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# *Car8* knockout in prefrontal cortex increases voluntary ethanol consumption in male mice

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We previously performed a genetic study on voluntary ethanol-drinking phenotypes across 636 male Diversity Outbred (DO) mice over five weeks of intermittent ethanol access (IEA), identifying *Car8* as one of 11 genes within a 90% Bayesian confidence interval for a significant bQTL for last week ethanol consumption on chromosome 4. We collected RNA-seq data from 220 prefrontal cortex (PFC) samples from these mice for eQTLs and correlations with ethanol consumption. Only *Car8* had a significant *cis*-expression QTL near the bQTL and a significant correlation with last week ethanol consumption (-0.22, $p = 0.008$). We hypothesize that knockout of *Car8* in PFC will increase voluntary ethanol consumption in mice.

Male and female C57BL/6JGpt-Car8$^{em1Cflox}$/Gpt mice were backcrossed to C57BL/6J mice and given injections of AAV8-hsyn-GFP-Cre to knockout *Car8* or AAV8-hsyn-EGFP into PFC (n = 8/sex/group). Mice were then exposed behavioral assays including five weeks of IEA via three-bottle choice ($H_2O$, 15% v/v EtOH, 30% v/v EtOH). ANOVA were used to compare differences between knockout/control and sexes.

Effects of sex ($p = 1.33*10^{-08}$) and sex-by-knockout-group interaction ($p = 2.93*10^{-02}$) were significant for ethanol consumption. *Car8* knockout male mice consumed significantly more ethanol than control male mice ($p = 3.07*10^{-02}$), but the same effect was not observed in females ($p = 9.87*10^{-01}$).

Our findings demonstrate that knockout of *Car8* in PFC neurons increases voluntary ethanol consumption in male mice. Given these results, *Car8* appears to be a strong candidate gene for modulating ethanol consumption.

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