59$, $p=0.12$), lower AUDIT scores ($t(40)= -1.36$, $p=0.18$), and less negative urgency($t(40)= -1.33$, $p= 0.19$) than participants that completed the study. Men were also more likely to drop out of the study than women ($\chi^2= 5.50$, $p= 0.02$). All participants were in good medical health and able to understand and complete questionnaires in English. The final sample had an average age of 24.81 (SD= 3.44), 20 women (57%), and 25 Caucasians (71%) (See Tables A1 and A2).

The larger project studied both a progressive work paradigm and a free access paradigm of IV alcohol (see Figure C1 for timeline of procedures). The first 22 participants (Sample 1) completed the progressive work paradigm and were recruited for heavy social drinking (consume at least 7 standard alcoholic drinks per week and at least one binge episode- 4 or more drinks at a time- per week). The remaining 14 participants (Sample 2) completed the free access paradigm (the two participants with insufficient saliva quantities completed the free access paradigm) and were recruited for social drinking (consume at least 4 standard alcohol drinks per week and at least two binge episodes per month) (NIH,2014). Sample 1 reported heavier drinking than Sample 2 (TLFB drinks per day: $t(34)= 2.87$, $p= 0.01$; Mean AUDIT scores: $t(34)= 2.49$, $p= 0.02$) (See Tables A3 and A4). Because of the differences in drinking variables between these groups, all analyses were conducted independently between groups. No differences existed between analyses, so only collapsed group results are reported.

Measures

During the screening session, participants completed a series of questionnaires and computer tasks for the larger study. The measures relevant to the current study are explained below:

Demographics

I collected demographic information on participants' age, gender, and race/ethnicity.

Screening Tools

The Timeline Followback Calendar-90 days (TLFB; Sobell & Sobell, 1992) is a self-report calendar of drinking behaviors in which participants list the number of standard alcoholic beverages consumed each day in the previous three months. It is designed to record both the frequency and amount of alcohol consumed. This method has good test-retest reliability for days abstinent (r =0.96), days drinking without a binge episode (r =0.95), and days with binge episodes (r =0.94) in social drinkers (Sobell & Sobell, 1992). It also has high concurrent validity with the Alcohol Dependence Scale (ADS; r=0.53) and the Short Michigan Alcohol Screening Test (SMAST; r=0.51) for heavy consumption days (Sobell & Sobell, 1992). For the current study, this measure was used to assess subject eligibility.

Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993) is a 10-item scale that assesses hazardous alcohol consumption, abnormal alcohol consumption behavior, and alcohol related problems. The AUDIT shows high test-retest reliability

(r=0.86) as well as concurrent validity with the Michigan Alcoholism Screening Test (MAST; r=0.88) and the CAGE test for alcohol addiction (r=0.78) (Hays, Merz, & Nicholas, 1995; Bohn, Babor, & Kranzler, 1995). The AUDIT can be used to discriminate between hazardous and non-hazardous drinkers (Saunders et al., 1993). AUDIT scores of 7 or below are considered low risk, scores between 8 and 15 indicate some risk, scores between 16 and 19 indicate increased risk, and scores 20 and above are considered high risk (Babor, Higgins-Biddle, Saunders, & Monteiro, 2001). The AUDIT was used as a screening tool to exclude participants scoring higher than 16.

The Semi Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994) is an interview that asks about alcohol and drug use, as well as mental and physical health. The SSAGA has good test-retest reliability with kappa values ranging from 0.62 to 0.84 for substance abuse and dependence as well as strong kappa values for depression (0.65) across various studies at the same center. There is also strong cross-center agreement, with a kappa value of 0.84 for alcohol dependence or abuse and a kappa of 0.74 for lifetime depression. The SSAGA was used as a screening tool, with individuals endorsing items that indicated current or prior alcohol or other substance dependence, suicide ideation, or criteria for DSM-IV Axis I disorders being excluded.

Measures Related to Study Procedures

Life Events Narratives (Abele, 1990) was used to induce either a negative or neutral mood. The negative life events narrative asks respondents to write about an event that made them particularly sad or upset in their lives. The neutral life events narrative asks respondents to write about their activities on a typical day for approximately twenty

minutes. Writing procedures are effective at inducing negative mood states (r_m= 0.522; e.g., Westermann, 1996). In order to increase the effect of the life events narrative, writing was paired with the musical mood induction (see below).

Musical Mood Induction Procedure (MMIP; Västfjäll, 2002) was used to maintain the negative or neutral mood. Initial song lists were taken from Västfjäll (2002). All songs were then rated by four trained raters, and songs that were not correctly categorized as negative or neutral were removed from the list. Negative songs are associated with a more negative subjective mood rating compared to neutral songs (p<0.05; Västfjäll, 2002), and neutral songs are associated with a more positive subjective mood rating than negative songs but more negative mood rating than positive songs (p<0.05; Västfjäll, 2002). Music was played continuously during the writing, priming, and working sessions (see full description in procedures). Songs and order of presentation can be seen in Table C1.

The Affect Grid (Russell, Weiss, & Mendelsohn, 1989) is a single-item scale designed to assess affect along the dimensions of pleasure-displeasure and arousal-sleepiness. It has adequate correlations with other, longer measures of current mood states such as the Mehrabian & Russell (1974) scale (r= 0.77), making it a more practical measure of current mood. In the present study, the affect grid was used as a check for the effectiveness of the mood manipulation.

Measures Related to Study Hypotheses

The UPPS-P Impulsive Behavior Scale- Revised (UPPS-P; Lynam et al. 2006) is a 59 item self-report scale, with responses ranging from 1 (agree strongly) to 4 (disagree

strongly). The UPPS-P is designed to measure five sub-facets of trait impulsivity: sensation seeking, lack of planning, lack of perseverance, positive urgency, and negative urgency. Because of study hypotheses, the present study only used the negative urgency subscale, which had adequate reliability ($\alpha = 0.86$). Items were coded so that higher mean scores represented higher levels of negative urgency.

Salivary Cortisol Collection. Saliva was collected using the Passive Drool method with the Saliva Collection Aid as described in the Salimetrics Saliva Collection Handbook (2013). Saliva samples were stored at -20ºC and sent to the Salimetrics Lab for analysis. The supplies and procedure used by the Salimetrics Lab (2013b) are outlined in Tables C2 and C3. Cortisol acts to inhibit release of vasopressin and corticotropin-releasing hormone (CRH), two hormones necessary for regulating many homeostatic functions, by the HPA axis (de Kloet, 2006). Greater cortisol levels indicate greater stress levels. The detection range of salivary cortisol is 0.007 to 1.80μg/dL, and the correlation between salivary cortisol and blood serum cortisol is 0.94 ($p<0.0001$) (Daniel et al., 2006). There is an average salivary cortisol increase of about 0.12μg/dL in response to negative mood inductions and cortisol increase of about 0.07μg/dL in response to neutral mood induction lasting 20 minutes in duration (Gadea et al., 2005). Cortisol levels return to daily circadian rhythms roughly 15 minutes following mood induction. In response to a 500kcalorie breakfast, there is an average salivary cortisol increase of approximately 0.08μg/dL, and it takes roughly 30 minutes for cortisol to return to daily circadian rhythms (Van Cauter, Shapiro, Tallil, & Polonsky, 1992). In response to noxious stressors, there is an average salivary cortisol increase of 0.10μg/dL, and it takes about 30 minutes for cortisol to return to daily circadian rhythms (Zimmer, Basler, Vedder, &

Lautenbacher, 2003). Based on a calorie content of 7 calories per gram of pure ethanol, and participants receiving 2.7mL of ethanol in the infusion session, we can expect a 0.003μg/dL increase in cortisol as a direct effect of the calories in the alcohol (Hamilton, Whitney, & Sizer, 1991). All cortisol measurements were within the expected range based on these increases and the daily circadian rhythm observed by Aardal & Holm (1995), suggesting there were no ceiling or floor effects (i.e., too high or low cortisol concentrations, respectively) within the cortisol data (see Figure C3).

Procedure

Informed consent was obtained before any study procedures began. All study documents and procedures were approved by the Indiana University Institutional Review Board and Human Subjects Office. Participants were given $30 for completion of the screening session, $100 for completion of the first self-administration session, and $150 for completion of the second self-administration session. At the beginning of each study session participants were asked to provide a urine sample, which was used for a drug screen and (women only) a pregnancy test. A positive test in any of the ten panels on the drug test resulted in exclusion from the study (see Table C4 for a full description for the targeted drugs, metabolites, and cutoff concentrations). Likewise, a positive pregnancy screen resulted in exclusion from the study.

Screening Session

Participants were recruited through the use of advertisements posted in public areas in the Indianapolis, IN area, on the campus of IUPUI, and on the Internet.

Participants were first administered a phone screen to assess eligibility. If the participant qualified after the phone screen, they were invited to complete a more in-depth screening at the Impulsivity Neuroscience Lab on the campus of IUPUI. At this session, they completed a series of questionnaires and computer tasks as part of the larger parent study, including study measures listed above, to assess subject eligibility and to measure negative urgency for main study hypotheses.

