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Relationship Between Level of Response to Alcohol and Acute Tolerance

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ABSTRACT

Background: A low level of response (low LR) to alcohol correlates with the later development of alcohol-related problems. Although some of the underpinnings of LR are understood, little is known about the potential relationship between LR and acute tolerance. The current analyses tested the hypothesis that a low LR will be explained in part by more intense acute tolerance to alcohol during a drinking session.

Methods: Data were generated through a reanalysis of data from 120 individuals who were 18- to 25-year-old, sex-matched pairs of low and high LR drinkers who at baseline did not yet meet criteria for an alcohol use disorder. Each subject participated in an oral alcohol challenge after consuming about 0.7ml ethanol per kg and acute tolerance was measured as the differences in alcohol's effects at similar breath alcohol levels (BrACs) during the rising and falling breath alcohol (BrAC) curve. Measures included aspects of the Subjective High Assessment Scale (SHAS) and body sway.

Results: Contrary to our hypothesis, but similar to results with other alcohol measures, acute tolerance was actually significantly attenuated in low LR compared with high LR individuals on most SHAS scores. Neither LR group demonstrated acute tolerance to alcohol for sleepiness or body sway. Men and women did not differ on any of these measures.

Conclusion: These data do not support a role of acute tolerance in the low LR to alcohol as measured by subjective feelings of intoxication or body sway in these subjects, findings that were similar across males and females. In addition, consistent with the literature, the analyses demonstrated differences in acute tolerance across measures such that this phenomenon was observed for most measures of subjective effects but not for body sway. Among the subjective effects, acute tolerance was observed for alcohol's intoxicating effect but not for feeling sleepy.

Keywords: alcohol sensitivity, level of response, acute tolerance, within-session tolerance; Mellanby effect

INTRODUCTION

There is compelling evidence from our group (Schuckit et al., 2016b, Schuckit, 2018) and others (Quinn and Fromme, 2011, Newlin and Renton, 2010, King et al., 2014) that characteristics related to how a person reacts to alcohol earlier in life correlates with the later development of heavy drinking, alcohol-related problems, and risk for alcohol use disorders (AUDs). These include our own low level of response (LR) model (Schuckit, 2018), Newlin and Thomson's Differentiator (D) Model (Newlin and Thomson, 1990), and King's Modified Differentiator (MD) Model (King et al., 2014). In our own work the less intense alcohol response at rising and peak alcohol levels is supported by a host of hormonal (Schuckit et al., 1987, Schuckit et al., 1988b) and electrophysiological data (Ehlers et al., 2004), with fMRI responses indicating potential LR group differences in some cognitive processes (Paulus et al., 2012, Schuckit et al., 2012). However, despite the robust correlation between the intensity of one's reaction to alcohol and the subsequent development of alcohol-related problems, it is not known if the level of response relates to another well-established phenomenon, tolerance. This is important because a better understanding of the underpinnings for phenotypes that contribute to an enhanced vulnerability to heavy drinking and alcohol problems can lead to prevention approaches that diminish that vulnerability (Schuckit et al., 2016a, Conrod et al., 2013).

The concept of tolerance is broad and has several components. These include pharmacodynamic, or functional, tolerance where the body develops less response, or more resistance, to a given level of the drug (Haass-Koffler and Perciballi, 2020, Kalant, 1998). Functional tolerance can be further characterized based on the duration and intervals between alcohol exposure. Acute tolerance, which develops during a single exposure to alcohol and is sometimes labeled as within-session tolerance or the Mellanby effect (Holland and Ferner, 2017), refers to the phenomenon whereby in a single drinking session one experiences less alcohol effect at a given blood level at falling alcohol concentrations as compared to an identical alcohol concentration at rising levels (Martin and Moss, 1993). Repeated bouts of alcohol

exposure can also produce chronic, or intersession, tolerance to the drug which might reflect both the pharmacodynamic and pharmacokinetic effects and is the usual tolerance definition that applies to the AUD criterion item in the recent versions of the Diagnostic and Statistical Manuals (DSMs) of the American Psychiatric Association (2013).

Acute tolerance in humans can be measured in a research laboratory by either having subjects ingest alcohol-containing beverages or by infusing ethanol intravenously (Cyders et al., 2020). While each method of administration has its strengths and limitations (see (Cyders et al., 2020) for a critical review), systematic reviews of the acute tolerance literature find that 60% (Holland and Ferner, 2017) to 80% (Comley and Dry, 2020) of these alcohol challenge studies yield evidence for acute tolerance to at least some of alcohol's effects. The reviews also find more consistent evidence of acute tolerance when subjective measures of intoxication are assessed at rising and falling alcohol concentrations as opposed to more objective measurements such as performance on neuropsychological tests or driving simulation (Holland and Ferner, 2017, Comley and Dry, 2020).

In summary, some studies have used alcohol challenges to document acute tolerance (reviewed in (Holland and Ferner, 2017) and (Comley and Dry, 2020)), and others have used alcohol challenges to evaluate the type and intensity of reaction to alcohol in individuals at higher risk for AUDs before repeated binge drinking or multiple alcohol problems develop. However, few, if any, studies have evaluated both acute tolerance and LR in the same population. When the relatively lower intensity of response to alcohol was first identified in young adult light-to-moderate drinking non-AUD offspring of individuals with AUDs, the phenomenon was labeled as a "low LR" because it was not possible to determine if the measure related to innate sensitivity or was the consequence of the development of some form of tolerance. Thus, there is a need to add evaluations of acute tolerance to alcohol challenge studies focusing on the low LR phenotype.

