

Supplemental Material

Research Articles | Behavioral/Cognitive

Anterior insula activity during alcohol and social reward self-administration and choice in male and female rats.

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Supplementary Figure Legends

Figure S1. Photos of the experimental chambers. DS, discriminative stimulus; CS, conditioned stimulus.

Figure S2. Comparison of aIC calcium during the cue period of the Early and Late choice sessions to the 'Middle' choice sessions (i.e. C3+C4). **(A)** Preference score comparing Early and Middle choice sessions. **(B, C)** Traces depicting aIC activity centred around the trial start (0s). Traces show aIC activity comparing Early and Middle sessions for trials where Alcohol **(B)** or Social **(C)** are chosen separately. **(D)** Preference score comparing Middle and Late choice sessions. **(E, F)** Traces depicting aIC activity centred around the trial start (0s). Traces show aIC activity comparing Middle and Late choice sessions for trials where Alcohol **(E)** or Social **(F)** are chosen separately.

Figure S3. Cue-associated aIC calcium from the experiment with traces separated by sex. **(A, B)** Traces depicting aIC activity centred around the trial start (0s) during all Early choice sessions. Traces show aIC activity from Male or Female subjects for alcohol **(A)** or social **(B)** separately. **(C, D)** Traces depicting aIC activity centred around the trial start (0s) during all Late choice sessions. Traces show aIC activity from Male or Female subjects for alcohol **(D)** or social **(D)** separately. **(E, F)** Traces depicting aIC activity centred around the trial start (0s) during all punished-alcohol choice sessions. Traces show aIC activity from Male or Female subjects for punished-alcohol **(E)** or social **(F)** separately. For all plots, coloured bars underneath the trace represent periods in time along the trial in which the bootstrapping test indicated that the trace was significantly greater than zero (i.e. baseline activity). The black bar represent periods in time where permutation test (Male vs. Female) was significant. If there is no bar then Male and Female activity was not different for the entire period of analysis.

Figure S4. Diagnostic trace plots for the group-level parameters from which the hierarchical model was built for each subject. Representative trace Left Panels show estimated posterior probability distributions. Each line represents one of the four MCMC chains, showing the density of plausible parameter values after model fitting. The overlap of these distributions indicates good agreement between chains. Right Panels show trace of samples for each of the four MCMC chains across 1000 post-warmup iterations. The "fuzzy caterpillar" appearance, with chains well-mixed and fluctuating around a stable mean, indicates good convergence and sampling from the stationary posterior

distribution. These visual diagnostics, along with $R^2 < 1.05$ and $ESS > 400$, confirmed model convergence for this dataset.

Figure S5. (A) Representative trace plots for the LBA model parameters from a single subject ('R23') during Early, Late, and Punished alcohol sessions. Left Panels show estimated posterior probability distributions. Each line represents one of the four MCMC chains, showing the density of plausible parameter values after model fitting. The overlap of these distributions indicates good agreement between chains. Right Panels show trace of samples for each of the four MCMC chains across 1000 post-warmup iterations. The "fuzzy caterpillar" appearance, with chains well-mixed and fluctuating around a stable mean, indicates good convergence and sampling from the stationary posterior distribution. These visual diagnostics, along with $R^2 < 1.05$ and $ESS > 400$, confirmed model convergence for this dataset. (B) Model comparison output.

Figure S6. Posterior predictive checks (PPC) of the LBA model. (A) correlation between the observed proportion of alcohol choices and the simulated proportion of alcohol choices. (B) Correlation between the observed mean response latency for alcohol choices in the Early, Late, and Pun sessions and the simulated mean response latencies for alcohol choices. (C) Correlation between the observed mean response latency for social choices in the Early, Late, and Pun sessions and the simulated mean response latencies for social choices.

Figure S7. Scatter plots depicting the relationship between the LBA model-derived mean accumulation rate (v) and mean z-scored $\Delta F/F$ aIC calcium activity during either (A-C) the 10-second cue period or (D-F) the 10-second period prior to the choice response. (G-I) depict relationships between mean response latency (in seconds) and mean z-scored $\Delta F/F$ aIC calcium activity during the 10-second cue period. Data are separated by the choice made. Each point represents the average value for a single rat within that specific session type and choice condition. Solid lines indicate the linear regression fit, with 95% confidence intervals shown. Pearson's correlation coefficient (R) and p -value are displayed for each panel. ($n=11$ males; diamond shapes, $n=12$ females, circle shapes.)