Infusion Sessions

Participants completed two counterbalanced IV alcohol administration sessions: one in which they engaged in a negative mood induction and one in which they engaged in a neutral mood induction (see Figure C1 for timeline of procedures). Participants arrived at the Indiana General Clinical Research Center at approximately 8 a.m. on study days. They had their height and weight measured (for calibration of the IV alcohol administration software) as well as their blood pressure, temperature, heart rate, and time awake recorded. Participants then gave a breath alcohol reading (BrAC), and gave a urine sample for drug and pregnancy screen; participants who tested positive for drug use (n= 1), were pregnant (n= 0), or who had a BrAC larger than 0mg/dL (n= 0) were dismissed. However, participants testing positive for marijuana were interviewed to ensure they were no longer under the effects of the drug. Thus, some participants tested positive for marijuana completed the study (n= 7), while others' whose reports indicated they may still be experiencing effects were dismissed (n= 4).

Participants then provided a saliva sample by allowing drool to pass into a 2mL cryovial with the help of the Saliva Collection Aid. Then, participants put on the

headphones, music was turned on, and they completed the life event narrative (either negative or neutral) for 20 minutes, which is comparable to the duration of previous mood induction studies (Gadea et al., 2005). At the end of the writing, they provided another saliva sample. Then, participants were given a standardized light breakfast (500kcal), monitored by the hospital staff. Thirty minutes after breakfast, a member of the nursing staff inserted the IV catheter in the participants' non-dominant arm, and the infusion hardware setup was completed, which included a control computer, infusion pumps, a response button pad (for participant responses) and two work buttons (one labeled "A" for alcohol, and one labeled "W" for water) (see Figure C2).

At the start of the infusion session, participants were given instructions for their infusion session (see Figure C3 for Sample 1 Instructions; Figure C4 for Sample 2 Instructions). Sample 1 was told that we were interested in their motivation to work for alcohol and water. These participants were told that they will complete a reaction time task called the continuous attention task (CAT; see Figure C2) in order to earn an alcohol (the equivalent of approximately half a standard alcoholic drink) or water (saline) reward. The CAT task is organized into work sets. At the beginning of each work set, the participant chose to work for either alcohol or water, which was the actual reward they received upon completing the work set. After choosing the reward, the participant saw a circle on the screen. To start each trial, they pressed and held the work button. When they did this, the ring on the button symbol on the screen turned yellow. A short time later, the ring turned blue. To have the trial count toward earning the reward, the participant had to release the button before a timer ran out on the blue ring. If they were fast enough, the center circle turned green; if not, the center circle turned red. The task was built so that

the participant would be fast enough about one-half of the time, and the number of required successful trials to get a reward increased throughout the session.

Sample 2 was told that we are interested in how much they *enjoy* alcohol. These participants did not have to complete the CAT task, and were able to simply press a button labeled either “A” for alcohol or “W” for water, which was the actual reward they received. All participants were told that they would be asked to give several saliva samples and answer questions about their experience of the alcohol infusion throughout the session.

Participants gave a saliva sample prior to the start of the priming dose. Sample 1 was told that they will first work for two alcohol rewards (the equivalent of one standard drink in all as a “priming” dose), and that after receiving the rewards, they will have a 15-minute break to experience the effects of the alcohol. Sample 2 was told to select alcohol for the first two rewards while Sample 1 had to complete two successful trials of the CAT task to get the alcohol reward in the priming dose; whereas Sample 2 had to press the button twice to get the alcohol reward. All participants were told that after this break, they would be asked to read aloud their life narrative. Participants then placed the headphones on, and the priming dose playlist began (see Table C1). Participants then completed the priming dose, gave another saliva sample and BrAC measurement, answered questions about their experience of the alcohol infusion, and self-reported their mood.

At the end of the 20-minute prime, participants were then told to continue with the 2-hour session. Sample 1 was told they can work for either water or alcohol while Sample 2 was told they can choose to receive either water or alcohol, and that they can

work as much or as little as they like, but that their session would still last 2 hours, and they would still be asked to stay in the unit until approximately 7pm that evening.

At the end of the infusion (roughly 12:30pm), participants had their IV removed and were required to stay on the unit until approximately 5pm-7pm, their BrAC was below 20mg/dl, and the nursing staff could no longer identify behavioral signs of intoxication. Participants were given lunch and dinner during their stay and were paid in cash at dismissal.

Hypothesis Testing

All analyses were conducted for the entire sample, with gender as a covariate because of differences in both negative urgency (Cross, Copping, & Campbell, 2011) and cortisol release (Kirschbaum, Wüst, & Hellhammer, 1992; Kudielka & Kirschbaum, 2005) typically observed across gender.

Hypothesis 1. There will be a main effect of mood condition on cortisol release, such that negative mood induction will result in an increased cortisol release as compared to neutral mood induction. To test this hypothesis, I conducted a repeated measures analysis of variance (ANOVA), with mood (negative/neutral) as the independent variable, cortisol level as the dependent variable, and two covariates: time awake and gender. A p-value less than 0.05 on the mood main effect variable was used to assess significance.

Hypothesis 1a. Negative urgency will be related to increased cortisol release in response to negative mood induction. To test this hypothesis, I conducted a repeated measure ANOVA with negative urgency as the independent variable,

cortisol level in the negative condition as the dependent variable, and two covariates: time awake and gender. A p-value less than 0.05 on the negative urgency main effect variable will be used to assess significance.

Hypothesis 1b. Negative urgency will partially mediate the relationship between mood induction and cortisol release. To test this hypothesis, I used the PROCESS macro provided by Hayes (2007). This macro estimates the direct effects of the independent variable on the dependent variable using a simple regression coefficient. I used the bootstrapping approach as this is robust to non-normal indirect effect distributions and offers higher power (Hays, 2007). To do this, the PROCESS macro took a random sample of cases from the original data, samples them with replacement, and estimated the indirect effects of the product of the regression coefficients generated to test the direct effects. This was repeated 10,000 times. Then, these effects estimates were sorted from lowest to highest. The 2.5^{th} percentile and 97.5^{th} percentile indirect regression coefficients were used to estimate the indirect effects confidence interval. I entered mood induction as the independent variable, negative urgency as the mediator, and post mood-induction cortisol level change as the dependent variable. Time awake, gender, and pre-mood cortisol were included as covariates. An indirect regression coefficient for negative urgency that had a 95% confidence interval not containing zero was used to determine a significant indirect effect.

Hypothesis 2. There will be an interaction between acute IV alcohol administration (2 drink prime) and mood condition (negative/neutral) on cortisol level, such that acute IV alcohol administration will result in increased cortisol levels in the neutral mood

condition, but decreased cortisol levels in the negative mood condition. To test this hypothesis, I conducted a repeated measures ANOVA, with mood (negative/neutral) as the independent variable, cortisol level as the dependent variable, and time awake and gender included as covariates.

Hypothesis 2a. Negative urgency will be related to the cortisol level changes in response to acute IV alcohol administration. To test this hypothesis, I conducted a repeated measure ANOVA with negative urgency as the independent variable, cortisol level as the dependent variable, and two covariates: time awake and gender. A p-value less than 0.05 on the negative urgency main effect variable was used to assess significance. As a sensitivity analysis, I separately examined an interaction between mood condition and negative urgency by conducting a second repeated measures ANOVA, with mood condition, negative urgency, and the interaction between the two factors as independent variables, cortisol level as the dependent variable, and three covariates: time awake and gender. I also probed the interaction and graphed the results as recommended by Frazier, Tix, and Barron (2004).

RESULTS

Data Cleaning and Screening

All analyses were conducted using SPSS 23.0. I first examined whether data from key variables were missing at random. There were no cases of missing negative urgency, gender, age, or race data. I then examined the data for outliers, using an absolute value z-score greater than 3.0 (Kline, 1998). There was one outlier in the pre-alcohol prime salivary cortisol data with a z-score of 4.62, but no other variables met criteria to be considered an outlier (all $|z| < 2.50$). All analyses were conducted with and without the outlier. Because the outlier did not affect the interpretation of any analyses, only analyses with the outlier included are reported. Using an absolute value of less than 3.0 for skewness and less than 10.0 for kurtosis (Kline, 1998), no variables met criteria for non-normal distribution (all $|W|<1.35$; all $|Kurt| <1.84$). I also used correlation and regression analyses to assess for excessive correlation ($r \geq 0.70$; Kline, 1998) between negative urgency and all cortisol measures. No cortisol measures had excessive correlation with negative urgency (all $|r| < 0.25$). Pre-mood induction cortisol levels were strongly correlated with post-mood induction cortisol levels for both negative mood induction ($r(34)= 0.70$; $p< 0.01$) and neutral mood induction ($r(34)= 0.73$; $p< 0.01$). Additionally, pre-prime cortisol levels were strongly correlated with post-prime cortisol levels for both the negative mood condition ($r(34)= 0.93$, $p< 0.01$) and the neutral mood condition

($r(34)= 0.92$, $p<0.01$). Cortisol levels reach their peak roughly 30 minutes after waking, sharply decline in the next two hours, and decline gradually for the remainder of the wake period, although there are individual differences (Smyth, 1997). Because of the nature of cortisol release throughout the day, cortisol data tend to be skewed to the right (Pani et al., 2013); however, in experimental designs, cortisol is released independent of its usual daily pattern (Smyth, 1998; Stalder, 2010). I conducted all analyses with and without controlling for time of day. Because there were no differences in these analyses, only analyses including time of day as a covariate are reported. On average, participants awoke at 6:30am, pre-mood induction saliva collection was at 8:22am, post-mood induction saliva collection was at 8:49, pre-prime saliva collection was at 10:01am, and post-prime saliva collection was at 10:17am. Based on these average times of saliva collection, we estimated the average daily circadian salivary cortisol levels according to Aardal and Holm (1995): pre-mood cortisol ($M= 0.50$, $SD= 0.06$), post-mood cortisol ($M=0.43$, $SD= 0.05$), pre-prime cortisol ($M=0.29$, $SD= 0.03$), and post-prime cortisol ($M=0.25$, $SD= 0.03$).

