Data supplement for Sinha et al., Moderation of Prazosin's Efficacy by Alcohol Withdrawal Symptoms. Am J Psychiatry (doi: 10.1176/appi.ajp.2020.20050609)

SUPPLEMENTAL METHODS

Participants

Individuals seeking treatment for alcohol use disorders (AUD) responded to advertising in newspapers, on-line websites and through specialized addiction treatment clinics for study participation. Those deemed eligible for study participation at screening were invited to sign written informed consent, conduct baseline assessments and then admitted to either the outpatient Clinical Research Program at the Yale Stress Center or the Clinical Neuroscience Research Unit (CNRU), an inpatient treatment and research facility of the Connecticut Mental Health Center (CMHC) for treatment initiation and study participation. Those requiring medication detoxification were provided the detoxification prior to study enrollment. All participants completed an assessment of alcohol withdrawal symptoms (AW) during the pre-treatment intake period using the Clinical Institute of Withdrawal Assessment-Alcohol revised (CIWA-Ar) (23) conducted by a clinical research staff member fully trained in conducting the CIWA-Ar observer-based assessment (see below). Additional demographic and clinical characteristics as well as physical health examination, including electrocardiogram (EKG), CBC/laboratory and liver function tests were assessed in the intake period. No assessments were completed unless the patient was alcohol abstinent as per a negative breathalyzer. Urine toxicology testing for ethyl glucuronide (EtG) and other illicit drugs and a breathalyzer was conducted to confirm recent alcohol use and objective assessment of drug use at baseline.

Study Procedures

Prazosin Dosing and Titration. Patients were initiated on study medication (prazosin/placebo) upon providing a negative breathalyzer on the day of initiation; thus, no minimum abstinence period was required for study initiation. Target medication dosing was three times/day (t.i.d. dosing) with 5 mg in the morning, 5 mg in the afternoon and 6 mg at night reached at the end of the 2-week period, and maintained until week 11, followed by a 5-day taper in week 12, as in previous research.(13, 16) The titration schedule was as follows: 1 mg dose at bedtime for 2 nights, followed by a 1mg dose morning and night (8 AM/8 PM) on day 3, then 2 mg dose t.i.d., on days 4-6, 3 mg dose (2 pills each) morning and afternoon, and 4 mg dose (2 pills) at night for days 7-9, increased to 4 mg dosing t.i.d. on days 10-13, and from day 14 through week 11, 5 mg (1 pill) each in the morning and afternoon, and 6 mg for the night (2 pills) dose. This was followed by a 5-day taper in week 12. Participants in the prazosin condition reported taking a mean of 93.6% (SD = 20.3%) of their doses per week, whereas the placebo group reported taking 96.1% (SD=14.2%) of their scheduled doses.

Assessment of Prazosin/Placebo Medication at Week 4 in Plasma: Plasma prazosin levels were measured using highly sensitive liquid chromatography/mass spectrometry (LC/MS; ABSciex QTRAP4000) with a low limit of quantification at 0.096 ng/ml. Blood samples were collected at the week 4 appointment at no specific time relative to medication dosing. After the blood draw, samples were immediately processed in a 4C centrifuge for plasma separation. Aliquoted plasma was stored at -80C until LC/MS assay processing at the end of the study. One sample was damaged in storage/transport from the placebo group (97% of available for processing) and 2 samples were found to have undetectable levels in the prazosin group (94% of available for processing).

Assessments

Sociodemographic Information. Demographic data, medical history, and family psychiatric history were assessed via interviews and self-reports. Collected data included age, race, socioeconomic status, marital status, psychiatric family history, educational and occupational levels, and significant medical history.

Structured Clinical Interview for DSM-IV-TR (SCID-I)(22) was used to obtain DSM-IV Alcohol Dependence and other lifetime and current psychiatric diagnoses.

Medical History and Physical Examination. All patients participated in a physical examination including a medical history, electrocardiogram (EKG), blood and urine laboratory assessments prior to initiation of prazosin to ensure eligibility for study protocol.