Figure S1. Experimental Chambers

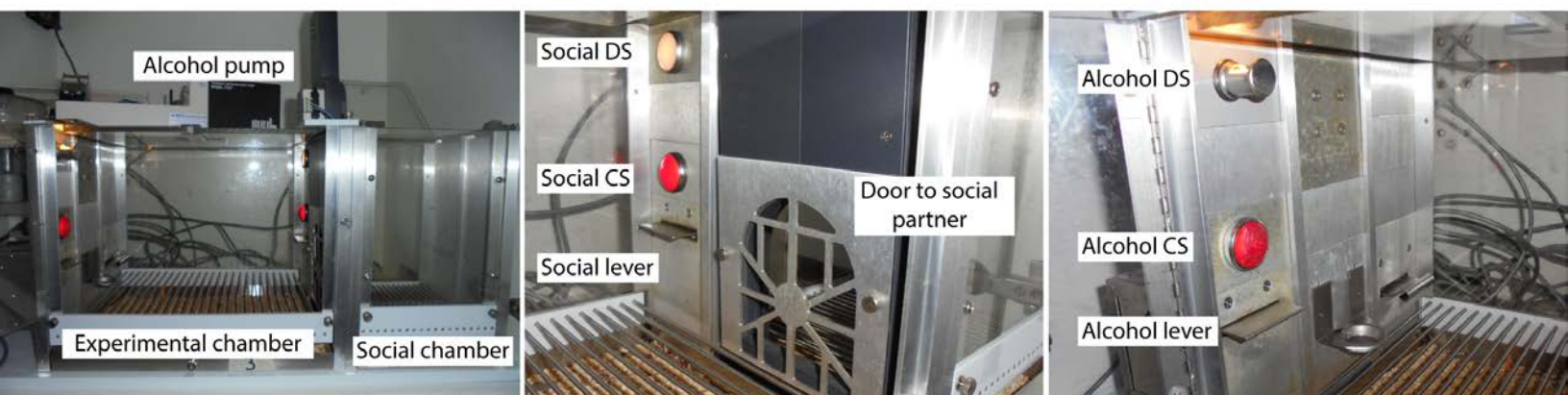
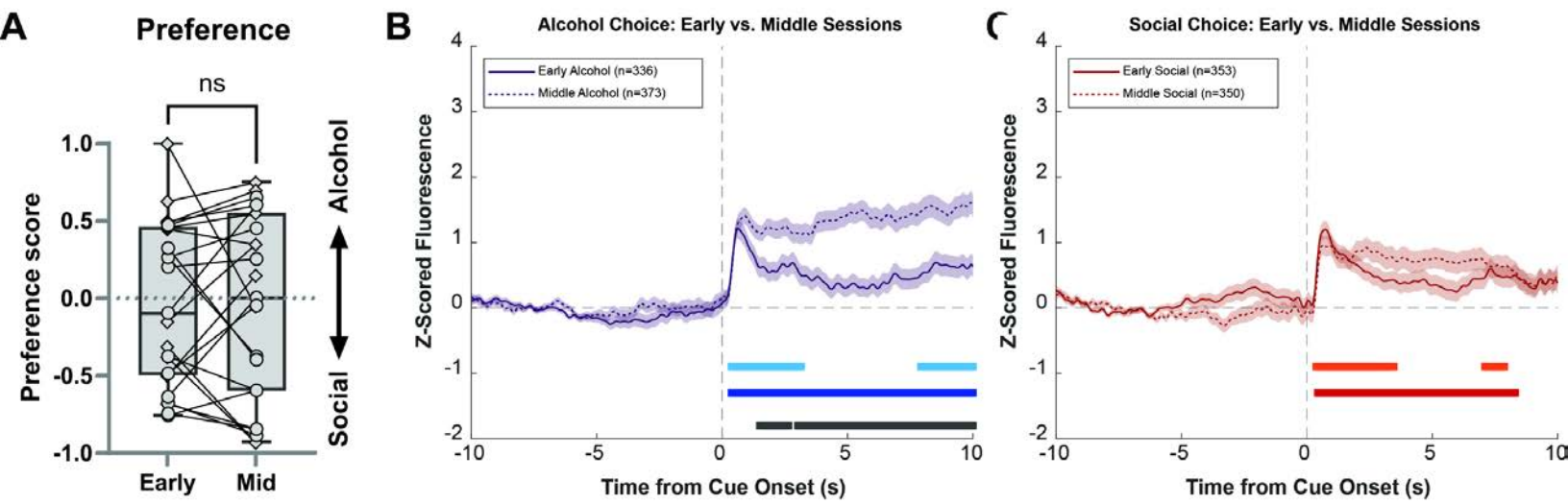


Figure S2. Cue-associated activity Middle choice sessions

Cue-associated activity - Early vs. Middle (C3+C4)