I conducted a repeated measures ANOVA to assess the effectiveness of the mood manipulations. I entered mood condition as the independent variable and pre and post mood induction self-reported affect scores as the dependent variables. Results of this analysis showed a significant main effect of mood induction condition, such that affect rating was significantly reduced following negative mood induction compared to neutral mood induction ($F(1, 40)= 25.45$, $p< 0.01$; see Table A5). These results show that the mood manipulations created the intended effects on self-reported mood.

I then conducted a repeated measures ANOVA to assess the self-reported affect changes in response to alcohol administration. I entered mood condition as the independent variable and pre and post alcohol administration self-reported affect scores as the dependents variables. Results of this analysis showed no difference in affect change to alcohol administration between conditions ($F(1,40)= 0.51$, $p= 0.48$; see Table A6). I also conducted independent samples t-test on the affect grid data before and after the alcohol prime in both the negative and neutral mood conditions. There was no difference between the pre-prime affect scores between neutral ($M= 6.15$, $SD= 1.20$) and negative mood ($M=6.05$, $SD= 1.38$; $t(35)= 0.73$), suggesting the expectation of reading a narrative aloud did not affect self-reported mood differently across conditions.

Study Hypothesis One: Negative mood induction will result in increased cortisol release compared to neutral mood induction

First, paired samples t-tests found that salivary cortisol levels were significantly decreased following negative mood induction (pre-induction: $M=0.41$, $SD= 0.19$; post-induction: $M= 0.35$, $SD= 0.21$; $t(35)= 2.49$, $p= 0.02$) and trended towards a significant decrease following neutral mood induction (pre-induction: $M=0.38$, $SD= 0.26$; post-induction: $M= 0.32$, $SD= 0.22$; $t(35)= 1.87$, $p= 0.07$; see Figure B1). An independent samples t-test showed no difference between the cortisol changes in response to mood induction in the current study ($M= 0.04$, $SD= 0.15$) and the typical circadian pattern change (Aardal & Holm, 1995; $N=197$; $t(231)= 0.37$, $p= 0.71$). I then conducted a repeated measures analysis of variance ANOVA, with mood induction (neutral/negative)

as the independent variable, salivary cortisol level as the dependent variable, and time awake and gender as covariates. Results of this analysis showed no main effect of mood-induction condition on salivary cortisol release ($F(1, 68)=0.02$, $p= 0.88$, see Table B1).

I then tested hypothesis 1a: *Negative urgency will be related to increased cortisol release in response to negative mood induction* using a repeated measures ANOVA. I entered negative urgency as the independent variable, salivary cortisol level as the dependent variable, and time awake and gender as covariates. Negative urgency was not related to cortisol release in response to negative mood induction ($F(19, 68)= 0.86$, $p= 0.63$, see Table B2).

To test hypothesis 1b: *Negative urgency will partially mediate the relationship between mood induction and cortisol release*, I used the PROCESS macro (Hayes, 2007). To do this, I entered mood induction as the independent variable, negative urgency as the mediator, post-induction cortisol level as the dependent variable, and pre-induction cortisol level and gender as covariates. I also selected the bootstrapping option with 10,000 iterations and a 95% confidence interval. Based on this analysis, there was no evidence of a partial mediation by negative urgency ($b= <0.001$, $SE= 0.01$, 95% CI[-0.01 – 0.01]; see Table B3.

Study Hypothesis Two: There will be an interaction between acute IV alcohol administration and mood condition on cortisol level

To test the second hypothesis, I first conducted paired samples t-tests on cortisol levels before and after the alcohol priming session, which showed a significant decrease in cortisol levels in both the negative mood condition ($M= 0.03$, $SD= 0.04$, $t(35)= 3.99$,

$p<0.01$) and the neutral mood condition ($M= 0.06$, $SD= 0.14$, $t(35)= 2.60$, $p= 0.01$; see Figure B2. Using a paired samples t-test, there was no difference in the decrease in cortisol release following the alcohol prime between the negative and neutral mood induction conditions ($t(35)= 1.31$, $p=0.20$). A paired samples t-test showed a possible trend difference in the mean cortisol level before the alcohol prime such that there were lower cortisol levels in the negative mood session ($M=0.26$, $SD= 0.11$) than the neutral mood ($M=0.32$, $SD= 0.26$; $t(35)= -1.46$, $p=0.15$). An independent samples t-test showed no difference between the cortisol changes in response to alcohol administration in the current study ($M= 0.06$, $SD= 0.21$) and the typical circadian pattern change (Aardal & Holm, 1995; $N=197$; $t(231)= 0.44$, $p= 0.64$). I then conducted a repeated measures ANOVA, with mood (negative/neutral) as the independent variable, cortisol level as the dependent variable, and time awake and gender as covariates. Results of this analysis showed no support for a mood by alcohol interaction ($F(1, 68)= 1.29$, $p= 0.26$; see Table B4).

To test hypothesis 2a: *Negative urgency will be related to cortisol level changes in response to acute IV alcohol administration,* I conducted a repeated measures ANOVA. To do this, I entered negative urgency as the independent variable, cortisol level as the dependent variable, and time awake and gender as covariates. There was a trend relationship between negative urgency and cortisol release in response to acute alcohol administration ($F(19, 28)= 1.59$, $p= 0.13$; see Table B5), such that those higher in negative urgency had greater cortisol release following alcohol administration. Though

results did not show a negative urgency by mood interaction on cortisol release in response to acute alcohol administration, this relationship was examined graphically as can be seen in Figure B3.

DISCUSSION

I hypothesized that the hypothalamic-pituitary-adrenal (HPA) axis would be differentially activated based on mood induction and acute intravenous (IV) alcohol administration. I also hypothesized that these changes would be affected by negative urgency. Results did not support study hypotheses. There was no main effect of mood induction or alcohol administration on HPA axis activation, evidenced by cortisol release. Similarly, cortisol release did not differ between negative and neutral mood induction, nor did it differ in response to alcohol administration in the negative and neutral mood conditions. Negative urgency was unrelated to cortisol release in response to mood induction, though there was a trend main effect of negative urgency on cortisol release in response to acute alcohol administration.

Despite study hypotheses being unsupported, there are several interesting relationships that can be taken from the current study. First, there was a trend main effect of negative urgency on salivary cortisol levels, such that those higher in negative urgency had higher salivary cortisol levels than those lower in negative urgency. Though this finding was not statistically significant, it is possible that this was because the present study was underpowered. If replicated in a larger sample, this would provide evidence to support the alcohol sensitivity hypothesis. According to this hypothesis (Schuckit, 1984), those with a familial history of alcoholism, and those with at-risk drinking behaviors,

display blunted responses (e.g., subjective intoxication rating, body sway) in response to alcohol compared to non-risk individuals (Lipscomb, Carpenter, Nathan, 1979; Schuckit, 1985; Heath et al., 1999). Furthermore, this blunted response to alcohol is responsible for developing alcohol dependence later in life (Schuckit & Smith, 1996). The idea is that those with less sensitivity to alcohol need to drink more in order to get expected benefits. Alcohol sensitivity may be a phenotype through which negative urgency, and thus the potential for an array of problematic behaviors, can be identified. Negative urgency is a well-established risk factor for problematic drinking (see Coskunpinar et al., 2013; see Cyders, Coskunpinar, & VanderVeen, *in press*). People with greater levels of negative urgency may be less physiologically sensitive to the effects of alcohol, leading them to consume alcohol at problematic levels. This conclusion must be taken with caution, as the study sample was small and the robustness of this finding is unknown. However, this could suggest a role of alcohol sensitivity for how negative urgency might influence alcohol use behaviors. In order to examine this further, future studies should examine cortisol (and other biomarkers) reactivity to alcohol in both short term and longer term drinking. The alcohol sensitivity hypothesis would be supported by those greater in negative urgency displaying less reactivity in short term drinking compared to those lower in negative urgency, but equal reactivity in longer term drinking. Future research should also use a mood induction that more reliably affects cortisol levels (e.g., Velten (1968) method, discussed below) and a larger sample, as this would cause more detectable elevations in cortisol level in negative mood, providing a better understanding of physiological responses to alcohol in certain mood states.

