GENE DELIVERY

Transgene delivery via the transverse sinus in neonates

Hamodi, A.S., Sabino, A.M., Fitzgerald, N.D., Moschou, D., and Crair, M.C. *eLife* (2020). https://doi.org/10.7554/eLife.53639

The brain, in effect, builds itself from nothing. Spontaneous neural activity eventually combines with sensory inputs to guide the brain to its fully functional form, but capturing those earliest moments in newborn animals can be challenging.

Ali Hamodi, a postdoctoral fellow at Yale University, studies the developing visual system. For their first two weeks of life, rodent pups are blind and deaf; this period is developmentally equivalent to the 3rd trimester in primates, but the rodents develop outside the confines of their mothers' wombs. This helps researchers study spontaneous brain activity patterns before the onset of visual and auditory sensation.

To visualize neural activity during this time, Hamodi performs calcium imaging, which involves expressing GCaMP6, a genetically encoded calcium indicator, in the brains of the pups. Transgenic animals are an option, but the process to make them involves costly and time-consuming animal crosses. An alternative, adeno-associated virus vectors (AAVs) can deliver transgenes postnatally, but current injection methods have limitations. Some require excess volumes of the viral vector, which can be disruptive and yet inefficient at transducing cells—some parts of the brain will appear bright, while others remain dim. Others that involve direct injections into the brain risk cellular damage, especially in small newborns, while still yielding non-uniform expression.

But, there turns out to be an alternative injection site that avoids touching the brain: the transverse sinus, one of two blood vessels at the edge of the forebrain. Hamodi and his colleagues recently published details of the approach, the neonatal sinus injection method (n-SIM), in *eLife*.

n-SIM involves making a small incision above a transverse sinus and using a glass pipette to deliver a viral vector and its cargo, which then cross the developing blood-brain barrier. Using n-SIM and 4 µL of AAV9, the team observed uniform GCaMP6 expression in the mouse cortex, thalamus, midbrain, and hippocampus within as little as four days following the delivery procedure on postnatal day 1; expression persisted into adulthood with no ill effect on the animals. They also show that multiple constructs can be delivered in the same animals to label and/or manipulate different cell types in the brain, and that the method can be used with rat pups as well.

Hamodi says he is now using n-SIM for calcium imaging studies in neonatal rodents while colleagues at Yale and elsewhere are considering it for use with other species—including ferrets and marmosets—that don't have as readily available transgenesis tools as mice. He also has plans to try n-SIM to deliver other cargo, including CRISPR/Cas9 and anatomical mapping tools. "Anything you can put inside an AAV, you can put inside the brain," says Hamodi.

Ellen P. Neff

Published online: 22 June 2020
https://doi.org/10.1038/s41684-020-0583-4