Cue-associated activity - Middle (C3+C4) vs. Late

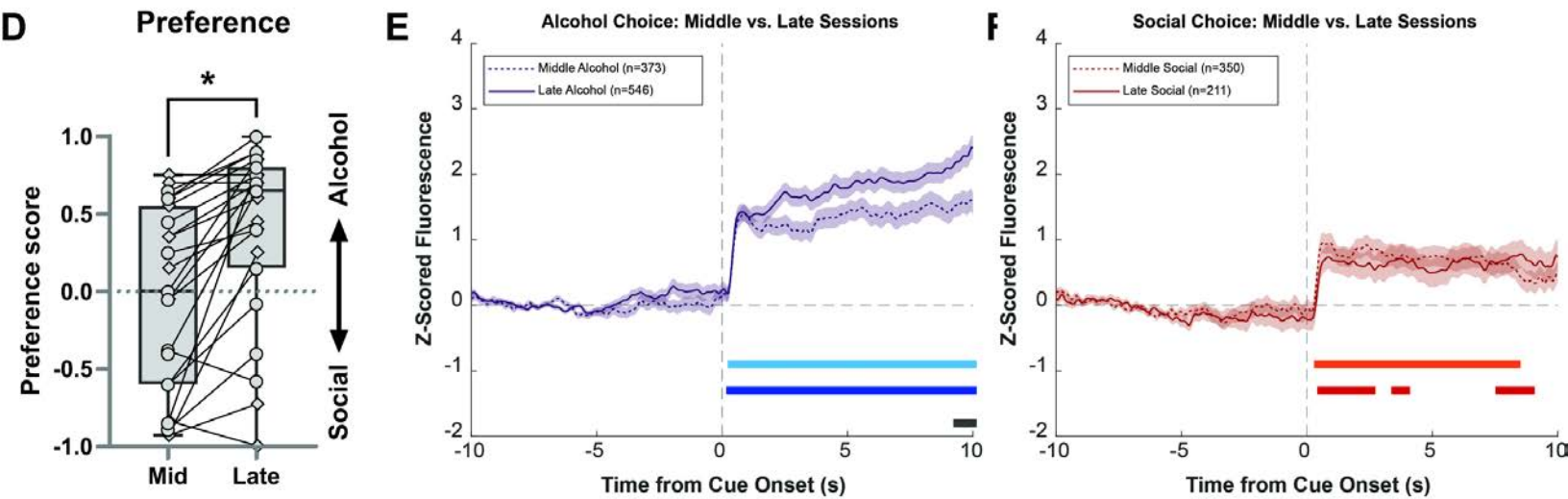