This paper presents the results of secondary data analyses from one of our prior alcohol challenge studies to directly test whether moderate drinking low and high LR individuals differ in the development of acute tolerance. The data compare alcohol challenge scores at similar breath alcohol concentrations (BrACs) along the ascending and descending limbs of the BrAC curve. Data are available on changes in scores for subjective responses to alcohol and alterations in the amount of body sway. Our Hypothesis 1 is that low LR individuals, who have been shown to demonstrate less intense subjective feelings and body sway during the alcohol challenge, will also demonstrate greater levels of acute tolerance (the Mellanby effect) than their sex- and age-matched high LR counterparts. In addition, Hypothesis 2 predicts that, the relationship of LR to acute tolerance will be similar across the sexes (Plawecki et al., 2019, Morzorati et al., 2002).

MATERIALS AND METHODS

Participants

As described in detail in our prior work (Paulus et al., 2012, Schuckit et al., 2012, Schuckit et al., 2016b), participants in the present secondary data analysis were 18- to 25-year-old Anglo and white Hispanic students enrolled at the University of California, San Diego (UCSD) who took part in a multistage experiment examining fMRI differences in subjects with low and high responses to alcohol. Following approval by the UCSD Human Research Protection Program, a random cohort of students was first asked to respond to an email survey requesting information on demography, physical health, drinking and other drug use characteristics, as well as their family history of alcohol and other drug related problems. Their survey responses were used to identify an initial cohort of healthy, right-handed students who had experience with alcohol but who never met criteria for an alcohol use or illicit substance use disorder; were not pregnant; and to be eligible for this functional Magnetic Resonance Imaging (fMRI) study, had no irremovable body metal and no history of traumatic brain injury.

The survey also included the Self-Report of the Effects of Alcohol questionnaire, a retrospective measure of LR, as a preliminary screen for the low LR phenotype (Schuckit et al., 1997, Schuckit et al., 2019). The SRE uses 12-items that ask individuals to recall the number of standard (10 to 12 grams of ethanol) drinks it took to feel four effects of alcohol across three time frames. The effects are: first feeling any effect; feeling as if speech was slurred; feeling unsteady walking; and unwanted falling asleep (Schuckit et al., 1997, Schuckit et al., 2019). The three time periods included the first five times one ever consumed at least a full drink, most recent three months where drinking at least once a month, and during one's period of heaviest drinking. The score for each period was the sum of the number of drinks for the effects actually experienced with alcohol for that timeframe, divided by the number of the up to four experiences reported to generate the average drinks needed per effect. In the present analysis, the First-5 (SRE-5) metric was used to preliminarily categorize participants into low (SRE scores indication averaging 4+ drinks per effect) and high LR subgroups (average scores of 3 drinks or less) (Schuckit et al., 2012). Each low LR individual was matched to a high LR subject on other characteristics that might affect LR including age, sex, recent six-month pattern of intake of alcohol, nicotine use and their use of other drugs (Schuckit et al., 2012).

Respondents who completed the survey, met the initial inclusion criteria, and who completed the SRE were contacted by phone to confirm their continued interest in participating in the laboratory portion of the protocol. Selected participants were invited to come to the laboratory where a trained interviewer administered the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994) interview to review their personal and family history of psychiatric and substance use disorders. Participants who still met the recruitment criteria were instructed to fast overnight before coming to the laboratory at 8AM and to refrain from using alcohol or other drugs for at least 48 hours prior to their first alcohol challenge session in our laboratory as part of the final screen for the subsequent fMRI placebo

and alcohol challenges. The data reported here came from that laboratory-based alcohol challenge as the fMRI-based sessions did not include the full usual laboratory measures.

Alcohol Challenge

Upon arrival at the laboratory, participants underwent a breathalyzer test (Intoximeters ™ Inc., St. Louis, MO) to confirm a zero-breath alcohol concentration (BrAC). They were seated in a recliner, allowed to acclimate to the lab environment, and fed an isocaloric snack. After approximately one hour, they were given 10 minutes to imbibe an alcoholic beverage mixed as a 20% by volume solution in a carbonated, non-caffeinated sugar-free soda flavored to their choice. Male participants received 0.75 mL/kg ethanol while female participants ingested a drink containing 0.70 mL/kg to adjust for sex differences in body water (Baraona et al., 2001). The average resulting BrAC peak was approximately 60 milligrams/dL at about 60 minutes post-ingestion as shown in Table 1 (Paulus et al., 2012, Schuckit et al., 2012). As per the standard procedure performed in our lab over the years, the beverage was consumed through a straw extending from a thermos that obscured the actual beverage offered.

At baseline prior to administering the drink, and at 30-minute intervals thereafter for up to 210 minutes, participants completed the Subjective High Assessment Scale (SHAS) (Schuckit and Gold, 1988). For these secondary analyses, to assess SHAS items most comparable to subjective measures used in other labs that perform human laboratory alcohol research (Cyders et al., 2020), we focused on the SHAS-7 items (Eng et al., 2005, Schuckit et al., 2000) of feeling High, Clumsy, Confused, Dizzy, Drunk, Alcohol's Effects, and Difficulty Concentrating. Notably, the SHAS-7 score correlates highly with the complete 13-item measure that the Schuckit lab has used widely in their research and it uses the same visual analog marking scales to measure an individual's subjective responses to alcohol (Eng et al., 2005, Schuckit et al., 2000). To compare our results more directly with reports from other human laboratories that measure subjective responses to alcohol and that use Biphasic Alcohol Effects Scale (BAES) (Martin and

Moss, 1993, Plawecki et al., 2019), we also analyzed the feeling Sleepy subscale of the SHAS which corresponds best with the Sedation subscale of the BAES. BrACs were also obtained every 30 minutes.