Clinical Institute Withdrawal Assessment-Alcohol revised (CIWA-Ar, 23) is a 13-item observer rated scale that measures alcohol withdrawal signs and symptoms (AW). The assessment was administered at intake and weekly during the trial by clinical research staff who were specifically trained on observer ratings of AW, as specified by Sullivan et al. (23). Items on this scale include objective measures (e.g., pulse), participants' responses to questions, such as orientation to time and space ("What day is this? Where are you? Who am I?"), and observations by the interviewer (evidence of tremor assessed by having patient extend arms and fingers, or paroxysmal sweats by assessing moistness of body surfaces, such as palms, fingers, forehead etc.). Item responses range from 0 indicating no evidence of the symptom to 4 indicating highest severity of symptoms and possible continuous (AWcont.) total scores range from 0 to 67.

Adverse Events. A modified version of the Systematic Assessment for Treatment Emergent Effects (SAFTEE) interview (31) of side effects was used to assess expected and

unexpected side effects and serious adverse events weekly. To capture the full scope of potential side effects from prazosin, participants were asked open-ended questions about any physical or health problems experienced during the past week and this was followed by more detailed assessment of any specific treatment emergent event on an adverse events form.

Vital Signs. Systolic and diastolic blood pressure (SBP/DBP) and pulse were assessed at each visit twice weekly using the Critikon Dinamap (GE Systems, Inc).

Urine Toxicology and Breathalyzer Screening. A urine toxicology screen was conducted at intake to confirm alcohol use. During treatment, urines were collected 3x/weekly to confirm sobriety during the inpatient stay, and urine samples were taken 2 times weekly during outpatient treatment using on-site TESTCUP5 Drug Screen (Roche Diagnostics Inc., Totowa, NJ) to monitor opiate and other substance use. The TestCup5 kit provides results for opioids, cocaine, THC, PCP and barbiturates. In addition, breath alcohol levels were assessed at each face-to-face contact with the Alcosensor III Intoximeter, and urine EtG levels were assessed weekly.

The Substance Use Calendar, based on the Time-Line Follow-Back Method,(25) was used to assess alcohol and other drug use at each assessment. The SUC uses a calendar prompt and several other memory aids (e.g., holiday, payday, and other personally relevant dates) to facilitate accurate recall of daily drug use during the targeted period. In the current study, we obtained self-reported day-to-day use of alcohol and drugs using this methods for the 60 days prior to treatment and for the treatment period.

Daily Diary Monitoring. Daily prompts via interactive voice response or a smartphone application (MetricWire) were completed nightly to track outcomes. Participants responded to

these prompts at approximately 8 p.m. each day and reported alcohol use and the amount of alcohol consumed that day. When reporting drinking behavior, participants were asked how many glasses of beer, wine, and mixed drinks that they consumed that day. Quantity of each type of drink was summed for each day to create an index of total drinks consumed per day. In addition to the evening questionnaire, all participants received a reminder at 8:00 am, 2:00 pm, and 8:00 pm to take their dose of prazosin.

Additional Secondary Outcome Measures. Additional *secondary* outcomes were alcohol craving, as measured by the Obsessive Compulsive Drinking Scale (OCDS, 27) every four weeks, weekly anxiety (Hamilton Anxiety Scale, 28) and depression symptoms (Center for Epidemiologic Studies – Depression Scale, CES-D, 29), sleep quality monthly using the Pittsburgh Sleep Quality Index (PSQI, 30) and alcohol withdrawal symptoms (AW) as measured by the CIWA-Ar.(23) Alcohol craving, depression, anxiety, sleep quality, and AW assessed throughout the study were baseline-corrected to control for person-level basal differences in these measures.

