NEUROSCIENCE

Putting a stamp on single cells

Virus stamping can target single cells in complex tissues, both in culture and *in vivo*.

Genetic access to single cells can benefit connectivity or functional studies in the brain or peripheral sensory systems, since it allows the use of fluorescent proteins or optogenetic tools. However, targeting a single cell for genetic manipulation within a complex tissue is currently either highly inefficient or technically complex. It is "a really big problem that one cannot address single cells with viruses," says Botond Roska from the Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland. Fortunately, Roska discussed the problem with Daniel Müller from the ETH next door. Together, the two researchers and their teams came up with a method for infecting single cells with viruses by using a stamping technique.

"We came up with a very complicated scheme," says Roska, but this turned out to be "a huge over-design." The final method is actually quite simple. For targeting single cells in culture or at tissue surfaces such as in retinas, the team coated the tips of glass pipettes with viruses and simply touched the cells of interest to infect them. Müller explains that they initially used an atomic-force microscope to approach the cells, which worked very well. But the team, including PhD student Rajib Schubert as a major driving force, wanted to make the technology as simple as possible, leading to the implementation of the method with glass pipettes and a micromanipulator.

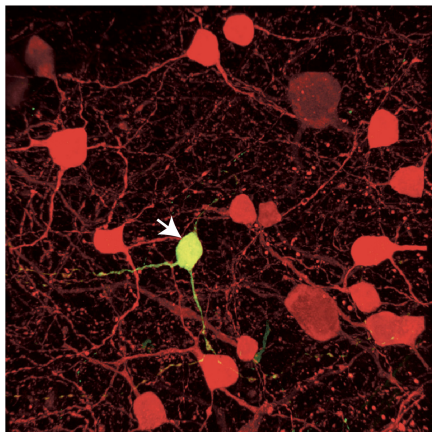
"And then we realized that it is extraordinarily simple to generalize this to *in vivo*," says Roska. Instead of coating pipettes, the team coated magnetic beads with virus. They filled the beads into a glass pipette and approached the target cells. They then steered the beads towards the cells by applying a magnetic field.

While the virus-stamping technology itself works very well, Müller points out that "your virus sample must be superclean." If there is debris in the virus preparation, the smaller particles absorb faster to the surfaces of the beads or pipettes and effectively block these surfaces. Thus, the team now invests quite some effort in ensuring the purity of the virus sample, which they check with transmission electron microscopy.

Roska points out two advantages of the virus-stamping technology over other approaches. First, he mentions the high infectivity of the virus particles when attached to surfaces. Müller and Roska explain that virus infectivity is governed by on and off rates at the cell surface. However, in the virus-stamping approach, the virus is trapped and cannot unbind anymore. "You force it from an equilibrium situation to an out-of-equilibrium situation," says Müller, and thus even low-affinity viruses are highly infective. "There is nowhere to go, only inside [the cells]," says Roska.

According to Roska, another advantage of the virus-coated-bead approach is its ability to be controlled remotely. "We are very interested to steer viruses in the brain by totally external forces," he says. Some of the applications Roska mentions go beyond applications in single cells and involve pulling the virus-coated particles out of the blood stream and targeting different nuclei in the brain.

The researchers developed the virus-stamping approach with the goal to trace circuits of single cells in the retina. "We thought that this method actually would be interesting for many other applications, too," says Müller. The researchers demonstrated the versatility of the virus-stamping approach in a variety of examples involving different viruses and target cells. In one example, they expressed a genetically encoded calcium indicator in single cells in the brain of a mouse and determined the cells' orientation tuning in response to visual stimuli.



Viral stamping targets a single cell in the explanted mouse retina. Image reprinted with permission from Schubert *et al.* (Springer Nature).

In the future, Roska and Müller want to continue developing the technology. Roska thinks that they can further improve the efficiency of the technology and maybe even automate it. Furthermore, he wants to target cells in deeper brain regions without relying on imaging-based guidance. Instead, he thinks about combining the technology with optogenetic identification of target cells, which involves channelrhodopsin expression, illumination and electrical recording from candidate cells before they are targeted by virus stamping.

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RESEARCH PAPERS

Schubert, R. *et al.* Virus stamping for targeted single-cell infection *in vitro* and *in vivo*. *Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.4034 (2017).