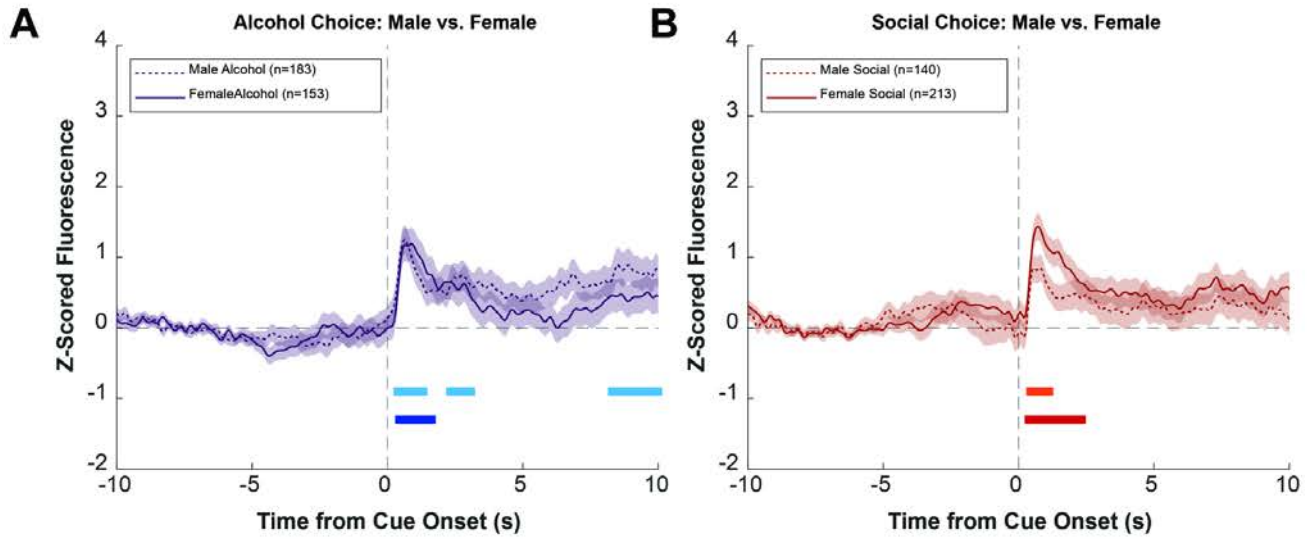
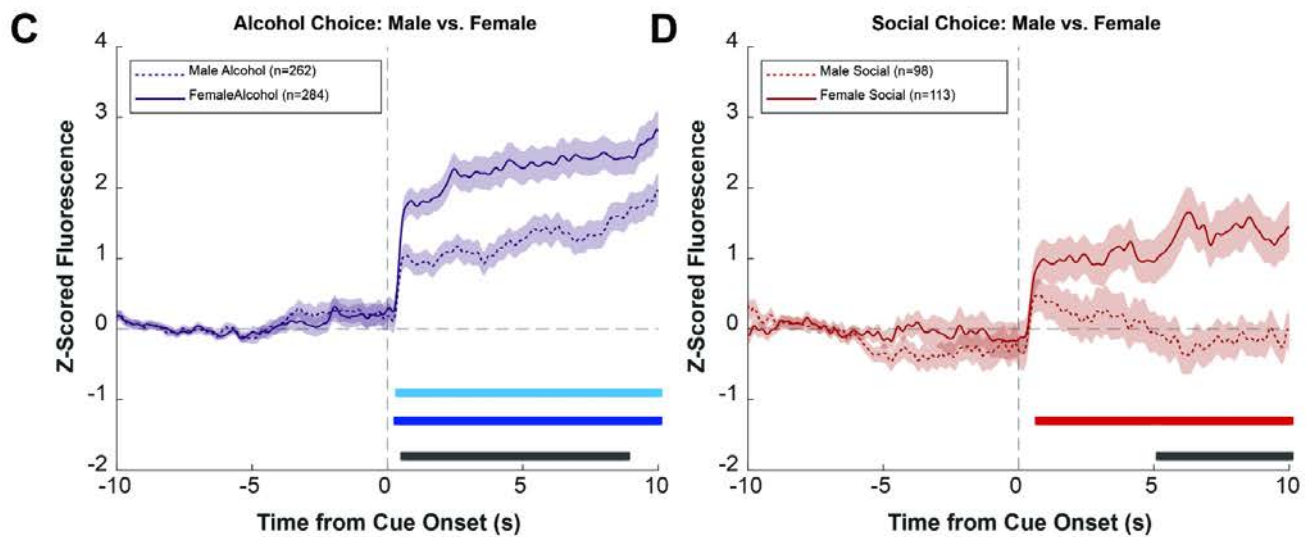