Apart from this one effect, other study hypotheses were largely unsupported. There are several explanations for the null results of the present study. First and likely most importantly, the small sample size resulted in a study unpowered to find effects, especially in regards to mediation and moderation effects, which are often smaller effects that require larger samples to detect. These underpowered effects suggest lack of certainty as to the reliability of the null results in a larger, properly powered sample. Second, the time of day of the study corresponded with typical decrease in cortisol release and circadian pattern, which likely masked my ability to increase cortisol with my study manipulation.

First, I was unable to detect reliable cortisol changes in response to my mood manipulation, which suggests a failure in mood manipulation. However, I did find a significant change in self-reported mood in the negative mood condition, suggesting that either 1) the mood manipulation was sufficient to change self-reported mood, but not physiological cortisol levels or 2) the mood manipulation was not an effective manipulation of mood or cortisol levels and self-reported mood changes were only affected due to the subject's anticipating the study and experimenter goals. Additionally, I will discuss the three most plausible methodological factors that likely affected my ability to detect reliable cortisol mood changes, in addition to power and timing of day factors discussed above.

First, there was an average of 27 minutes between the pre-mood induction and post-mood induction saliva collections. It is possible that in this time, there was an elevation of cortisol following the initial reading of the mood induction but the remaining time allowed cortisol levels to return to their typical circadian rhythm. Because cortisol

can fluctuate in a span of about 5 minutes in response to stressors and return to typical circadian levels in about 15 minutes (Gadea et al., 2005), it is possible that the time between pre-mood induction and post-mood induction was too large to detect any changes. If this is true, this long time span between cortisol measurements could have resulted in the appearance of no cortisol change in response to the mood manipulation. I used the passive drool collection method in an attempt to be the least invasive, but issues in obtaining saliva samples proved to be problematic for the present study.

Second, the type of mood induction used in the current study might not have been ideal to detect reliable cortisol changes. There have been several types of tasks used to manipulate salivary cortisol levels- namely stress tasks and mood manipulations. Stress tasks have consistently been shown to increase salivary cortisol levels (Al'Absi et al., 1997; Préville, Zarit, Susman, Boulenger, & Lehoux, 2008; Morris, Rao, Wang, & Garber, 2014). However, stress tasks may not be directly comparable to (negative) mood inductions and may not activate the HPA axis in a similar fashion. This is evidenced by the mixed findings of negative mood induction on salivary cortisol. While reading a series of sequentially more depressing statements for 20 minutes and talking to another person about a negative event both elevate cortisol levels (Gadea et al., 2005; Engert, Smallwood, & Singer, 2014), there are other negative mood inductions that have not changed salivary cortisol levels. Such inductions that had no effect on cortisol levels include a 20-minute negative rumination task and listening to sad music for 20 minutes (Kuehner, Huffziger, & Liebsch, 2009; Chopra, Segal, Buis, Kennedy, & Levitan, 2008). Mood inductions causing an elevation in cortisol involve constant effort by the participants (i.e., reading or speaking out loud) while those that did not change cortisol

did not require constant effort (i.e., ruminating or listening to music). The effort put forth on the mood inductions could have been responsible for the observed cortisol patterns. Alternatively, the mood inductions that required less direct effort could have afforded the participants an opportunity to fully process their emotions leading to the observed null change. Despite inconsistent findings with both writing and musical mood inductions, there is strong research evidence from several meta-analyses that supported the combination of these procedures to elicit an effect (Westermann et al., 1996; Bass, De Dreu, & Nijstad, 2008).

Third, writing tasks are often used in therapeutic settings and are a mainline strategy in the treatment of many problematic behaviors (see Pennebaker, 1997). For example, emotion-based writing tasks are related to a reduction in depression, anxiety, and posttraumatic symptom severity in women with substance use disorders (Meshberg-Cohen, Svikis, & McMahon, 2014) as well as a decline in craving intensity and self-reported cocaine use in a cocaine dependence treatment program (Grasing, Mathur, & DeSouza, 2010). A study of patients with rheumatoid arthritis showed a decrease in self-reported pain after writing an emotion-based story, independent of the mood condition (negative, neutral, or positive) to which participants were assigned (Lumley et al., 2011). With these studies in mind, the writing mood induction task used in the current study may have been “therapeutic.” In the neutral mood condition, writing had little effect on salivary cortisol, causing levels to decrease along with typical circadian patterns. In the negative mood condition, however, there may have been an initial elevation in salivary

cortisol as the participant considered their negative event, but cortisol was subsequently reduced (or returned to its natural circadian rhythm) while the participant engaged in therapeutic writing.

Additionally, I found salivary cortisol was reduced following acute alcohol administration in both the negative and neutral conditions; however, this pattern was not different from typical circadian patterns, suggesting that alcohol administration does not notably affect salivary cortisol level. I see three plausible explanations for the current findings. First, our small dose of alcohol might not be sufficient to cause physiological changes. Perhaps alcohol does not induce physiological effects until a certain quantity threshold is met. This is in line with the alcohol sensitivity hypothesis (Schuckit, 1984), which suggests that those at-risk for developing an alcohol use disorder show a blunted physiological response to alcohol. For instance, several studies have shown that those with a history of heavy alcohol use show an attenuated response to alcohol compared to lighter drinkers (Schuckit, 1994; King, Munisamy, de Wit, & Lin, 2006). The present study recruited all at-risk consumers of alcohol that may be less sensitive to the effects of alcohol. Thus, I might have chosen a group of alcohol drinkers with low alcohol sensitivity that are less physiologically responsive to alcohol, especially at such low doses. To properly examine this hypothesis, choosing alcohol drinkers across a wide range of sensitivity levels may better characterize potential physiological mechanisms affected by alcohol consumption.

Second, the lack of alcohol cues in the study might have led to failure to detect cortisol changes to acute IV alcohol administration. There have been several studies finding that alcohol-related cues affect alcohol-related behaviors, independent of actual

alcohol consumption, including the positive effects of alcohol (Field, Mogg, & Bradley, 2005), cravings (Field, Mogg, Zetteler, & Bradley, 2004) and salivation (Monti et al., 1993). Heavy drinkers and alcoholics show increased physiological responses, such as skin conductance and heart rate in response to the smell of alcohol without consuming alcohol (Kaplan, Cooney, Baker, Gillespie, Meyer, & Pomerleau, 1985), increased activation of the striatum, thalamus, insula, and inferior frontal, midfrontal, and cingulate gyri in response to alcohol pictures (Ewing, Filbey, Chandler, & Hutchison, 2010), and increased activation of the anterior cingulate, left prefrontal, and bilateral insular regions in response to alcohol-related words (Tapert, Brown, Baratta, & Brown, 2004). This body of work emphasizes the importance of alcohol cues in the physiological response to alcohol. Because the present study removed effects of smell, taste, and other environmental cues, it is possible that cortisol release is less effected by alcohol's pharmacological effects and more by cues or cues in conjunction with pharmacological effects. Despite the benefits of using IV alcohol administration to study the effects of alcohol on human behavior (e.g. control for rate of exposure to alcohol, studying physiological effects, etc.), previous work has suggested failure of HPA axis activation in response to IV alcohol administration (Danel et al., 2006).

Finally, I found no difference in the salivary cortisol levels before the alcohol prime between the negative and neutral mood conditions, which suggests that the expectation of having to read aloud a narrative was not a strong enough manipulation to induce physiological changes. Although previous work has shown that the expectation of having to read aloud is a stressful event (Al'Absi et al., 1997; Hostinar, McQuillan, Mirous, Grant, & Adam, 2014), these were done in front of an audience. It is possible

that social factors were also at play in these previous studies such as anxiety about public speaking, embarrassment, and social inhibition (performing worse in front of others on a difficult task; Gable, Reis, & Elliot, 2000). These factors may not have affected participants in the present study because they were speaking to a single researcher (as opposed to a peer), behind a curtain, on a subject of their own choosing. Also, as noted previously, stress tasks are likely not equal to mood induction tasks, at least in their ability to induce changes in cortisol release. Participants did not report a change in affect before the alcohol prime, suggesting that the expectation of the reading task did not alter mood. Thus, I see it most plausible that the manipulation prior to the alcohol prime in the current study was largely not effective.

