Long-Term Alcohol Drinking Reduces the Efficacy of Forced Abstinence and Conditioned Taste Aversion in Crossed High-Alcohol Preferring Mice

Supplemental Methods

Experiment 1

Statistical Analysis. Intakes over the final 3 days of initial consumption for each group were computed into their average and compared using a factorial ANOVA with factors of Abstinence, drinking Duration, and Sex. Although abstinence had not yet occurred, we wanted to assess whether groups differed prior to the onset of abstinence. Post-abstinence BEC and footslip data were analyzed using ANOVAs with between-subjects factors of Abstinence, Drinking Duration, and Sex (2 X 2 X 2) and a within subjects factor of either Hour (for Day 42 intakes) or Day (Post-Abstinence Days 2-14).

Experiment 3

Statistical Analysis. CTA induction data were analyzed using a mixed-model ANOVA with within-subject factors of Day and Flavor and a between-subjects factor of Sex (4 X 2 X 2), collapsing across sex in the absence of interactions with the other factors. Paired-samples t-tests were performed between Day 1 and Day 4 as in Experiment 2. A factorial ANOVA with factors of Duration and Sex was used to compare average g/kg/day intakes prior to CS+ adulteration. Bihourly Bin intake data were analyzed using ml/kg intake stratifying *a priori* by fluid access groups (Water only or Free Choice) due to different intake levels in these groups with factors of Duration (of baseline drinking) and bihourly Bin. Bihourly preference data, used to facilitate comparison between CS+ mixed with ethanol and controls receiving the CS+ in water,

were analyzed using two mixed-model ANOVAs, one on each day of testing, testing between-subjects factors of Duration of drinking, and test Fluid over the 6 Time bins (3 X 2 X 6). Independent-samples t-tests were used to assess planned comparisons between CS+/ethanol and CS+ alone consumption for all alcohol drinking durations at the time points of the bihourly preference data, and one-way ANOVAs analyzed potential differences between the durations at these time points.

Supplemental Results

Experiment 3

Four mice died due to injection-related complications during CTA induction, and their data were excluded from all analyses. CTA to almond and banana was successfully established, though group intakes did not reach floor as they did in Experiment 2 (Fig. S2). A mixed-model ANOVA revealed a large main effect of Day ($F(3,180) = 415.74$, $p < .001$), along with a more modest effect of Flavor ($F(1,60) = 54.83$, $p < .001$). Importantly, paired-samples t-tests revealed that Day 4 was significantly lower than Day 1 for each flavorant (banana: $t(61) = 25.96$, $p < .001$, almond: $t(61) = 21.40$, $p < .001$).

Supplemental Figures

Figure S1. Acquisition of taste aversion conditioning to a flavorant paired with LiCl injection (CS+) but not a flavorant paired with saline injection (CS-) in Experiment 2. Flavor did not show significant effects across days in either condition. Disparate points

plotted on each individual day of each treatment represent separate groups of mice. *n*s = 6-7, *** $p < .001$ compared to Day 1 collapsed across Flavor, paired-samples t-test

Figure S2. Successful aversion conditioning of two flavorants paired with LiCl injection in Experiment 3. Flavor did not show significant effects across days in either condition. Disparate points plotted on each day are within-subjects across two successive days of conditioning. *n*s = 62, *** $p < .001$ in both flavors compared to Day 1, paired-samples t-test