Genome Editing in the Adult Rat Brain

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Historically, the rat has been the preferred animal model for behavioral studies. Limitations in genome modification have, however, caused a lag in their use compared to the bevy of available transgenic mice. Here, we have developed several transgenic tools, including viral vectors and transgenic rats, for targeted genome modification in specific adult rat neurons using CRISPR-Cas9 technology.

Methods: A Cre-dependent Cas9 transgene was inserted into the rat Rosa26 locus of Long Evans rat by fertilized oocyte injections to create "LSL-Cas9" rats. A second transgenic rat, "LSL-nickase", was made using a Cre-dependent Cas9 nickase transgene targeted to the rat Rosa26 locus of rat spermatogonial stem cells. A third transgenic rat was made to express Cre-recombinase from the rat dopamine transporter promoter, "DAT-iCre" rat. LSL-Cas9 rats were crossed with DAT-iCre rats and combined with adeno-associated viral (AAV) vectors viral vectors to provide cell-specific genome editing.

Results: Starting from wild-type rats, knockout of tyrosine hydroxylase was achieved with expressing Cas9 or guide RNAs (gRNAs). We subsequently created an AAV vector for Cre-dependent gRNA expression as well as three new transgenic rat lines to specifically target CRISPR-Cas9 components to dopaminergic neurons. One rat represents the first knock-in rat model made by germline gene targeting in spermatogonial stem cells.

Discussion: The rats developed in the current study serve as a versatile platform for making cell-specific and sequence-specific genome modifications in the adult brain and potentially other Cre-expressing tissues of the rat.

References: Bäck et al. Neuron. 2019 Apr 3;102(1):105-119.e8. doi: 10.1016/j.neuron.2019.01.035.

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