Figure S3. Cue-associated activity separated by sex
Cue-associated activity - Early Choice



Cue-associated activity - Late Choice



Cue-associated activity - Punished-alcohol

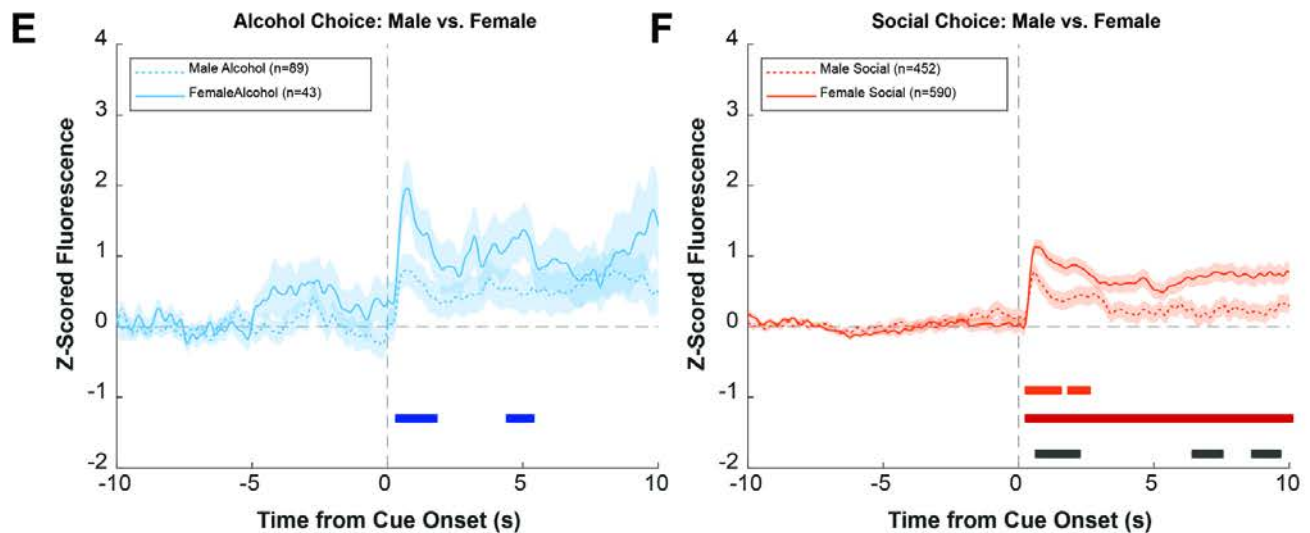
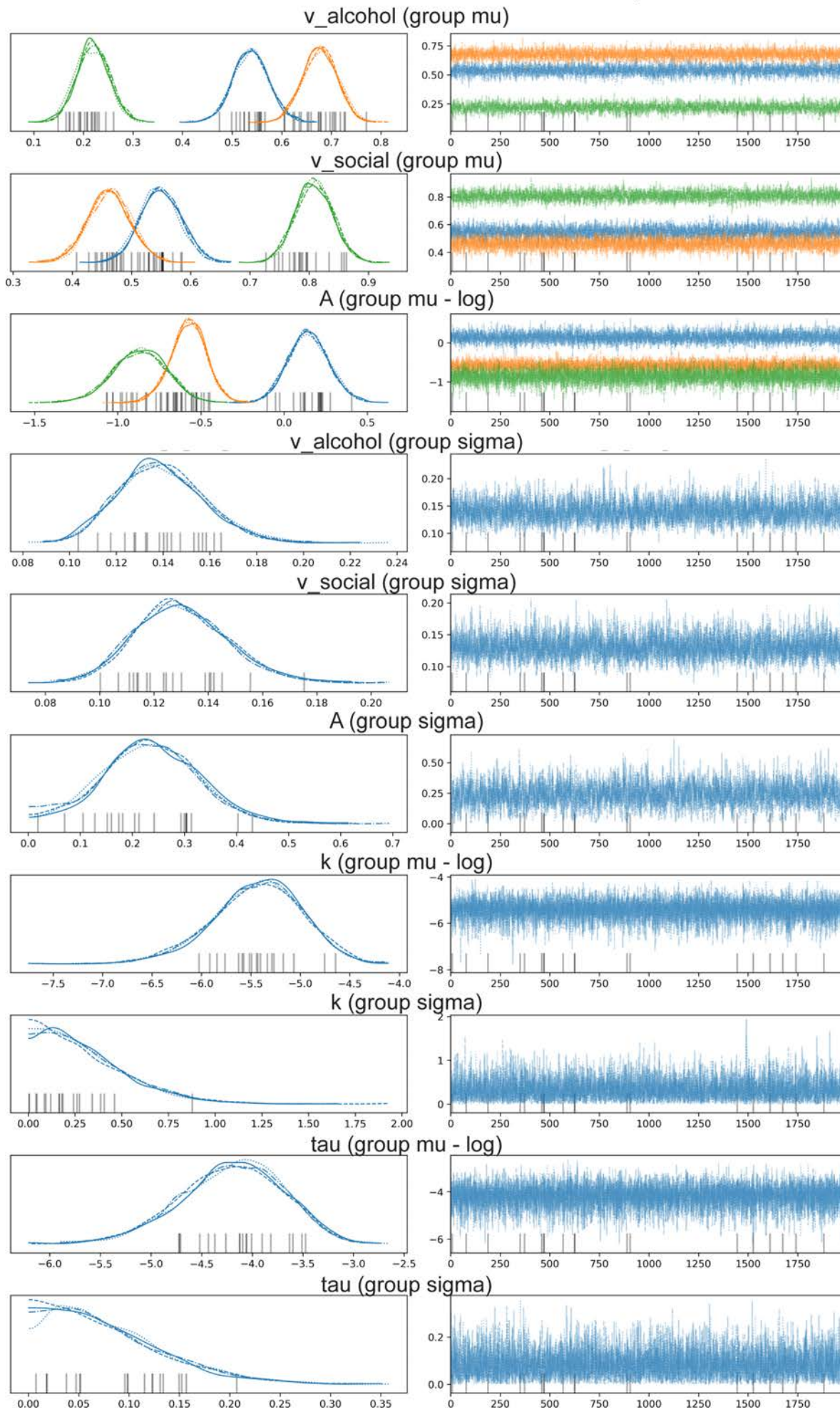


Figure S4. Diagnostic trace plots for the group-level parameters



Posterior distributions

Trace plots



Parameter Value

Iteration Number

Figure S5. Example Trace Plots and Model Comparison Plot.

