Cas9 cuts to the quick in the cortex

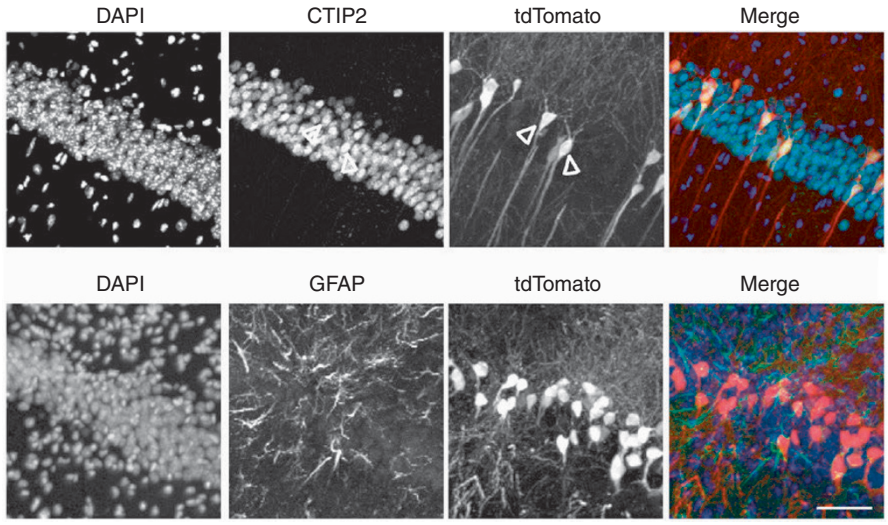
A method for direct delivery of Cas9 complexes to the brain enables efficient and targeted genetic manipulation in post-mitotic neurons.

In 2012, Brett Staahl was finishing up his doctorate at Stanford University, studying chromatin remodeling during neural development, when Aaron Gitler's lab moved in down the hall. The Gitler lab was searching for human genes involved in non-familial amyotrophic lateral sclerosis and discovered *de novo* mutations in a gene Staahl had been characterizing for his thesis. This happenstance not only made for a natural collaboration, but also sparked Staahl's interest in the field of gene therapy. "The idea that you could inactivate or correct a disease-causing allele was really intriguing to me", says Staahl.

Around that same time, Jennifer Doudna's lab at University of California, Berkeley, published a seminal paper in *Science* on a programmable DNA nuclease, called Cas9, that offered a highly efficient and easy-to-use method of genetic engineering. "When I saw that paper", recalls Staahl, "I thought it would be something I'd like to work on and try to develop into a therapeutic to treat the underlying cause of genetic disease."

Since Cas9 came onto the scientific scene, the clinical potential has been clear, but meeting that potential will require improvements for delivering Cas9 *in vivo*. This is particularly true for the disorders that interest Staahl, like neurodegenerative diseases, where direct manipulation of developed tissues and specific cell types in the brain might be needed for therapeutic intervention.

So, Staahl set up another collaboration, this time with the Doudna lab and Anirvan Ghosh, then Head of Neuroscience Research at Roche, Switzerland, with the goal of developing a method to deliver Cas9 ribonucleoprotein (RNP) complexes directly to the brain for gene editing. For Doudna, whose lab specializes in basic biochemistry research, the idea of an applied research project using Cas9 was attractive. "When thinking about how working in a biochemistry lab, like mine, could lend itself to a very applied project for Brett, combined with Anirvan at Roche…we thought it could be an interesting partnership."



Neurons in the hippocampus expressing tdTomato after Cas9-based gene editing. Note co-localization with CTIP2 marker of CA1 neurons, but no overlap with GFAP, a marker of astrocytes. Adapted from *Nat. Biotechnol.* doi:10.1038/nbt.3806.

In new work performed in mice and published in *Nature Biotechnology*, the first fruits of this collaboration are demonstrated with a method for direct and efficient gene editing in adult, post-mitotic neurons in multiple brain regions, including cortex, hippocampus, and the striatum (*Nat. Biotechnol.* doi:10.1038/nbt.3806, published online 13 February 2017).

Because Cas9 itself does not have any cell penetrating ability, the research team first had to devise a strategy to get Cas9 RNP complexes across cell membranes. Working from a previous report showing enhanced cell penetration using another type of nuclease (zinc fingers), the group attached arrays of Simian vacuolating virus 40 nuclear localization sequence (SV40-NLS) onto the Cas9 protein, and found significantly enhanced cell penetration and gene editing with up to 4xNLS repeats attached, first tested *in vitro* and then confirmed *in vivo* with stereotaxic injections directly into adult mouse brains.

As shown in their paper, the Cas9 complex edits brain cells in a dose dependent manner, enabling the team to accurately estimate the number of cells that undergo gene editing per *in vivo* injection. Additionally, the group found that Cas9 cell penetration and action were restricted to neurons, with astrocytes remaining unedited. "We're very keen on seeing if we can get even greater cell specificity with Cas9 editing in the future", remarks Doudna, "for example by using a receptor-mediated uptake method." The team also hopes to apply the method to a mouse model of Huntington's disease to see if they can achieve sufficient editing to alleviate symptoms, creating a path for clinical development.

But for Doudna, the success of this project reflects something bigger about the integration of basic and translational science. "I think that this is a great example of how someone like Brett, who had a very applied interest in a technology, was able to embed himself in a research laboratory doing fundamental, curiosity driven research, and lead to a really exciting outcome that we hope is going to have clinical impact in the future. I'd love to see more of that kind of partnership in science."

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