Submitter Name: Tamara J. Richards
Submitter email: phillipt@ohsu.edu

A Single Nucleotide Replacement Restores Methamphetamine Traits to Low Addiction Risk Levels

Tamara J. Phillips[1,4], Cheryl Reed[1], John R.K. Mootz[1], Lev Fedorov[3], Aaron Janowsky[2,4], and Kim A. Neve[1,4]

[1]Departments of Behavioral Neuroscience and [2]Psychiatry, and [3]Transgenic Mouse Models Shared Resource, Oregon Health & Science University; [4]VA Portland Health Care System

A spontaneous mutation arose in the trace amine-associated receptor 1 gene, *Taar1*, in DBA/2J (D2) mice at The Jackson Laboratory in 2001-2003. Homozygosity for the mutant allele (*Taar1*^{m1J}) corresponds with high methamphetamine (MA) intake on multiple genetic backgrounds, including pure D2, mixed C57BL/6J x D2 and heterogeneous stock collaborative cross x D2. Low sensitivity to aversive effects of MA corresponds with high MA intake and also appears to be impacted by this mutation. MA is a direct agonist at TAAR1 and this mutation eliminates receptor function. We hypothesized that restored function would reverse these high MA addiction risk phenotypes. To examine this, the native *Taar1*$^{+}$ allele was inserted into the genome of mice bred for high MA intake, which are homozygous for the *Taar1*^{m1J} allele. CRISPR-Cas9 was used to first excise the *Taar1*^{m1J} variant and then replace it with the *Taar1*$^{+}$ variant, thus restoring TAAR1 function. These mice were compared to non-altered controls for 3 MA phenotypes. All phenotypes were restored to levels found in mice with the *Taar1*$^{+}$ allele that codes for a functional protein. Thus, MA intake was low and sensitivity to aversive and hypothermic effects of MA was high, compared to controls. These results confirm a causal role for *Taar1* in MA intake, and suggest that functional TAAR1 maintains sensitivity to effects of MA that may protect from excessive use. Some human *TAAR1* variants have been identified that impact TAAR1 function and may alter sensitivity to effects of MA that are relevant to risk for MA use.