Study Limitations and Future Directions

This study should be evaluated in the context of its limitations. First, this study was likely underpowered to find meaningful relationships. With 36 participants, I was unlikely to find relationships that may exist, particularly when conducting analyses requiring the control of other factors. Because of the issue of power, the present study was unable to fully explore the possibility of negative urgency impacting alcohol sensitivity. Future studies should examine negative urgency and multiple measures of physiological reactivity (e.g. heart rate, skin conductance, etc.) in response to alcohol consumption to further elucidate these effects. If found, these responses could serve as physiological phenotypes of negative urgency thus providing important targets for interventions. For example, exercise based interventions that help to regulate physiological processes, including heart-rate, could be important to explore. A recent

review suggests exercise interventions may be useful for treating alcohol use disorders (Giesen, Deimel, & Bloch, 2015), though negative urgency was not assessed and could be responsible for the mixed findings. Potential research could also consider treating affective lability as a target in substance use research. If negative urgency is found to play a strong role in activating the HPA axis, this would provide evidence for further drug treatment development. For instance, mood stabilizers, such as lithium, could potentially be beneficial in treating alcohol use disorders, but have been largely understudied in this domain.

Other limitations affect the generalizability of findings. I collected a sample of individuals in the Indianapolis area, so it may not be generalizable to individuals living outside of an urban Midwestern city. Also, we did not set out to compare low social drinkers with heavy social drinkers.

There is also a limitation of time of day. It is possible that drinking in the morning would have different effects on the HPA axis than drinking in the evening. Because salivary cortisol fluctuates so much throughout the early parts of the day, it may be more useful to examine cortisol changes due to alcohol in the evening. This would be important to examine because: 1) salivary cortisol levels are more stable so potential changes could be directly attributable to experimental procedures and 2) evening drinking would more closely resemble typical drinking patterns of participants. The current study was limited by the time of day required by the larger parent study. Future studies should examine the effects of alcohol on HPA axis activation at times more closely related to participants' regular drinking times.

Next, there were limitations specific to study methodology. The use of salivary cortisol and mood induction has yielded inconsistent results in previous studies (Gadea et al., 2005; Kuehner, Huffziger, & Liebsch, 2009). Future studies need to clarify and justify the use of stress induction compared to mood induction, although studies of cortisol change to lab stress tasks have shown this relationship is mediated by negative affectivity (Al'Absi et al., 1997). It is possible though, that there is some type of interaction between negative affectivity and stress in activating the HPA axis. For instance, a negative mood itself may not be enough to elicit such a change, but paired with the stressful thoughts about the past and future combined with this negative mood may be responsible. Additionally, there are other markers of HPA axis functioning beyond salivary cortisol, including adrenocorticotropic hormone (ACTH) and vasopressin (Pariante & Lightman, 2008; Symons, Wolff, Stone, Lim, & Bodfish, 2011), though the analysis of these biomarkers tends to be more expensive and less consistently related to psychological processes (Chandola, Heraclides, & Kumari, 2010; Symons, Wolff, Stone, Lim, & Bodfish, 2011). Future studies should investigate the use of multiple HPA axis biomarkers, rather than relying solely on cortisol measurement. Also, recent research studying HPA axis reactivity to IV alcohol has used blood serum measures taken every 15 minutes (Stangl & Ramchandani, 2015). It is possible that taking more measurements over the course of a study could allow for a better understanding of HPA axis reactivity to IV alcohol, despite this approach being more invasive. Additionally, some studies have found that the biggest predictor of cortisol reactivity is genetic disposition (e.g., homozygous *s* allele) (Gotlib, Joormann, Minor, & Hallmayer, 2008). Because we did not conduct genetic analyses, we may have been

unable to identify cortisol differences based on these factors. Future studies may benefit from some form of genetic analyses so as to identify changes in cortisol based on biological dispositions.

Furthermore, I may not have observed changes in cortisol simply based on the nature of cortisol itself. For instance, cortisol is highly reactive to changes in homeostasis including things such as fear, pain, stress, and food consumption (Goldstein & Kopin, 2007). Also, our writing prompt may have actually been therapeutic, as this is a main-line therapy option for the treatment of depression (Freely, 2004). Cortisol may have elevated quickly after reading the negative mood induction prompt, but writing about it could have had therapeutic effects, thus causing cortisol to be decreased by the time we collected saliva. Future studies using life events narratives should collect several cortisol measurements throughout the manipulation to identify when the most physiological effects are being exerted, if at all. Future studies should also distinguish between the use of stress inductions (Al'Absi et al., 1997; Gadea et al., 2005) and mood inductions (Kuehner et al., 2009). These types of inductions likely have disparate effects on HPA axis activation.

Finally, the use of IV alcohol administration may have been unable to cause HPA axis activation in the absence of alcohol cues. It is possible that the experience of oral alcohol consumption, with smell, taste, and environmental cues, causes HPA axis activation. Future studies should compare the physiological responses amongst IV alcohol administration with and without cues, and with oral alcohol consumption. This could help to parse the effects of mood due directly to the physiological effects of alcohol and those due to alcohol-related cues.

Conclusions

The present study examined the effects of mood induction on HPA axis activation, the effects of mood induction on HPA axis responses to IV alcohol administration, and the role of negative urgency in these relationships. Results showed no difference between cortisol response to writing tasks or acute IV alcohol administration and typical circadian patterns of cortisol, likely due to methodological factors. Despite being largely underpowered, the present study provides preliminary evidence for the alcohol sensitivity hypothesis, such that those with greater negative urgency appeared to be less physiologically reactive to alcohol than those with less negative urgency. I believe this suggests that future research should design properly powered studies to further test this hypothesis. The current study also highlights differences between stress and mood inductions, as they might differentially affect mood and physiological responses. The use of salivary cortisol as compared to other measures of HPA axis reactivity, as well as the use of oral compared to IV alcohol administration are likely responsible for differing results across studies.

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APPENDICES

Appendix A: Differences among Variables

Table A1

Differences in categorical variables between participants that completed the study, participants that qualified and dropped out, and excluded participants.

		Excluded		Completed Study		Completed and Excluded			Dropped Out		Completed and Dropped Out		
		Frequency	Percent	Frequency	Percent	χ^2	df	*p*	Frequency	Percent	χ^2	df	*P*
Gender	Male	11	55%	15	43%	0.61	1	0.43	5	83%	5.50	1	0.02
	Female	9	45%	20	57%				1	17%			
Race	Caucasian	11	55%	25	71%	5.65	3	0.13	6	100%	2.88	3	0.41
	Black	9	45%	6	17%				0	0%			
	Hispanic	0	0%	2	6%				0	0%			
	Asian	0	0%	2	6%				0	0%			

Note. Differences were tested using chi-square test of independence.

Table A2

Differences in continuous variables between participants that completed the study, participants that qualified and dropped out, and excluded participants.

	Excluded (*n*=22)		Completed (*n*=36)		Completed and Excluded			Dropped Out (*n*=8)		Included and Dropped Out		
	M	*SD*	*M*	*SD*	*t*	df	*p*	*M*	*SD*	*t*	df	*p*
Age	25.05	2.84	24.81	3.44	0.27	56	0.78	23.13	3.44	-0.86	42	0.39
Drinking Days	41.05	25.71	34.47	13.93	1.24	56	0.22	22.63	9.99	-1.59	42	0.12
Drinks Per Day	4.35	2.32	5.24	3.53	-0.99	56	0.33	5.20	1.99	-0.28	42	0.78
AUDIT	10.45	6.17	10.06	3.35	0.32	56	0.75	8.38	1.85	-1.36	42	0.18
Negative Urgency	2.03	0.90	2.05	0.56	-0.10	56	0.92	1.72	0.28	-1.33	42	0.19

Note. Differences were tested using independent samples t-test.

Table A3

Differences in categorical variables between Sample 1 and Sample 2

		Sample 1 (*n*=22)		Sample 2 (*n*=14)				
		Frequency	Percent	Frequency	Percent	χ^2	df	*p*
Gender	Male	10	45%	5	36%	0.33	1	0.56
	Female	12	55%	9	64%			
Race	Caucasian	14	64%	12	86%	8.81	3	0.03
	Black	6	27%	0	0%			
	Hispanic	0	0%	2	14%			
	Asian	2	9%	0	0%			

Note. Differences were tested using chi-square test of independence.

Table A4

Differences in continuous variables between Sample 1 and Sample 2.

	Sample 1 (*n*=22)		Sample 2 (*n*=14)				
	M	*SD*	*M*	*SD*	*t*	df	*p*
Age	24.77	3.73	24.86	2.83	-0.07	34	0.94
Drink Days	36.73	15.24	30.93	11.19	1.23	34	0.23
Drinks Per Day	6.47	4.05	3.31	0.74	2.87	34	0.01
AUDIT	11.09	3.31	8.43	2.82	2.49	34	0.02
Negative Urgency	2.11	0.63	1.94	0.43	0.87	34	0.39

Note. Differences were tested using independent samples t-test.

Table A5

Differences in self-reported affect rating following mood induction.