Additional Description of Data Analysis. Linear piecewise growth models for continuous outcomes and generalized linear mixed effect models (LME/GLME) for binary outcomes were used to evaluate the interactive effect of treatment and AW(Cont.) over time (each day for drinking outcomes, and by week or month for secondary outcome measures) during the study. Each model for primary and secondary outcome was implemented in a piecewise fashion with a "breakpoint" centered at the beginning of week 3, when full-dose of the medication was achieved. The rationale for using piecewise growth models is that by modeling time based on specific aspect of the independent variable (i.e., treatment medication) such as the period prior to achieving full dose (PreFD: weeks 1-2) versus the full odse period (PostFD: weeks 3-12) can be

modeled separately to account to variation in outcomes for each dosing phase. A similar set of models were conducted using cardiovascular measures from baseline including systolic blood pressure, diastolic blood pressure, and heart rate.

SUPPLEMENTAL RESULTS

Adverse Events

TABLE S1. Adverse events reported during the trial

Adverse Event	Prazosin (n=55)		Placebo (n=45)		*p*-value[a]
	n	%	*n*	%	
Infection	0	0%	3	7%	0.09
Shortness of Breath	4	7%	0	0%	0.12
Eye Irritation	3	5%	0	0%	0.25
Dizzy/Lightheaded	6	11%	2	4%	0.29
Drowsiness	1	2%	3	7%	0.32
Dry Mouth	4	7%	1	2%	0.37
Headache	5	9%	7	16%	0.37
Racing Heart	5	9%	2	4%	0.45
Pain	10	18%	10	22%	0.63
Nausea/Vomit	7	13%	4	9%	0.75
Cold Symptoms	9	16%	6	13%	0.78
Fatigue	3	5%	2	4%	1.00
Sleep Problems	3	5%	2	4%	1.00

[a] Group frequency rates were assessed via chi-square test and tested for significance via Fisher's exact test.

Baseline AW Continuous Scores and Relationship with Drinking and Abstinence Symptoms at Intake

Frequency of specific AW in the sample divided by those with high vs. low AW (median cut-off) at baseline are presented in the main manuscript in Table 2. As expected, higher baseline AW predicted greater depression (R^2=.13, *B*=.547, *p*=.002), anxiety (R^2=.45, *B*=1.07, *p*<.00001), alcohol craving (R^2=.20, *B*=.502, *p*<.0001) and sleep problems (R^2=.15, *B*=.33, *p*<.001) (Figure S1). AW was also significantly associated with intake drinking levels for average number of drinks consumed/day (R^2=.09, *B*=.307, *p*=.008, *Figure S1*) and marginally with percent drinking

days (R^2=.06, *B*=1.21, *p*=.051), but not the percent of heavy drinking days (HDD; R^2=.007 *B*=.55, *p*=.417) or the number of days that elapsed since the last drink relative to the CIWA-Ar assessment (R^2=.04, *B*=-.09, *p*=.295) at intake. Baseline blood pressure (SBP: *p*=.072; DBP: *p*=.536) and heart rate (*p*=.488) were not significantly associated with AW.

