# Chrna5-expressing neurons in the interpeduncular nucleus mediate aversion primed by prior stimulation or nicotine exposure

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Understanding the receptors and neural pathways underlying the reinforcing and aversive effects of nicotine may suggest new treatments for tobacco addiction. Genetic studies have shown an association between smoking behavior and specific haplotypes at the *CHRNA5/A3/B4* gene locus, a conserved genomic region that encodes the α5, α3 and β4 nicotinic receptor subunits. The α5 subunit has been specifically implicated because the smoking-associated haplotypes contain a functional mutation in the *CHRNA5* gene. α5 null mice exhibit reinforcement with increased doses of nicotine that are normally aversive. The *Chrna5/a3/b4* locus is conserved across species, and the expression of these subunits in rodents suggests neural pathways through which the reinforcing and aversive properties of nicotine may be mediated. Here we show that in α5 null mice, neurons of the interpeduncular nucleus (IP), the site of highest α5 mRNA expression, have markedly reduced electrophysiological responses to acetylcholine and nicotine. We then used BAC recombineering to generate transgenic mice that express Cre-recombinase from the *Chrna5* locus without overexpression of the associated genes. Reporter expression driven by Chrna5$^{Cre}$ demonstrates that *Chrna5* is regulated independently from the *Chrna3/b4* genes, transcribed on the opposite strand. α5-IP neurons are GABAergic, but project to distant targets in the mesopontine raphe and tegmentum, rather than forming local circuits. Optogenetic stimulation of α5-IP neurons is aversive in a shuttle box assay, and this response is primed by recent prior activation of this nucleus, or recent exposure to a single dose of nicotine. These results support the idea that natural variants and induced mutations in the α5 receptor disrupt the balance between nicotine reward and aversion, and that the normal aversive responses are mediated by the IP. They are not consistent with a model in which changes in the α5 receptor directly affect the medial habenula, which lies upstream of the IP.