Body sway, or standing ataxia, was recorded using a harness attached to the participant at the level of the axilla, from which ropes extended to the front and side at an approximate 90-degree angle from one another. Each rope passed over a pulley and anterior-posterior (AP) and lateral sway were recorded as the total number of centimeters of back-and-forth movement of the rope. Subjects completed three 1-minute trials at each time point with eyes open, feet together, and hands at their sides, with scores recorded as the mean values of the three trials. This is the same approach that has been used in our laboratory since about 1980. Body sway scores were adjusted for baseline differences before analyses were conducted.

In keeping with NIAAA guidelines, participants were released from the laboratory when their BrAC fell below 0.01 g %. Following the completion of the laboratory-based alcohol challenge individuals went on to participate in the fMRI portion of the study the results of which have been reported previously (Paulus et al., 2012, Schuckit et al., 2012, Schuckit et al., 2016b).

Evaluations of Acute Tolerance

The following paradigm was used to compare low and high LR participants on their patterns of within-session acute tolerance. Using the methods of Plawecki et al. (2019) (Plawecki et al., 2019), the half-peaks on the ascending and descending BrAC arms, as well as the peak of the individual's BrAC curve, were calculated. Specifically, we used the Spline function in MATLAB® to determine the latencies corresponding to a session's peak BrAC and to the same half-peak BrAC on the ascending and descending arms of the BrAC curve. We then computed corresponding subjective responses on the SHAS-7, Sleepy subscale, and Body Sway measures at those latencies, using linear interpolation between the nearest data

collection time points. In keeping with procedures used in our lab for decades, participants were instructed to rate their subjective feelings on the SHAS visual analog scale as “none” prior to consuming the beverage. Thus, the baseline SHAS value was always a score of zero.

Statistical Analyses

The combined SHAS-7 total of scores were calculated by summing the scores for the seven individual items that comprise the scale that included the feeling High, Clumsy, Confused, Dizzy, Drunk, Alcohol’s Effects, and Difficulty Concentrating subscales. SHAS-7 total and individual item scores, the Sleepy subscale score, and baseline-corrected anterior-posterior (AP) and lateral body sway data were analyzed using a series of two-way, 3 within-subjects factors-by-2 groups mixed effects analysis of covariance (ANCOVAs), with Greenhouse-Geisser corrections for sphericity violations. The 3-level within-subjects factor was *Time* (ascending limb half-peak, peak, and descending limb half-peak time points) and the 2-level between-subjects factor was either *LR* group (low LR versus high LR) or *Sex* (men versus women). Separate analyses examining acute tolerance were performed utilizing one-way ANCOVAs between *LR* and *Sex* groups. Here, we defined the dependent variable, acute tolerance, as the difference score (i.e., descending limb response minus the ascending limb response) for each SHAS item at half-peak BrACs. In both sets of analyses, we covaried for the usual number of drinks per typical drinking occasion for the prior 6 months given that the low- and high-LR groups differed (see Table 1) on this measure of recent drinking history prior to the alcohol challenge. The covariate was centered around the population mean before entry into the ANCOVA models as a main effect and as an interaction term with *Time* (Schneider et al., 2015). All analyses were done in SPSS version 26 (2019). The results also include the effect size using partial η^2 as a measure of the strength of the independent effects.

RESULTS

Table 1 displays the demographic and physical characteristics as well as the drinking and other drug use patterns of the 60 pairs of low and high LR participants categorized based on their scores on the SRE-5. Consistent with prior reports on subsets of this sample (Paulus et al., 2012, Schuckit et al., 2012, Schuckit et al., 2016b), the two groups were well matched on demographic and physical characteristics and most measures of drinking and other drug use frequency occurring in the past six months. Given that low LR participants reported a higher number of drinks per typical drinking occasion than high LR participants in the six months preceding the study, and since other groups have reported that recent heavy drinking influences subjective perceptions of alcohol along the ascending and descending limbs of the BrAC curve (Wetherill et al., 2012), this variable was used as a covariate in our analyses.

Subjective Response to Alcohol's Effects: As illustrated in the top panel of Figure 1, there was a significant Time-by-LR group interaction effect for total of the items in the SHAS-7 ($F_{1.7,193.3} = 10.36$, $p < 0.001$, partial $\eta^2 = 0.08$) such that the low and high LR subjects differed in the changes in the total of SHAS-7 scores on rising versus falling time points. Post-hoc Time-by-LR difference contrasts were significant when comparing the descending limb half-peak time point to the average of the other time points ($F_{1,117} = 14.48$, $p < 0.001$, partial $\eta^2 = 0.11$). To better understand the pattern of the interaction as it relates to acute tolerance, we examined the difference in SHAS-7 total scores between the descending limb minus the ascending limb with a one-way ANCOVA. As illustrated in the lower panel of Figure 1, compared with the high LR group, the low LR group exhibited a significantly attenuated degree of acute tolerance in their subjective response to alcohol ($F_{1,117} = 11.41$, $p < 0.001$ partial $\eta^2 = 0.09$). Additionally, examination of acute tolerance scores for each group showed that both low LR (mean difference = 12.55, standard error = 3.17, 95% Confidence Interval = 6.20-18.90) and high LR (mean difference = 35.72, standard error = 5.59, 95% Confidence Interval = 24.52-46.91) groups demonstrated acute tolerance, although the magnitude of the Mellanby effect was greater for the high LR group.