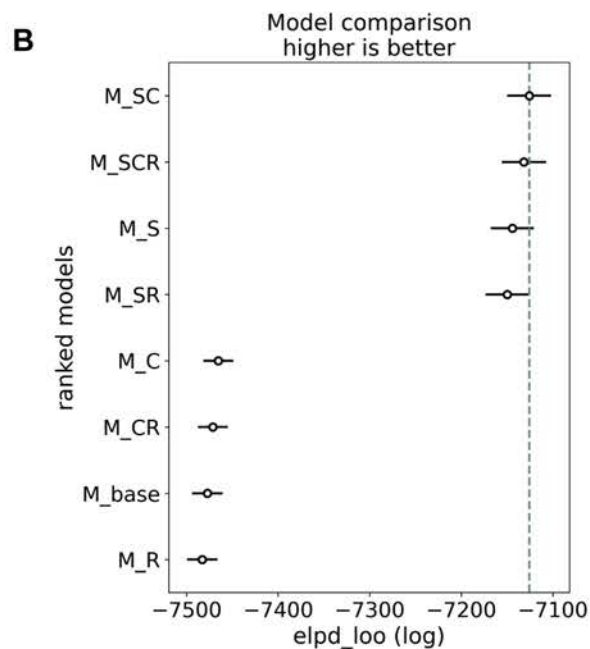
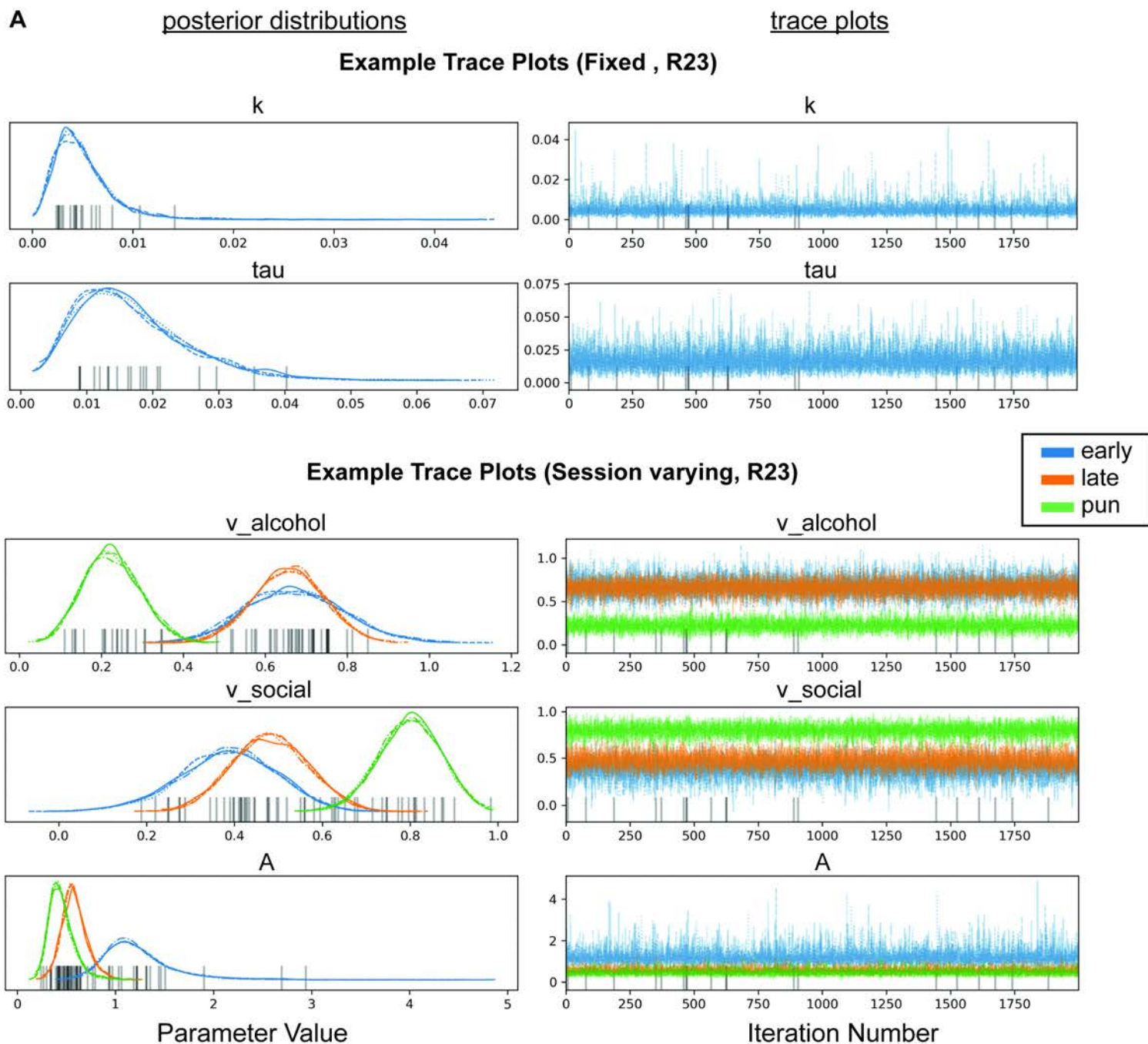


Figure S6. Posterior Predictive Checks (PPCs)

