Suitability of hCMV for viral gene expression in the brain

To the editor: Viral gene delivery is a powerful tool that is rapidly gaining popularity in experimental neuroscience. I believe that the near future will see rapid expansion of this remarkably versatile and adaptable method. Recent communication by Wickersham *et al.*[1] describes yet another interesting implementation of this technology for retrograde tracing of neurons from their target areas. The key point of the paper is demonstration of a high level of enhanced GFP (EGFP) expression using a rabies virus mutant with glycoprotein gene substituted by *EGFP*. After injecting the vector into the thalamus, the authors showed many bright EGFP-expressing cortical pyramidal neurons. As a reference, they used a lentivirus with rabies virus envelope glycoprotein in which EGFP expression was driven by human cytomegalovirus (hCMV) promoter and found a very low level of EGFP expression with it.

I would like to draw the attention of *Nature Methods*' readers to the issue of suitability of hCMV-driven constructs for targeting brain neurons. According to the experience of our laboratory, hCMV expression in brain is only stable in some neuronal phenotypes, for example, cholinergic neurons such as those found in the nucleus of the hypoglossal nerve or vagal motor neurons. Other neurons, for example, in the adjacent nucleus of the solitary tract (which are glutamate, GABA and noradrenergic neurons) yield extremely poor expression with hCMV-containing adeno- and lentiviruses[2]. Hence, the assumption that hCMV-driven viral constructs are guaranteed to express in all neurons is incorrect. Such constructs are, however, very active in glia, sometimes creating a confusing impression of high level of expression (see **Supplementary Fig. 1** online). A striking example of a lack of hCMV activity in medullary neurons was recently given[3]. Therefore, for the comparison to be fair, it is critical that stable and high-level EGFP expression in pyramidal neurons be demonstrated using the same lentivirus (presumably this would require using viruses coated with vesicular stomatitis glycoprotein rather than rabies virus glycoprotein). Incidentally, we also had demonstrated bright unstained EGFP-expressing noradrenergic neurons targeted using conventional adenoviral vectors injected into spinal cord[2]. Such cells are easily identifiable in acute slices prepared from pretransfected animals and can be studied using patch clamp. Curiously, it seems that the phenotype of neurons affects their suitability for retrograde transduction, as we never succeeded in achieving the same with glutamatergic neurons, such as cortical pyramidals, which were the target in the study by Wickersham *et al.*[1]. Finally, it is likely that the expression profile of hCMV-containing vectors based on adeno-associated virus may obey different rules owing to the effects of the viral inverted terminal repeats[4].

Note: Supplementary information is available on the Nature Methods website.

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COMPETING INTERESTS STATEMENT
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Wickersham and Callaway reply: Dr. Kasparov draws attention to the idiosyncrasies of the widely used human cytomegalovirus promoter, and we agree that viral vectors using this promoter cannot reliably drive strong transgene expression in neurons of particular types. His observations provide additional examples of the drawbacks of previously available viruses proposed for use as retrograde tracers. We are happy to say that we have detected no such expression difficulties using the deletion-mutant rabies virus described in our paper, which infects a wide variety of cell types in every brain region in which we have tested it. In contrast to commonly used viral vectors for which, as Dr. Kasparov points out, the choice of promoter is crucial, rabies virus transcribes its genes with its own polymerase, conferring complete independence from the host-cell transcriptional environment and allowing high-level transgene expression regardless of cell type. Also unlike typical vectors, the virus described in our paper retains the ability to replicate its core to high copy number within infected cells, leading to EGFP expression levels that are impressive in the extreme.

Finally, we would like to take this opportunity to point out another advantage, described in detail in our recent paper in *Neuron*[1], of this glycoprotein-deleted rabies virus. When complemented by expression of its glycoprotein in trans within infected cells, this virus spreads to cells directly presynaptic to them. This should allow tracing not just of cells that project to an injection site but also of the cells presynaptic to the ones that are initially infected. This initial infection furthermore can be directed to a genetically specified population of cells, allowing tracing of direct inputs to particular neuron types or even to single neurons.

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