Figure 2 extends these analyses by examining the individual SHAS-7 items and the Sleepy subscale item. This evaluation revealed a pattern of results for each item that was similar to the results in Figure 1 that had created a total score for the combined seven items. There were significant Time-by-LR group interactions for ascending versus descending BrAC scores for six of the seven SHAS-7 items: the degrees of feeling High ($F_{1.8,204.2} = 7.00$, $p = 0.002$, partial $\eta^2 = 0.06$), Clumsy ($F_{1.9,216.9} = 7.84$, $p = 0.001$, partial $\eta^2 = 0.06$), Confused ($F_{1.6,185.3} = 4.56$, $p = 0.02$, partial $\eta^2 = 0.04$), Dizzy ($F_{1.8,211.6} = 4.50$, $p = 0.02$, partial $\eta^2 = 0.04$), Drunk ($F_{1.8,210.9} = 15.41$, $p < 0.001$, partial $\eta^2 = 0.12$), and Alcohol's Effects ($F_{1.8,204.2} = 12.89$, $p < 0.001$, partial $\eta^2 = 0.10$), but not for Difficulty Concentrating ($F_{1.9,222.8} = 2.34$, $p = 0.10$, partial $\eta^2 = 0.02$). Regarding the Sleepy subscale on the SHAS, there was only a main LR group effect ($F_{1,117} = 31.33$, $p < 0.001$, partial $\eta^2 = 0.21$) reflecting the fact that the low LR group reported feeling less Sleepy than the high LR group.

In separate analyses, a direct examination of acute tolerance, calculated by subtracting ascending half-peak values from descending half-peak scores for the SHAS-7 and Sleepy subscale items, are presented in Figure 3. There were significant group differences between low LR and high LR groups in the magnitude of acute tolerance observed for all SHAS-7 items (High: $p = 0.014$; Clumsy: $p = 0.002$; Confused: $p = 0.036$; Dizzy: $p = 0.011$; Drunk: $p < 0.001$; Alcohol's Effects: $p = 0.001$; and Difficulty Concentrating: $p = 0.038$) such that the low LR group demonstrated attenuated acute tolerance compared to the high LR group. There was no LR group difference for the SHAS Sleepy item ($p > 0.05$).

***LR Group Effects on Body Sway (Standing Ataxia)*:** As depicted in Figure 4, for anterior-posterior (AP) body sway measurements, there was no significant main effect of Time or a Time-by-LR group interaction effect (p's > 0.05), but there was a trend towards significance for a LR group effect ($F_{1,117} = 7.37$, $p = 0.08$, partial $\eta^2 = 0.06$; Fig. 4); the high LR group demonstrated slightly greater AP body sway than the low LR group. Examining the marginal means for LR group

differences at each of the three time points revealed only significant LR group differences at the peak BrAC ($p = 0.01$) where, again, low LR participants exhibited less standing ataxia than the high LR group.

For lateral body sway measurements, there were no significant Time-by-LR group interaction or main effects of time (p's > 0.05), but there was a trend towards significance for a LR group effect ($F_{1,117} = 3.29$, $p = 0.07$, partial $\eta^2 = 0.03$). However, examination of the interaction pattern using difference contrasts revealed a trend for a significant time-by-LR group interaction comparing peak to ascending half-peak time points ($F_{1,117} = 3.14$, $p = 0.08$, partial $\eta^2 = 0.0$) such that the low LR and high LR groups differed at peak BrACs ($p = 0.02$) and not at the ascending half-peaks of the BrAC curve (p's > 0.05; see Figure 4).

Sex Effects: There were no significant interactions between Sex and Time on the SHAS-7 ($F_{1.7,189.2} = 2.28$, $p = 0.11$, partial $\eta^2 = 0.04$), Sleepy subscale score ($F_{1.9,217.8} = 0.11$, $p = 0.89$, partial $\eta^2 = 0.001$), lateral body sway ($F_{1.6,187.3} = 2.12$, $p = 0.13$, partial $\eta^2 = 0.04$), or A/P body sway measures ($F_{1.6,181.8} = 1.78$, $p = 0.18$, partial $\eta^2 = 0.02$). Similarly, there were no main effects of sex on the SHAS-7 ($F_{1,117} = 0.06$, $p = 0.80$, partial $\eta^2 = 0.001$), Sleepy subscale score ($F_{1,117} = 3.72$, $p = 0.06$, partial $\eta^2 = 0.03$), lateral body sway ($F_{1,117} = 0.80$, $p = 0.37$, partial $\eta^2 = 0.01$), or A/P body sway measures ($F_{1,117} = 0.72$, $p = 0.40$, partial $\eta^2 = 0.01$). However, for the SHAS Sleepy subscale score, there was a trend toward women reporting increased levels of sleepiness compared with men at each time point.

DISCUSSION

These analyses are the first to directly examine the potential relationship between the low level of response to alcohol and acute tolerance. The results offered no support for Hypothesis 1, which had predicted that the low LR to alcohol would be explained, at least in part, by a more robust acute adaptation to alcohol's effect in participants with low LR. None of the measures

tested here supported that prediction including results from the SHAS-7, the subjective report of feeling sleepy, or the body sway measures. These negative results regarding acute tolerance complement the evaluation of light drinkers with lower LR who were evaluated in their early teens but who were unlikely to have developed chronic tolerance (Schuckit et al., 2008). The combination of the current results and the prior data regarding drinking 13-year-old UK sample is not consistent with a conclusion that either form of functional tolerance is central to the low LR phenomenon.