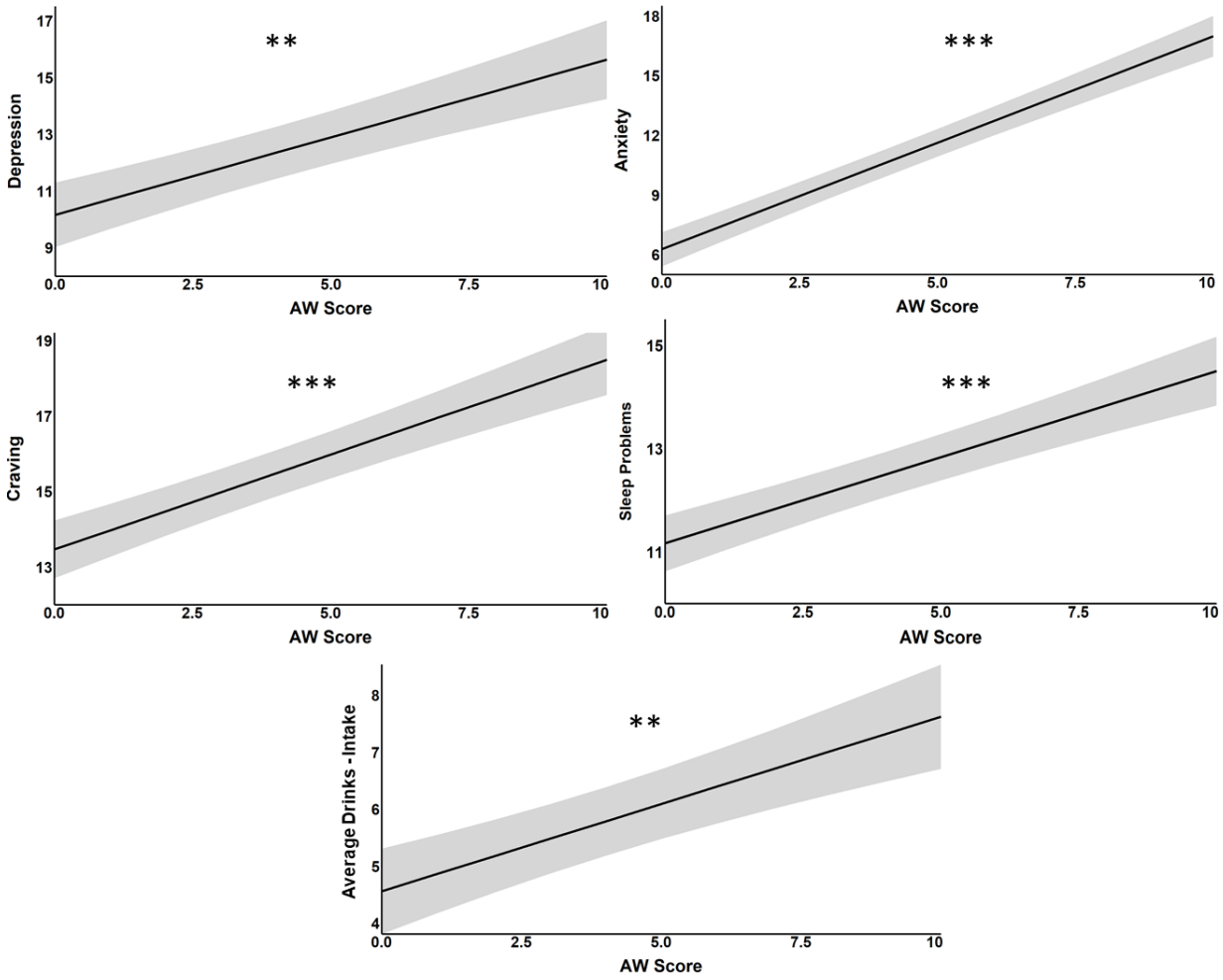

FIGURE S1. Baseline AW(Cont.) at treatment entry and depression, anxiety, craving, sleep problems and average drinks/day at intake. Higher the AW(cont.) scores, greater the difficulties in depression (p<.002), anxiety (p<.00001), craving (p<.0001), sleep problems (p<.001) and average number of drinks/day (p<.008) during the intake period.

Alcohol use outcomes at Baseline week 0 and during the trial (weeks 1-12) for Prazosin/Placebo Treatment Groups by High and Low AW (Median Split)