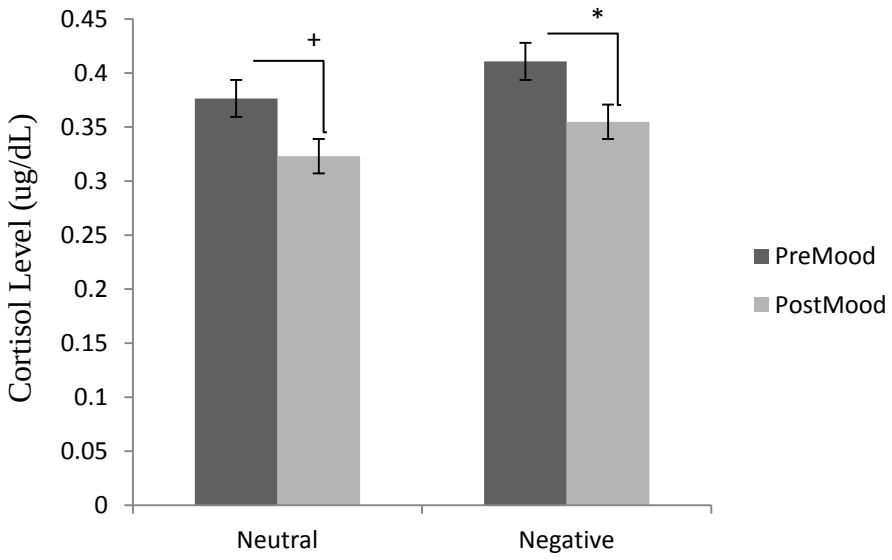
	Source	SS	df	Mean Square	F	*p*
Within-Subjects Effects	Mood	31.60	1	31.60	25.45	<0.01
Between-Subjects Effects	Intercept	5808.39	1	5808.39	1907.82	<0.01
	Error	243.56	40	3.05		

Table A6

Differences in self-reported affect in response to alcohol administration.

	Source	SS	df	Mean Square	F	*p*
Within-Subjects Effects	Mood	0.39	1	0.39	0.51	0.48
Between-Subjects Effects	Intercept	6195.51	1	6195.51	2417.19	<0.01
	Error	205.05	40	2.56		

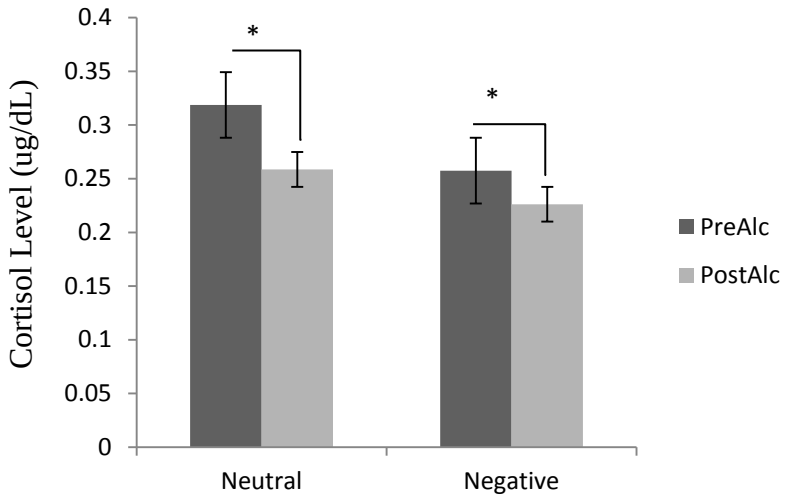
Appendix B: Main Results



Note. Differences were assessed using independent samples t-tests.
[+] trend- Neutral: $t(35)= 1.87$, $p= 0.07$
[*] significant at $p<0.05$- Negative: $t(35)= 2.49$, $p= 0.02$

Figure B1.

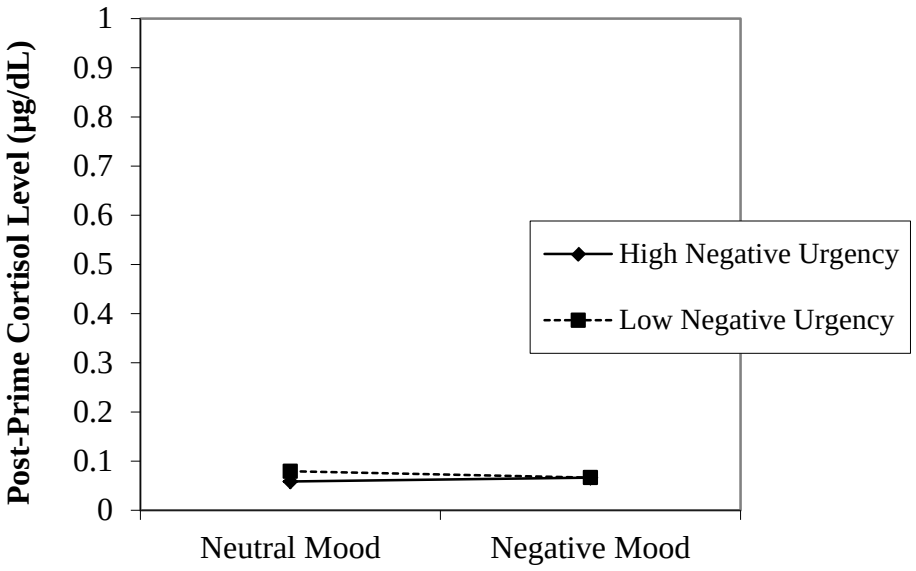
Differences in cortisol release in response to mood induction.



Note. Differences were assessed using independent samples t-tests.
* significant at $p<0.05$- Neutral: $t(35)= 2.60$, $p= 0.01$; Negative: $t(35)= 3.99$, $p< 0.01$

Figure B2.

Differences in cortisol release in response to the alcohol prime.



Note. Negative urgency*mood interaction was assessed using repeated measures ANOVA, $F(19)= 0.70$, $p=0.79$.

Figure B3.

Interaction between negative urgency and mood induction in response to alcohol prime on cortisol release.

Table B1.

Results of repeated measures ANOVA mood induction effects on salivary cortisol levels.

	Source	SS	df	Mean Square	F	*p*
Within-Subjects Effects	Mood	0.00	1	0.00	0.02	0.88
Between-Subjects Effects	Gender	0.00	1	0.00	0.05	0.83
	Time Awake	0.02	1	0.02	1.35	0.25
	Error	0.89	68	0.01		

Table B2.

Repeated measures ANOVA negative urgency effects on salivary cortisol release in negative mood induction.

	Source	SS	df	Mean Square	F	*p*
Within-Subjects Effects	Negative Urgency	1.21	19	0.06	0.86	0.63
Between-Subjects Effects	Gender	0.05	1	0.05	0.64	0.44
	Time Awake	0.01	1	0.01	0.04	0.85
	Error	0.16	68	0.01		

Table B3.

PROCESS macro results of negative urgency mediating mood induction-cortisol release.

Predictor	*b*	*SE*	*t*	*p*	LLCI	ULCI
Model 1						
Constant	0.17	0.77	0.22	0.82	-1.37	1.71
Mood	-0.04	0.12	-0.31	0.76	-0.29	0.21
Gender	0.50	0.13	3.85	<0.01	0.24	0.75
Time Awake	0.01	0.01	1.76	0.08	-0.01	0.01
Pre-Mood Cortisol	-0.48	0.30	-1.60	0.11	-1.08	0.12
Model 2						
Constant	-0.10	0.22	-0.45	0.65	-0.53	0.33
Negative Urgency	0.07	0.03	1.92	0.06	-0.01	0.14
Mood	0.01	0.03	-0.26	0.79	-0.08	0.06
Gender	0.02	0.04	0.48	0.64	-0.06	0.09
Time Awake	0.01	0.01	0.08	0.94	-0.01	0.01
Pre-Mood Cortisol	0.67	0.09	7.84	<0.01	0.49	0.84
Direct Effects	-0.01	0.03	-0.26	0.79	-0.08	0.06
Indirect Effects of Negative Urgency	-0.01	0.01			-0.03	0.01

Table B4.

*Repeated measures ANOVA for a mood*alcohol interaction on salivary cortisol release.*

	Source	SS	df	Mean Square	F	*p*
Within-Subjects Effects	Mood	0.07	1	0.07	1.31	0.26
	Mood*Alcohol	0.01	1	0.01	1.41	0.24
Between-Subjects Effects	Sex	0.06	1	0.06	0.87	0.33
	Time Awake	0.03	1	0.03	0.61	0.44
	Error	0.37	68	0.01		

Table B5.

Repeated measures ANOVA results of negative urgency related to cortisol release following alcohol prime.