The current results actually indicated the opposite of Hypothesis 1 in that low LR participants had significantly lower acute tolerance responses on the SHAS-7 in response to the alcohol challenge than high LR individuals. . That finding of a relative resistance to change in the presence of alcohol is consistent with the less intense alcohol reaction seen for most measures of alcohol response including those related to EEG (Ehlers et al., 2004), ERP (Schuckit et al., 1988a), hormonal (Schuckit et al., 1987), and fMRI measures (Paulus et al., 2012, Schuckit et al., 2012). As reported elsewhere (Paulus et al., 2012), some data indicate that the low LR might reflect a general need for greater cognitive effort to recognize relatively subtle differences in some sensory inputs, a phenomenon that might relate to sensitivity rather than tolerance. Prior to the current analysis it was not possible to evaluate whether acute tolerance also contributed to the low LR phenomenon.

In addition to the hypothesis that low LR reflects a diminished sensitivity to interoceptive cues that does not highly correlate with intra-session tolerance *per se* (Paulus et al. 2012; Schuckit et al 2008), the present results shine new light on several older theories intended to explain the relationship between an individual's initial sensitivity to a drug and the development of acute tolerance. Regardless of whether these models evoked theories involving classical Pavlovian conditioning principles (Siegel et al., 2000, Siegel, 1983), the opponent-process theory of acquired motivation (Solomon, 1980), the

regulatory model of addictive vulnerability (Ramsay et al., 2020, Ramsay and Woods, 1997) or Koob's and Le Moal's allostatic model of addiction (Koob and Le Moal, 2001), a key element that cuts across these four models is the view that acute tolerance likely represents an individual's counter-regulatory response to a drug's pharmacodynamic effects. Viewed through this lens, another interpretation of our results might be that low LR individuals exhibit diminished acute tolerance compared with the high LR group because their compensatory physiological responses underwent more rapid, long lasting, or intense adaptation after their initial exposures to alcohol earlier in their histories. Alternatively, at the moderate dosage of alcohol tested, the magnitude of the pharmacodynamic stimulus may be diminished in low LR individuals, thus, eliciting an attenuated counter-regulatory response in LR participants compared with high LR individuals. While the present results cannot answer these considerations, our current studies are probing alcohol's pharmacodynamic effects at higher dosages. We are also currently examining the drug's effects on the stress axis and in relation to functional connectivity networks governing stress and reward processing to further explore the relationship between alcohol sensitivity and tolerance. The finding that at least high LR individuals demonstrated acute within-session tolerance to a single dose of alcohol is consistent with a large body of literature over the past 70 years. Both Holland and Ferner (2017) (Holland and Ferner, 2017) and Comley and Dry (2020) (Comley and Dry, 2020) in their systematic reviews of the topic reported that 63% to 81% of the dozens of studies they reviewed found evidence for acute tolerance to alcohol. What is novel about the present study, however, is our demonstration that LR groups differed in their development of acute tolerance such that intra-session adaptation to alcohol effects was not related to the low LR phenotype.

Also consistent with the literature, self-ratings of intoxication were more closely linked to acute tolerance than a performance-based measure such as of body sway is also consistent with the broader literature (Holland and Ferner, 2017, Comley and Dry, 2020). In contrast to the results observed on the SHAS-7, while low LR individuals reported lower rating of sleepiness overall, acute tolerance was not observed for this subjective measure of a sedating effect of alcohol, sleepiness. Similar to the data on sleepiness, while low LR subjects tended to demonstrate lower body sway data than high LR subjects our data did not reveal evidence of acute tolerance for standing steadiness for either LR group. Finally, also consistent with the bulk of the literature (Plawecki et al., 2019, Morzorati et al., 2002), we did not find sex differences or level of response by sex interaction effects (Eng et al. 2005) in the development of acute tolerance or sensitization to alcohol's effects.

As is true of almost all research, the results presented here must be interpreted in light of the study limitations. First, the laboratory-based alcohol challenge session from which the current data were extracted was structured to verify participants' LR status and the safety of testing these subjects in an fMRI scanner, goals that did not require a placebo laboratory session. Thus, no placebo data were available for these analyses and we cannot rule out that other aspects of the experimental paradigm (e.g., alcohol expectancies) influenced the results. Additionally, since demonstrations of acute tolerance using the Mellanby effect typically require a placebo condition to rule out practice effects along the descending limb, it is important to replicate these results in other datasets that had a placebo control group. Second, for reasons relevant to the original protocol, all subjects reported Anglo or White Hispanic ethnicities and it is not clear if the current results will generalize to other ethnicities including Asian American and African American groups. Third, similar caveats relate to the fact that the 120 participants were originally selected from a Southern California university population. Fourth, the lower intensity of the alcohol response in participants with low LR might have produced a "floor effect" making it more difficult to observe differences between rising and falling BrAC timepoints for lower LR subjects. Finally, all of the

participants in these secondary analyses were 18- to 25-year-old, right-handed individuals who had no alcohol or other substance use disorder diagnoses. While this step was important from a matching and fMRI methodologic standpoint, the homogeneity of the sample might have also reduced the generalizability of the results.