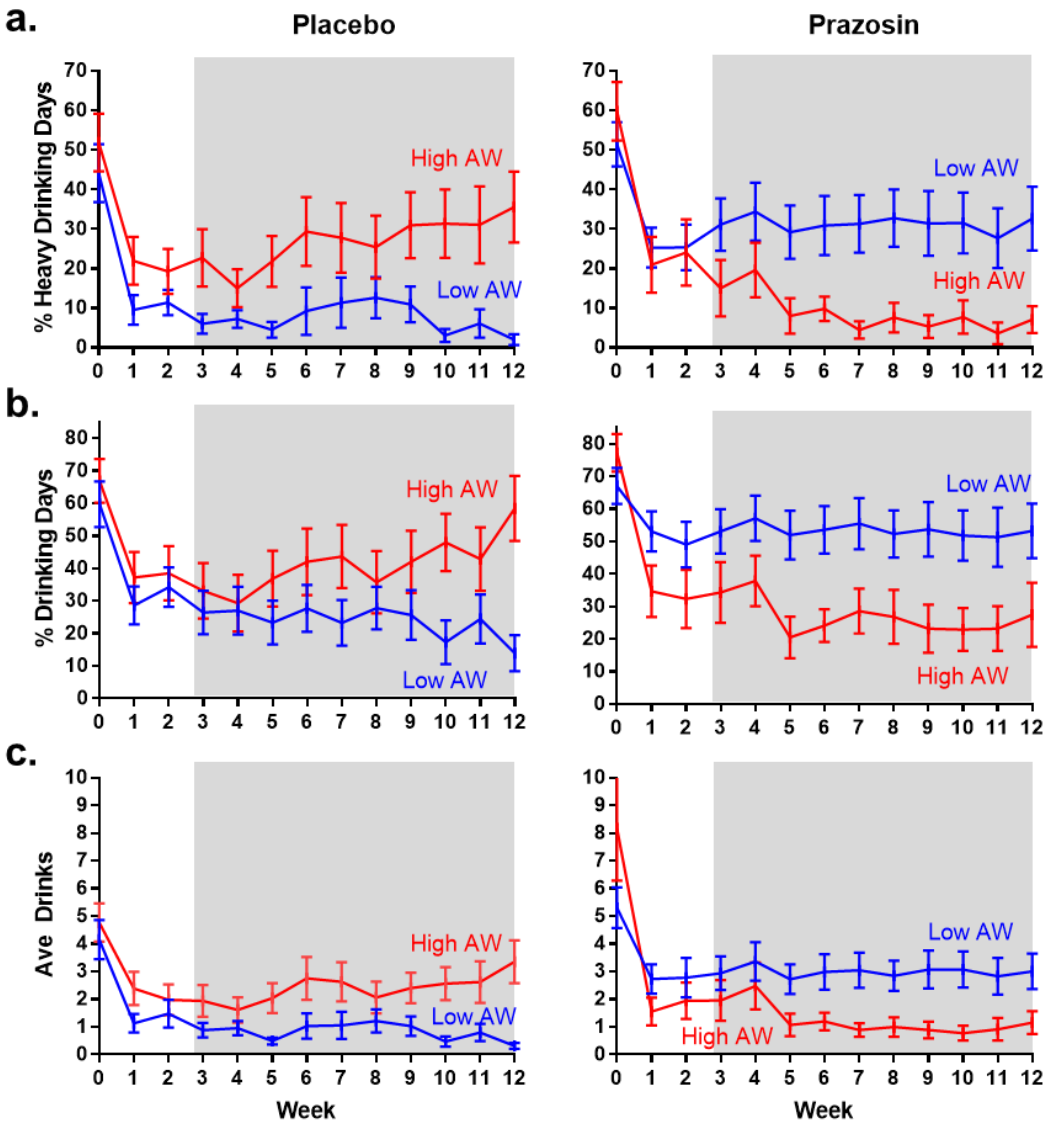

FIGURE S2. Significant interaction between AW(Cont.) continuous AW scores X Prazosin/Placebo treatment X Post full Dose (PostFD: weeks 3-12) was observed for HDD (χ^2=6.02, *p*=.01), DD(χ^2=9.43, *p*=.002) and AvgD (F[1,76]=5.00, p<.03): **a**: Percent Heavy Drinking Days (HDD); **4b**: Percent Drinking Days (DD); and **4c**: Average Number of Drinks/day (AvgD). Alcohol withdrawal (AW) symptom scores shown here with AW median split for Low AW (CIWA-Ar ≤ 2 [Placebo: N=22; Prazosin: N=34]) and High AW (CIWA ≥ 3 [Placebo: N=23; Prazosin: N=21]) for illustration purposes only. Baseline drinking prior to treatment are shown at the Week 0 point on the x-axis. All analyses were conducted using CIWA (cont.), and assessing drinking outcomes during week 1 through week 12 (see *ST2* for full model results). Note: DD and HDD are binary outcomes, and for depiction purposes, the percentage of drinking days and heavy drinking days was calculated for each participant in each week and then averaged across the subgroups. Error bars are ± SEM.

