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Gene mapping and editing of an intronic variant in *Gabra2* in methamphetamine sensitivity and naloxone-induced aversion behaviors in a reduced complexity cross

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Substance use disorders have a genetic component that remains poorly understood. Rodent forward genetic studies complement human GWAS, providing statistical power, easy access to brain tissue for expression QTL (eQTL) analysis, and the molecular genetic tools necessary to demonstrate causality *in vivo*. Reduced complexity crosses between nearly identical substrains offer rapid gene mapping of complex traits, given their drastically simplified genetic architecture (typically monogenic), and ability to immediately fine map and validate quantitative trait nucleotides on nearly isogenic backgrounds. We identified robust C57BL/6 substrain differences in the locomotor stimulant response to methamphetamine (2mg/kg, i.p.) and conditioned place aversion in response to the opioid receptor antagonist naloxone (4mg/kg, i.p.) between C57BL/6J (B6J) and C57BL/6NJ (B6NJ) substrains. To map the genetic basis of these traits, we tested 208 B6J x B6NJ-F2 mice alongside 213 saline control F2 mice. We mapped a single QTL underlying methamphetamine and naloxone behaviors. A significant *cis*-expression QTL for *Gabra2* (alpha2 subunit of the GABA-A receptor) was identified via RNA-seq analysis of striatal tissue from F2 mice (N=23; $p=1.66 \times 10^{-30}$). Mulligan and colleagues recently validated a functional single nucleotide deletion within intron 4 of *Gabra2* in the B6J strain that underlies this eQTL at the RNA and protein level using CRISPR/Cas9-engineered mice containing the inserted (“corrected”) B6NJ nucleotide. Mice harboring this corrected *Gabra2* B6NJ nucleotide showed a complete rescue of reduced methamphetamine sensitivity and reduced naloxone-induced aversion behaviors. These results identify novel gene functions for *Gabra2* in psychostimulant sensitivity and endogenous opioid regulation of basal mood state.