In summary, we found that the development of more robust acute tolerance to alcohol is unlikely to contribute significantly to the low LR. Second, the results regarding acute tolerance were not significantly different in males and females. Finally, while acute tolerance was observed for high LR subjects for alcohol's intoxicating effects, this adaptation was not significant for alcohol-related sleepiness or for body sway, an observation that underscores how acute tolerance differs for different effects of alcohol.

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FIGURE LEGENDS

Figure 1. Top Panel: Total Scores on the 7-Item Subjective High Assessment Scale (SHAS-7) in Low LR and High LR Participants at Peak Breath Alcohol Concentration (BrAC) and Half-Peak BrACs Along the Ascending and Descending Limbs of the BrAC Curve. Lower Panel: Difference in SHAS-7 Total Scores Between the Descending Limb Minus the Ascending Limb. Error bars represent standard error of the mean.

Figure 2. SHAS-7 Individual Items + Sleepy Subscale Scores in Low LR and High LR Participants at Peak Breath Alcohol Concentration (BrAC) and Half-Peak BrACs Along the Ascending and Descending Limbs of the BrAC Curve. Error bars represent standard error of the mean.

Figure 3. Difference Scores in Subjective Responses (SHAS-7 items + Sleepy Subscale Score) to Alcohol at Half-Peak Breath Alcohol Concentrations (BrAC) Along the Descending and Ascending Limbs of the BrAC curve in Low LR and High LR Participants. Error bars represent standard error of the mean.

Figure 4. Anterior-Posterior (left panel) and Lateral (right panel) Body Sway Measurements in Low LR and High LR Participants at Peak Breath Alcohol Concentration (BrAC) and Half-Peak BrACs Along the Ascending and Descending Limbs of the BrAC Curve. Error bars represent standard error of the mean.

Table 1. Characteristics of Participants with Low and High LR to Alcohol

	Low LR (n = 60)	High LR (n = 60)	p-value[c]
Age (yrs)	19.77 (1.4)	19.97 (1.5)	0.46
% Female (n)	50%	53.30%	0.72
Yrs of Education Completed	13.58 (1.1)	13.60 (1.2)	0.94
Height (Inches)	68.30 (4.2)	68.31 (3.9)	0.99
Weight (Pounds)	154.27 (27.2)	150.88 (24.3)	0.47
Days/Months Used Alcohol[a]	7.20 (4.4)	6.27 (4.8)	0.27
Usual Drinks/Occasion[a]	3.95 (1.8)	3.17 (1.7)	0.02[d]
% Ever Used Tobacco	66.7%	48.3%	0.04[d]
% Regular Tobacco User[b]	0%	8.3%	--
% Ever Used Cannabis	66.7%	50%	0.07
Lifetime Cannabis Use Occasions	27.43 (76.2)	23.27 (83.3)	0.78
BrAC at Peak (mg/dL)	0.06 (0.01)	0.06 (0.01)	0.14
BrAC at Ascending Half-Peak (mg/dL)	0.05 (0.02)	0.05 (0.02)	0.33
BrAC at Descending Half-Peak (mg/dL)	0.05 (0.01)	0.05 (0.01)	0.18

Values given are mean (standard deviation), unless otherwise indicated.
BrAC: Breath Alcohol Concentration; LR: Level of Response.
[a]Data for prior 6 months.
[b]Regular user defined as smoking a total of 100 cigarettes in lifetime.
[c]p-values for independent samples t-test or χ^2 test.
[d]$p < 0.05$.

Figure 1.