Baseline Cardiovascular Measures as a Moderator of Treatment Outcome

Baseline SBP, DBP, and HR were used as moderators on the prazosin by interaction effect. DBP moderated the postFD effect of prazosin on HDD (p=.017), but not DD (p=.140) or AvgD (p=.667). Individuals who had higher baseline DBP and randomized to prazosin were less likely to have a HDD postFD as compared to those on placebo with a similarly high DBP (*OR*=.972, p=.017), whereas preFD these same individuals were more likely to have a DD (*OR*=1.076, p=.041). Higher baseline heart rate for individuals on prazosin was associated with less postFD AvgD (*B*=-.005, p=.014) and a lower likelihood of a postFD DD (*OR*=.974, p=.007). Overall, individuals with higher SBP and DBP treated with prazosin had higher levels of AvgD (SBP: *B*=.257, p=.053, DBP: *B*=.285, p=.033) and DD (SBP: *OR*=2.956, p=.034) than those on placebo (*Supplemental Table S3* below).

TABLE S2. Interaction effect of prazosin/placebo treatment and AW on primary drinking outcomes

	Drinks/Day (AvgD) (log-transformed)			Drinking Day (DD)			Heavy Drinking Day (HDD)		
Fixed Effects	F pvalue	Est	95% CI	X^2 pvalue	OR	95% CI	χ^2 pvalue	OR	95% CI
Baseline AW	**4.334***	**0.287**	**0.063, 0.512**	3.615	2.451	0.973, 6.176	**5.737***	**3.052**	**1.225, 7.607**
Days pre-FD	0.167	-0.004	-0.017, 0.01	1.272	0.971	0.923, 1.022	0.281	0.982	0.919, 1.05
Days post- FD	0.003	0.002	-0.001, 0.004	**3.955***	**1.014**	**1.000, 1.028**	0.205	0.996	0.979, 1.013
Treatment	**4.434***	**0.268**	**0.019, 0.517**	**4.509***	**3.017**	**1.089, 8.358**	3.683	2.804	0.978, 8.036
AW x Treatment	**4.450***	**-0.286**	**-0.551, -0.02**	2.230	0.435	0.146, 1.297	2.774	0.391	0.13, 1.18
AW x pre-FD	0.362	-0.004	-0.02, 0.012	0.192	0.986	0.925, 1.051	0.015	0.996	0.934, 1.062
AW x post-FD	**8.469****	**0.005**	**0.002, 0.008**	**14.099*****	**1.035**	**1.016, 1.053**	**8.932****	**1.026**	**1.009, 1.044**
Treatment x pre-FD	1.506	0.011	-0.007, 0.03	2.004	1.050	0.982, 1.123	1.483	1.05	0.97, 1.137
Treatment x post-FD	2.538	-0.003	-0.007, 0.001	3.605	0.983	0.965, 1.001	1.205	0.988	0.967, 1.009
AW x Treatment x pre-FD	0.058	0.002	-0.017, 0.022	0.002	0.998	0.927, 1.076	0.001	0.999	0.92, 1.085
AW x Treatment x post-FD	**5.001***	**-0.005**	**-0.009, -0.001**	**9.431****	**0.967**	**0.947, 0.988**	**6.023****	**0.972**	**0.951, 0.994**

Note: Findings from the linear mixed piecewise growth models, accounting for gender, age, and inpatient versus outpatient. Pre-FD=Pre-Full Dose (weeks 1-2); Post-FD=Post-Full Dose (weeks 3-12); AW=Clinical Institute Withdrawal Assessment for Alcohol-Revised, z-scored; Treatment=Prazosin versus Placebo; OR=Odds Ratios. Significant results are flagged with bold typeface. * p<.05, ** p<.01, *** p<.001.