	Source	SS	df	Mean Square	F	*p*
Within-Subjects Effects	Negative Urgency	1.42	19	0.08	1.59	0.13
	Mood	0.07	1	0.07	1.56	0.22
	Negative Urgency*Mood	0.62	19	0.03	0.70	0.79
Between-Subjects Effects	Sex	0.07	1	0.07	1.53	0.23
	Time Awake	0.02	1	0.02	0.35	0.56
	Error	0.15	28	0.01		

Appendix C: Supplementary Figures and Tables

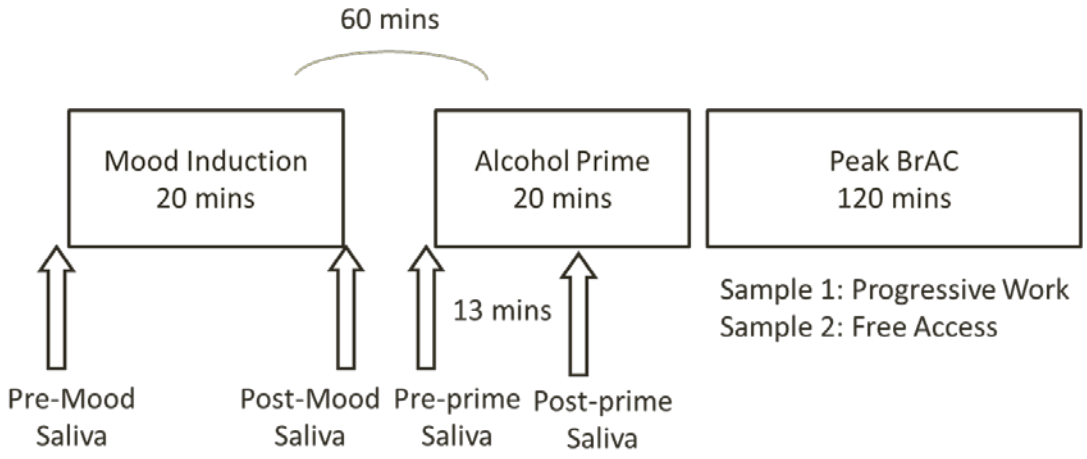


Figure C1.

Timeline of Study Procedures.

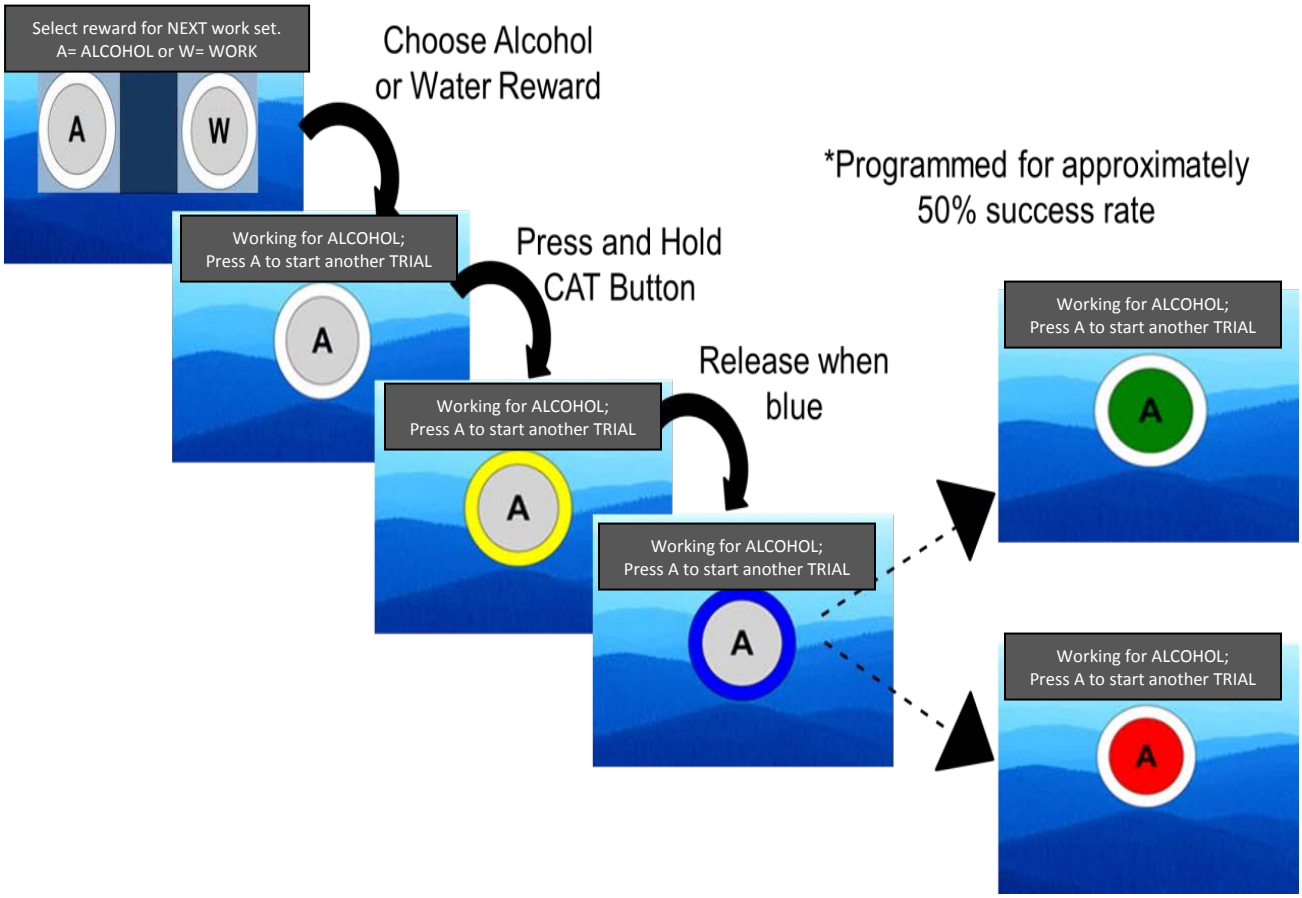


Figure C2.

Constant Attention Task- Sample 1 Only.

The goal of this experiment is to see how much work you are willing to perform in order to earn alcohol and whether you prefer alcohol over water as a reward. To measure your willingness to work, you'll be completing trials of the constant attention task (CAT) that you practiced in your screening session.

As you might remember, the task is organized into work sets. At the beginning of each work set, you will see an option to select "alcohol" or "water" for your reward. At this time, we'll have you complete an affect grid, and then choose your reward. Once you choose the reward, you have to complete the work set before you will be able to choose another reward.

After choosing the reward, you will see a circle on the screen. To start each trial, you press and hold the CAT button. When you do this the ring around the button symbol on your screen will turn yellow. A short time later, the ring will turn blue. To have the trial count toward earning your reward, you must release the button before a timer runs out on the blue ring. If you are fast enough, the center circle will turn green. If not, the center circle will turn red. You must pay close attention to complete the task successfully. Although the system is built so that you will be fast enough about one-half of the time, the task is measuring your actual reaction time, and, as such, receiving a reward does depend on your attention and responses on the task.

The number of correct trials required in each work set increases throughout the session, and we anticipate that at some point you might decide that the work is too much to continue. It is entirely up to you whether you want to continue working for additional rewards or not. You can wait as long as you wish before starting any trial in a work set and pause whenever you wish. There is no obligation to complete a work set, even if it has already been started. If you choose to stop working, your session will still last 2 hours and you will still be required to stay here until approximately 7pm.

Every now and then throughout the experiment we will ask you to answer questions on the screen about how you are feeling. Please read these questions out loud as you answer them. Additionally, we'll need to obtain occasional BAL readings from a breath meter that you will blow into. If you need to take a bathroom break, please tell us as far ahead as you can so we can pause the experiment. We do not expect you to experience any nausea or discomfort, but if you do, please tell us right away. If you need to stop for any reason, you will still get paid for your participation. Any questions?

Ok, great. Go ahead and put on your headphones and we will start the music. Shortly, we will have you start with the CAT task, to earn your first two rewards (please choose alcohol for each of these first two trials). After that, we will have you sit for a few minutes and just experience the music and the effects of the alcohol. During this time, we will have you give a breath alcohol reading, answer the questions on the screen (remember to read them out loud), and take a saliva sample, as you did earlier. At the end of this, we will have you read aloud what you wrote earlier, and then we will have you start working by choosing to work for alcohol or water. Please remember to complete an affect grid each time before you choose water or alcohol. Any questions?

Figure C3.

ASAP CAT Instructions- Sample 1

Welcome to the IV Bar! Your task is to "drink" and enjoy alcohol. But in this case, the "drinking" is done by giving you alcohol intravenously (that is, through a vein in your arm). You will be able to order intravenous "drinks" by pressing a button. For our purposes here today, it is important that you order just the right amount of alcohol so that it is ***most enjoyable*** to you (that is, so that it feels best). Most specifically, this is not an occasion to see how intoxicated you can become, or to test how much alcohol you can tolerate. Rather, you should "order drinks" so that the effect is ***most enjoyable*** to you. Here's how this will work

At the start of session, we will prompt you to order your first bit of alcohol. This is to be sure you know how the system works and to help you know what IV alcohol feels like. After that, we will wait for about 20 minutes before letting you take charge for the next 2 hours.

After you complete the 20 minute priming session, we will ask you to read your narrative out loud.

You will be able to order more drinks by pressing the button at your leisure. That is, just because you see that the bar is open, and that you can press the button for more alcohol, does not mean that you *have to* press and order more. The button will wait for you.