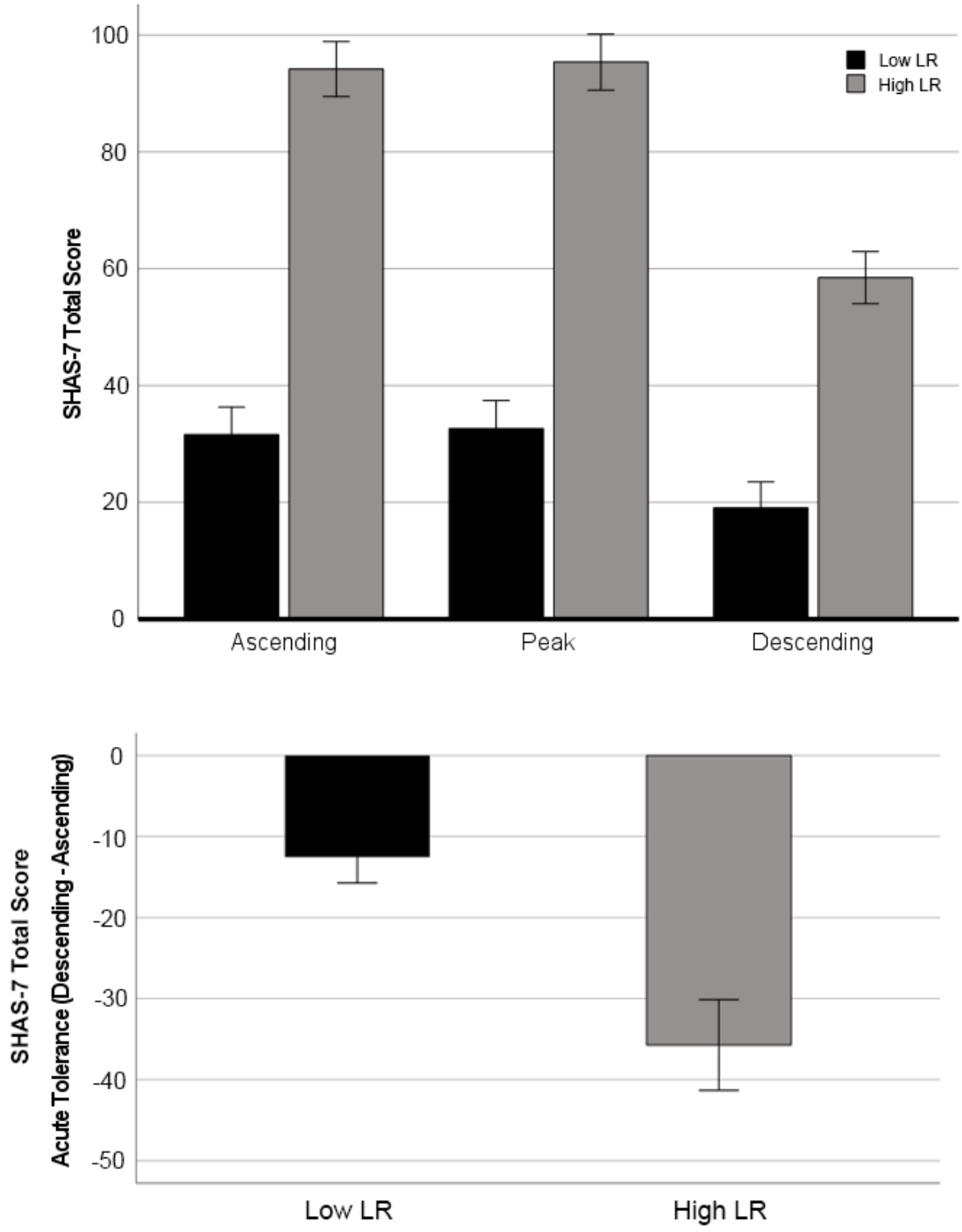

SHAS-7 Total Score
100
80
60
40
20
0
Low LR
High LR
Ascending
Peak
Descending
SHAS-7 Total Score
Acute Tolerance (Descending - Ascending)
0
-10
-20
-30
-40
-50
Low LR
High LR

Figure 2.

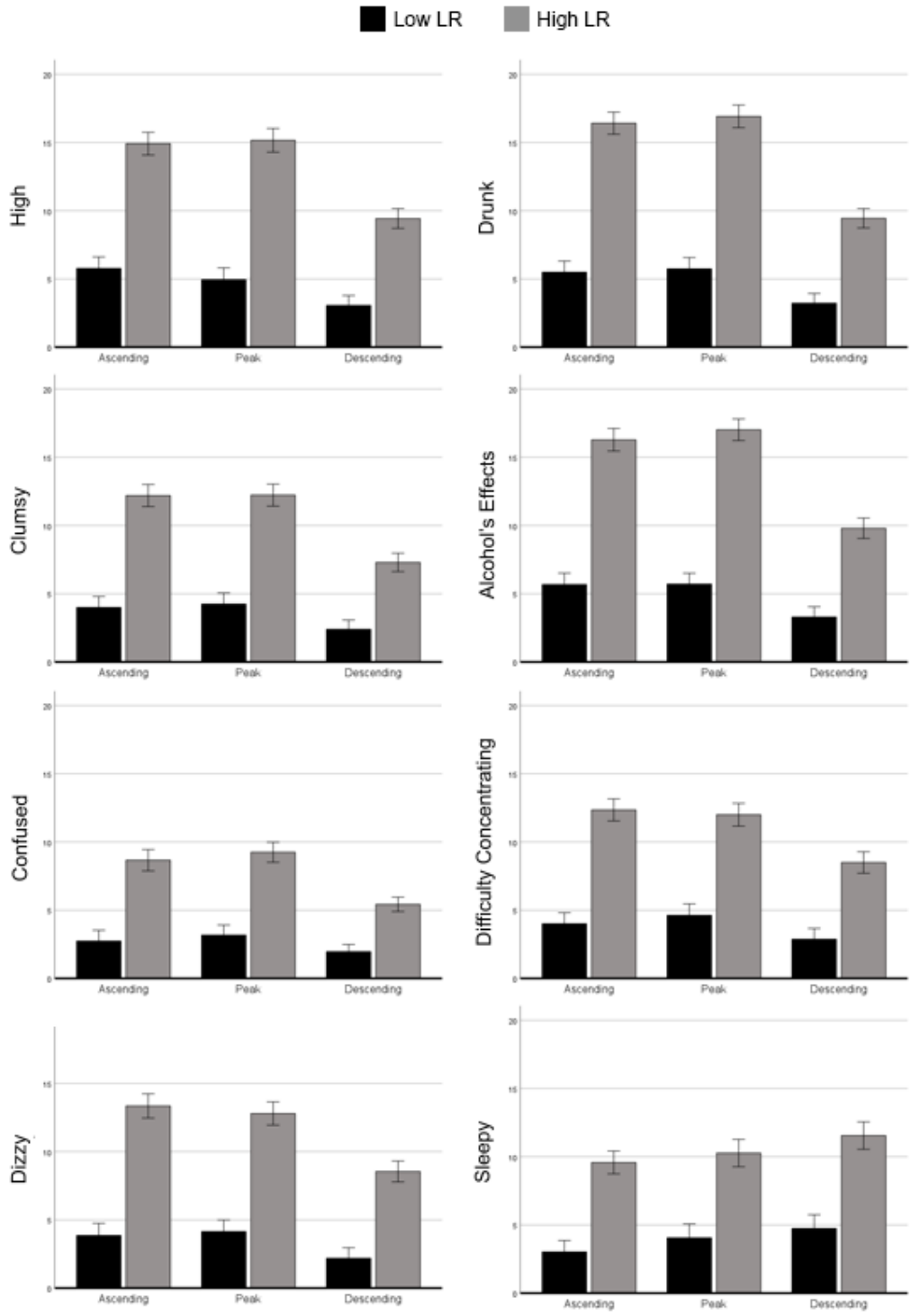

Low LR
High LR
High
Drunk
Clumsy
Alcohol's Effects
Confused
Difficulty Concentrating
Dizzy
Sleepy
Ascending
Peak
Descending

Figure 3.