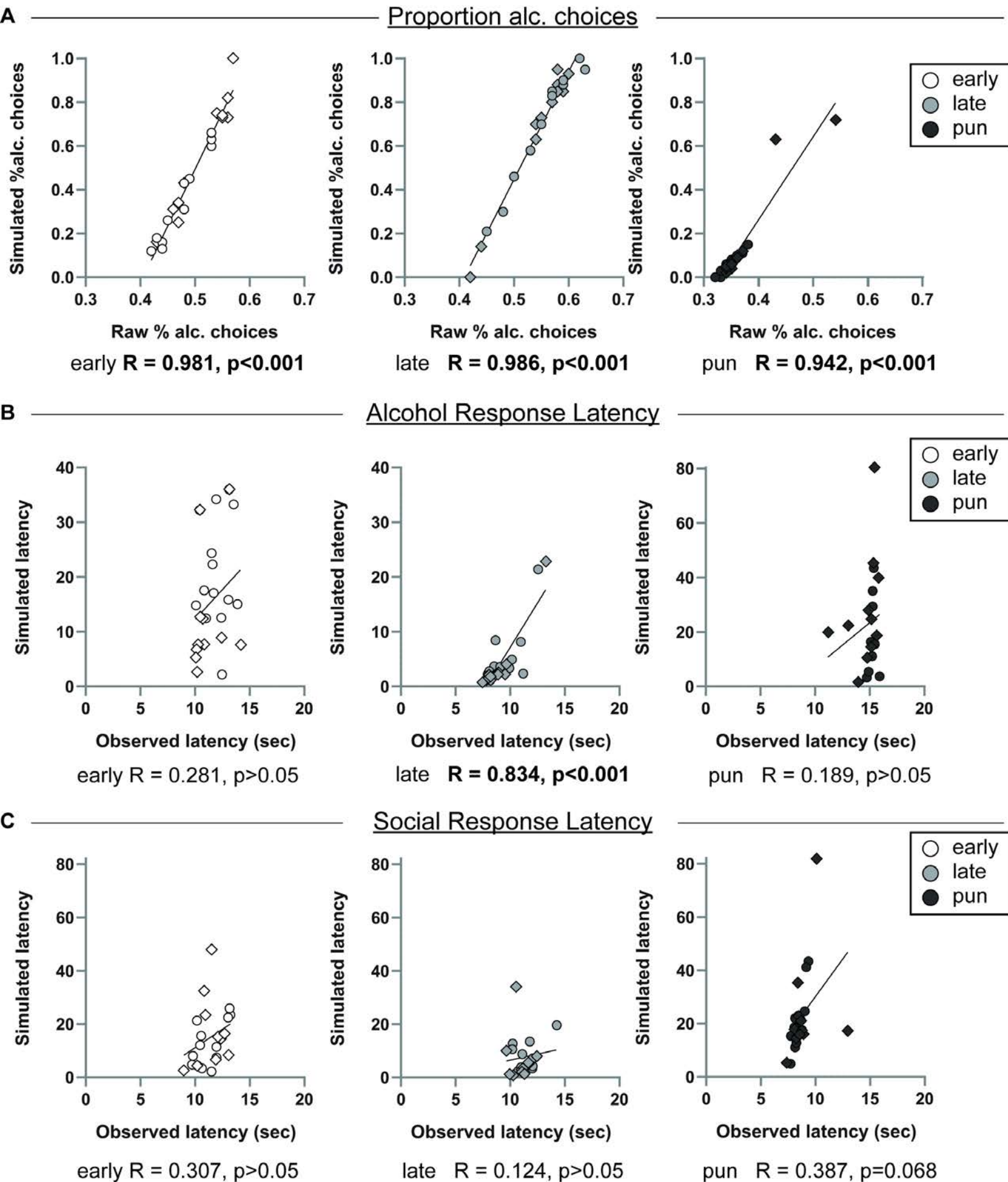
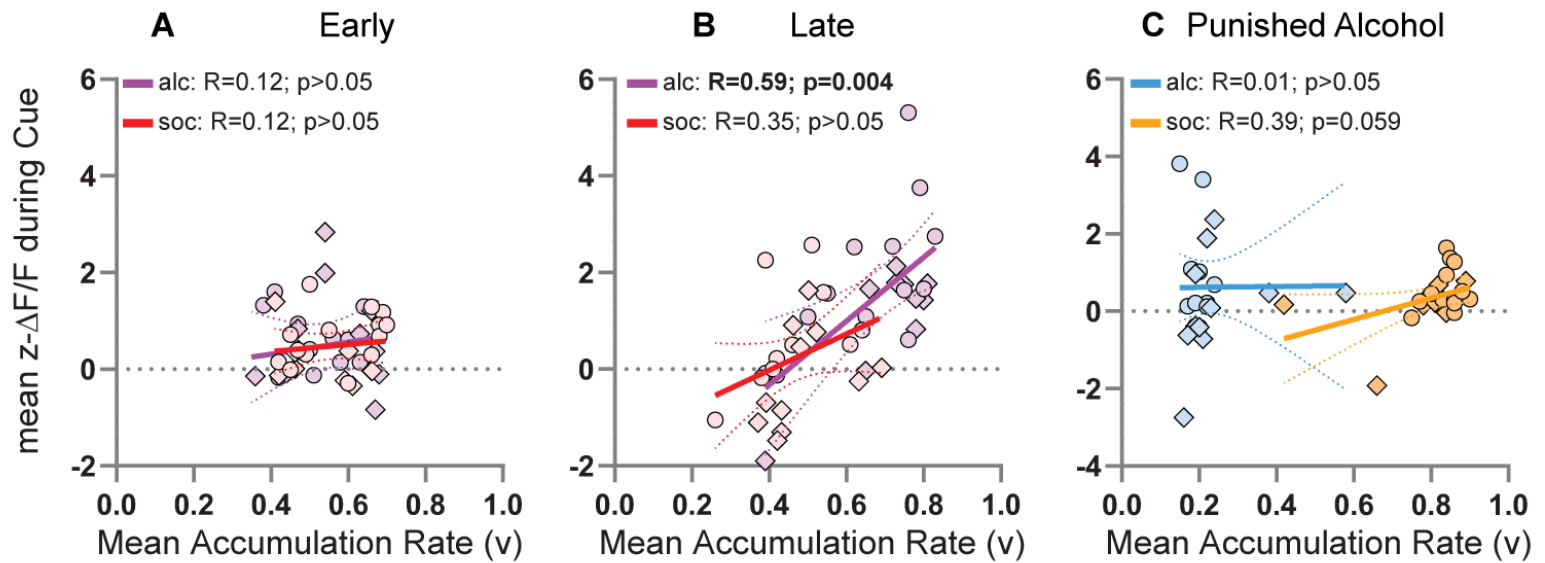
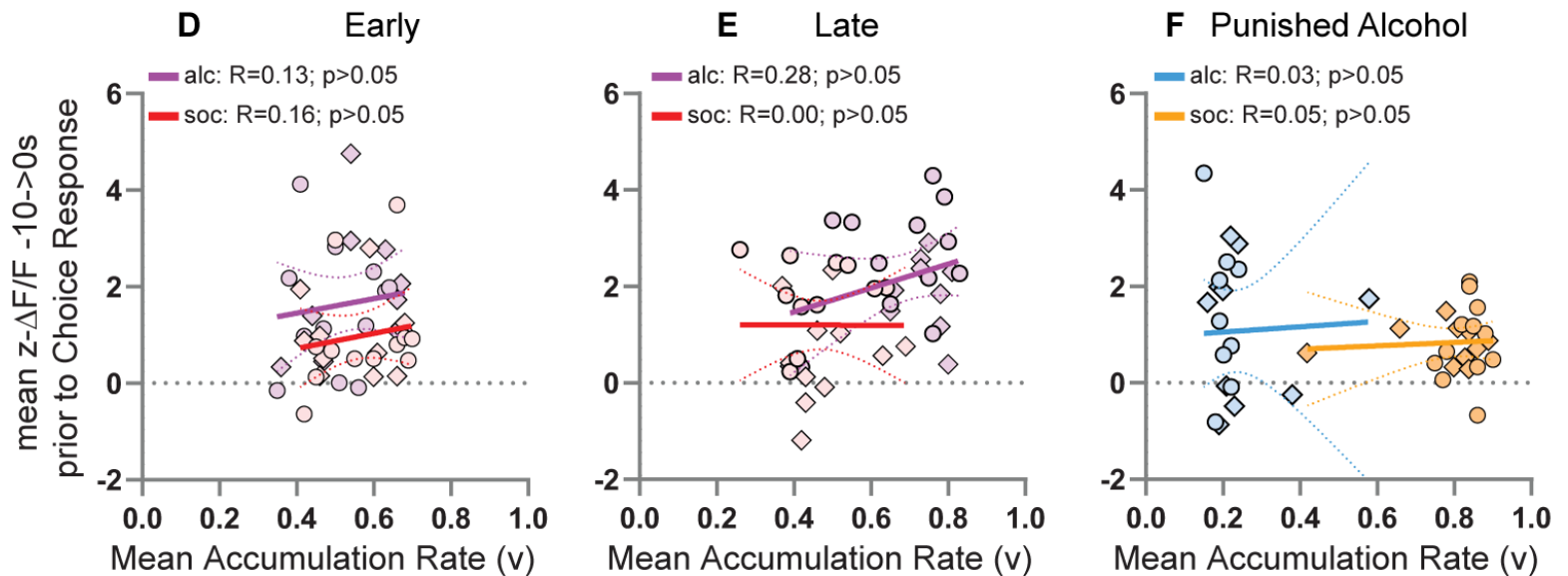


Figure S7. Additional correlations

Relationship between accumulation rate (v) and aIC calcium during cue



Relationship between accumulation rate (v) and aIC calcium 10s-->0s prior to response



Relationship between Response Latency and aIC calcium