During the couple of minutes that any of your alcohol orders are being delivered, button pushing will not order any alcohol. Your computer screen will tell you when you can make another order for more alcohol.

There is a safety limit to how much alcohol you can order; we close the bar whenever the next drink would take you past that safety limit.

From time to time we will ask you to complete a computerized questionnaire about how the alcohol makes you feel.

From time to time we will ask you to complete an affect grid. We ask that you complete this before pressing the button to order your drink.

Once in a while, we will ask you to wait while we measure your breath alcohol, but you will not be told the result of those measurements. At those times we will also be asking you to perform a brief finger-to-nose and hand-clapping task in order to judge if your motor coordination has been impaired. If we think your coordination is too impaired for safety, or that you are uncooperative, we will stop the experiment, but you will still be paid for the session.

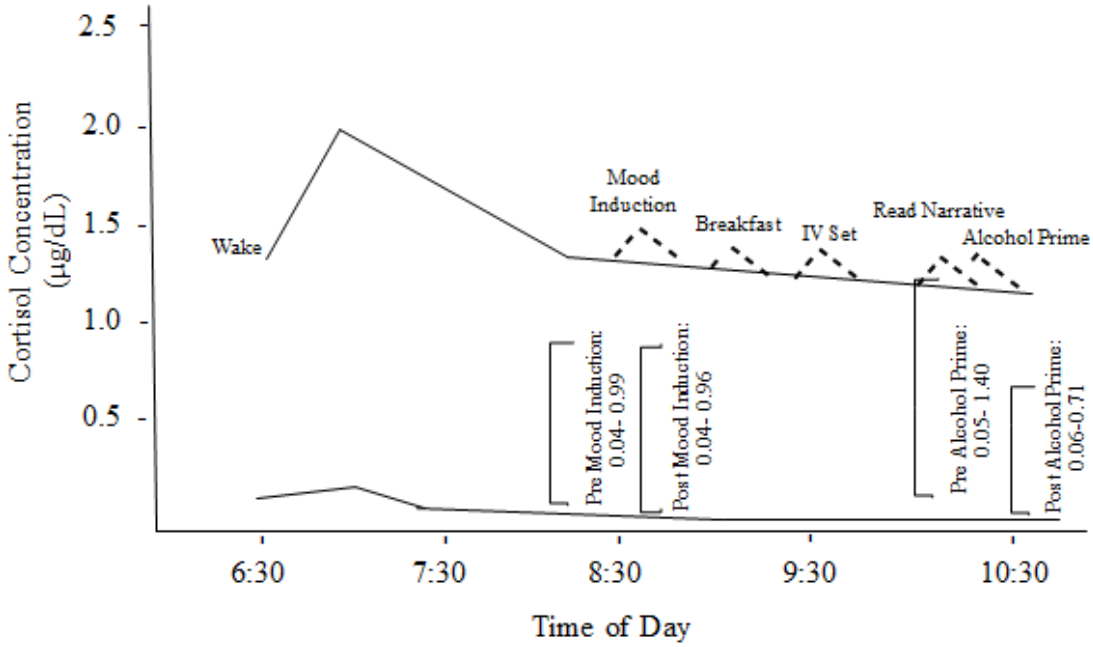
You can stop or pause at any time and take bathroom breaks as needed. You will be staying on the CRC until approximately 7pm, no matter how much or how little alcohol you order, so don't worry about time.

If you have questions at any time, please ask the technician. If you experience any discomfort or nausea, please tell the technician right away. If you need to use the bathroom at any time, please tell the technician ahead of time.

Any Questions? Ready?

Figure C4.

ASAP CAT Instructions- Sample 2.



Note. Solid line is cortisol daily circadian rhythm based on participant average wake time of 6:30am (SD=34.43). Dotted lines are hypothesized cortisol increases based on study procedures. Brackets represent obtained salivary cortisol concentration ranges.

Figure C5.

Expected and observed cortisol concentrations throughout procedure.

Table C1.

Musical mood induction playlists for negative and neutral sessions.

NEGATIVE	Duration
Writing Exercise	Total: 19m 54s
Beethoven – Sonata 14	15m 0s
Eagles- "I Can't Tell You Why"	4m 54s
Progressive Work	Total: 2hr 28m 02s
Willie Nelson- "Blue Eyes Crying in the Rain"	3m 32s
Raag Basant- "Classical Wonder of India"	18m 30s
Edvard Grieg- "Peer Gynt Suite"	14m 49s
Gustav Holst- "The Planets- Mars, Bringer of War"	7m 26s
Chopin- "Funeral March"	8m 25s
Keith Jarret- "Spheres"	12m 9s
Albinoni- "Adagio"	8m 57s
Luther Vandross- "Superstar"	7m 49s
Alessandro Marcello- "Oboe Concerto in D Minor"	4m 19s
Bonnie Raitt- "I Can't Make You Love Me"	4m 43s
Mussorgsky- "Nigh On Bald Mountain"	9m 37s
Beethoven- Sonata 3	9m 55s
Faure- "Pie Jesu"	4m 43s
Alan Stivell- "Renaissance of the Celtic Harp"	10m 0s
Sinead O'Connor- "Nothing Compares to You"	5m 15s
Paradise Lost- "Forever Failure"	4m 18s
Samuel Barber- "Adagio pour Cordes"	8m 20s
Kenny G- "Ester"	5m 25s

NEUTRAL	Duration
Writing Exercise	Total: 20m 37s
John Adam- "Common tones in simple time"	20m 37s
Progressive Work	Total: 2hr 34m
Reymond Lefevre- "Le Canon de Pachelbel	3m 27s
Antonin Dvorak- "New World Symphony"	42m 37s
Debussy- "La Mer"	9m 9s
Steve Reich- "Variations for winds, strings, and keyboards"	2m 17s
Chopin- Waltz no.11	1m 54s
Gustav Holst- "The Planets Op.32"	7m 34s
Faure- "Ballade for piano and orchestra, op.19"	14m 29s
Kraftwerk- "Pocket Calculator"	4m 57s
Mozart- "Symphony No.40"	26m 25s
Chopin- "The Waltzes no. 12"	3m 0s
Claude Debussy- "Prelude"	10m 51s
Michael Hedges- "Aerial Boundaries"	4m 42s
Delibes- "Coppelia"	2m 31s
John Adam- "Common tones in simple time"	20m 37s

Table C2.

Salimetrics Salivary Cortisol Enzyme Immunoassay Supplies

Supplies

1) **Microtitre Plate**- coated with monoclonal anti-cortisol antibodies

2) **Cortisol Standards**- 500µL each of 3.0, 1.0, 0.333, 0.11, 0.037, 0.12 µg/dL of traceable NIST standard containing cortisol, buffer, and preservative

3) **Cortisol Controls**- High, Low in a saliva-like matrix contains cortisol, buffer, and preservative

4) **Wash buffer concentrate**- contains phosphate buffer, detergent, and preservative diluted to 10mL/L

5) **Assay Diluent**- contains phosphate buffer, pH indicator, and preservative

6) **Cortisol Enzyme Conjugate**- cortisol conjugated to HRP and preservative, diluted using 15µL of conjugate to 24mL of assay diluent

7) **Tetramethylbenzidine (TMB) Substrate Solution**

8) **Stop solution**- contains 3 M sulfuric acid

9) **Non-specific binding (NSB) wells used as blanks.**

Table C3.

Salimetrics Salivary Cortisol Enzyme Immunoassay Procedure

1. Bring all reagents to room temperature and mix before use.
2. Bring plate to room temperature and prepare for use with NSB wells. (Use of NSB wells is optional.)
3. Prepare 1X wash buffer.
4. Prepare tube with 24 mL of assay diluent for conjugate dilution, which will be made later.
5. Pipette 25 µL of standards, controls, and unknowns into appropriate wells.
6. Pipette 25 µL of assay diluent into zero and NSB wells.
7. Make 1:1600 dilution of conjugate (15 µL into 24 mL assay diluent), mix,
8. Mix plate for 5 minutes at 500 rpm. Incubate for an additional 55 minutes at room temperature.
9. Wash plate 4 times with 1X wash buffer. Blot.
10. Add 200 µL TMB solution to each well.
11. Mix plate for 5 minutes at 500 rpm. Incubate in dark at room temperature for 25 additional minutes.
12. Add 50 µL stop solution to each well. Mix for 3 minutes at 500 rpm.
13. Wipe plate bottom clean and read within 10 minutes of adding stop

Table C4.

Targeted drugs, metabolites, and cutoff concentrations in drug test

Test	**Calibrator**	**Cut off (ng/mL)**
Amphetamine	Amphetamine	1,000
Barbiturates	Secobarbital	300
Benzodiazepines	Oxazepam	300
Cocaine	Benzoylecgonine	300
Marijuana	Marijuana	50
Methadone	Methadone	300
Methamphetamine	Methamphetamine	1000
Opiate	Morphine	2,000
Phencyclidine	Phencyclidine	25
Oxycodone	Oxycodone	100