TABLE S3. Interaction effect of prazosin/placebo treatment and baseline cardiovascular measures on primary drinking outcomes

Outcomes	Treatment-Related Effect	F / χ2	p	Est	Description
Systolic Blood Pressure					
AvgD(Drinks/Day)[a]	Treatment	3.879	.052	.257	Prazosin > Placebo (trend)
DD[b]	Treatment	4.526	.033	2.956	Prazosin > Placebo: ↑ DD on average
HDD[b]	SBP x post-FD	5.296	.021	1.020	↓ baseline SBP = ↓ HDD post-FD
Diastolic Blood Pressure					
AvgD (Drinks/Day)[a]	Treatment	4.705	.033	.285	Prazosin > Placebo: ↑ daily drinks on average
DD[b]	Treatment x DBP x pre-FD	4.143	.042	1.076	Prazosin > Placebo: ↑ baseline DBP = ↑ in DD pre-FD
HDD[b]	Treatment x DBP x post-FD	5.651	.017	.972	Prazosin < Placebo: ↑ baseline DBP = ↓ in HDD post-FD
Heart Rate					
AvgD(Drinks/Day)[a]	Treatment x HR x post-FD	2.475	.014	-.005	Prazosin < Placebo: ↑ baseline HR = ↓ in daily drinks post-FD
DD[b]	Treatment x HR x post-FD	7.168	.007	.974	Prazosin < Placebo: ↑ baseline HR = ↓ in DD post-FD
HDD*[b]	HR x post-FD	6.075	.014	1.021	↓ baseline HR = ↓ in HDD post-FD

Note: [a] Est=B. [b] Est=Odds Ratios. Findings from the linear mixed piecewise growth models, accounting for gender, age, and inpatient versus outpatient. Pre-FD=Pre-Full Dose (weeks 1-2); Post-FD=Post-Full Dose (weeks 3-12); Treatment=Prazosin versus Placebo; DD=Drinking Day; HDD= Heavy Drinking Day; SBP=Systolic Blood pressure (continuous); DBP=Diastolic Blood Pressure (Continuous); HR = Heart Rate (Continuous); OR=Odds Ratios. Significant results are flagged with bold typeface. *In the model using HR to predict HDD, the random slope for PreFD was removed due to issues with convergence.* * $p<.05$, ** $p<.01$, *** $p<.001$.

TABLE S4. Prazosin/placebo treatment effects across weeks for secondary outcomes

Outcomes (baseline-corrected)	n	Treatment-Related Effect	F	p	Cohen's f
Anxiety	96	**AW x Treatment x Week**	**2.80**	**.002**	**.226**
Depression	96	**AW x Treatment x Week**	**2.44**	**.006**	**.195**
Craving	97	**AW x Treatment**	**9.67**	**.003**	**.278**
Sleep Quality	87	Treatment	.02	.883	.012
SBP	96	Treatment*	3.74	.056	.071
DBP	96	Treatment	1.69	.197	.047
HR	96	**Treatment x Week****	**1.93**	**.033**	**.069**
CIWA-Ar (week)	96	AW x Week***	3.36	<.001	.225

Note: AW=Baseline CIWA-Ar score; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; HR=Heart rate. Significant Treatment (prazosin vs. placebo) effects are denoted with bold typeface. Note: *denotes trend for prazosin<placebo for average SBP during treatment; **denotes higher HR for those on prazosin vs placebo in earlier weeks; and ***denotes reduction in CIWA-Ar scores across weeks but only in those with higher AW(cont.) scores and no change by week in those with no/low levels of AW.