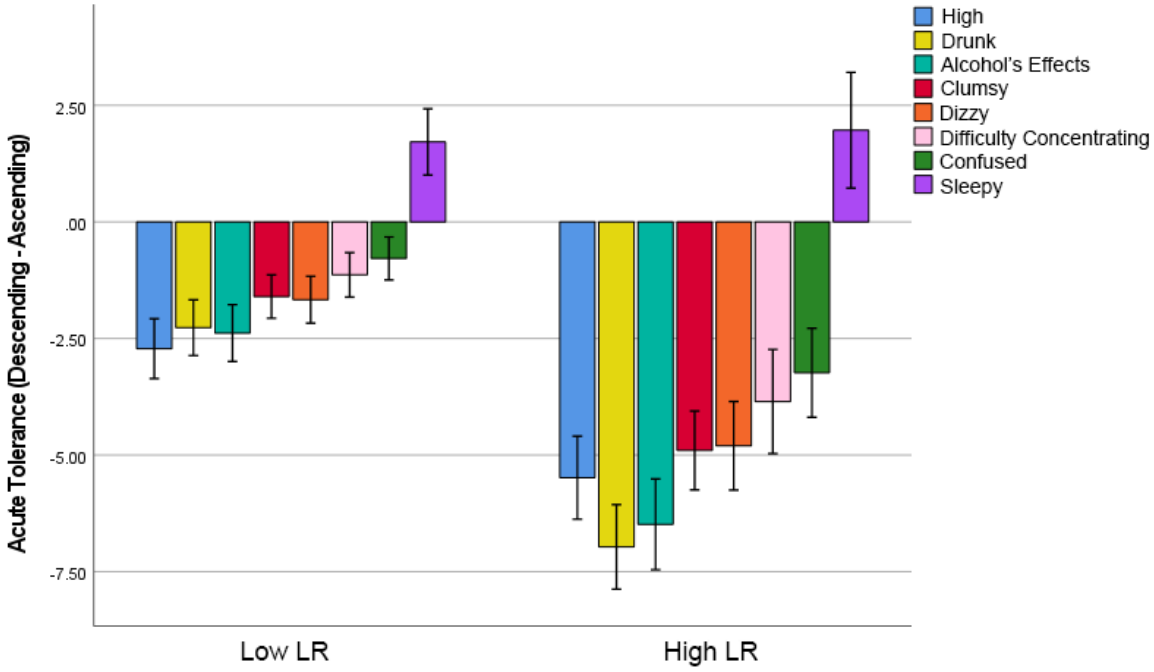
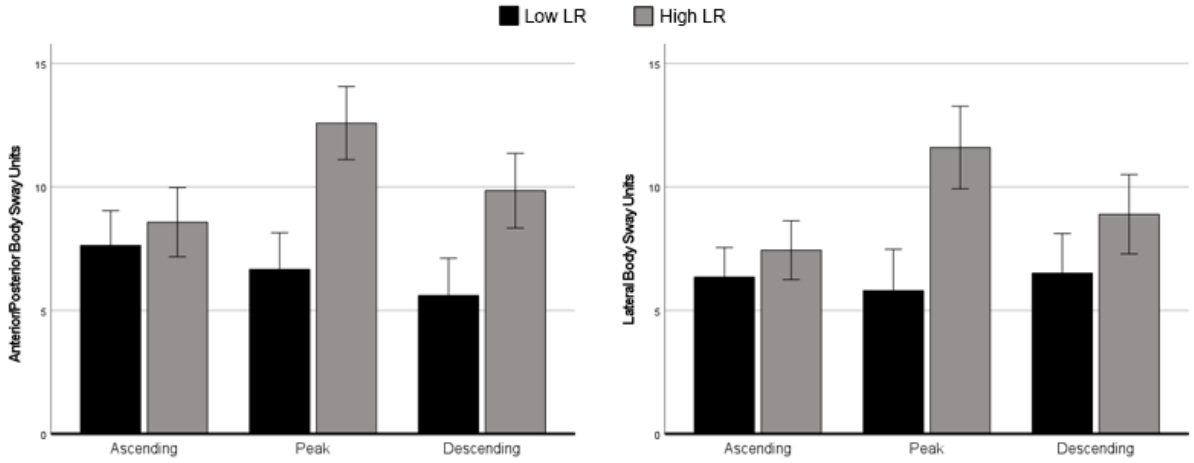

High
Drunk
Alcohol's Effects
Clumsy
Dizzy
Difficulty Concentrating
Confused
Sleepy
Acute Tolerance (Descending - Ascending)
2.50
.00
-2.50
-5.00
-7.50
Low LR
High LR

Figure 4.


Low LR
High LR
Anterior/Posterior Body Sway Units
Lateral Body Sway Units
15
10
5
0
Ascending
Peak
Descending