A Trojan horse could help get drugs past our brain's tough border patrol

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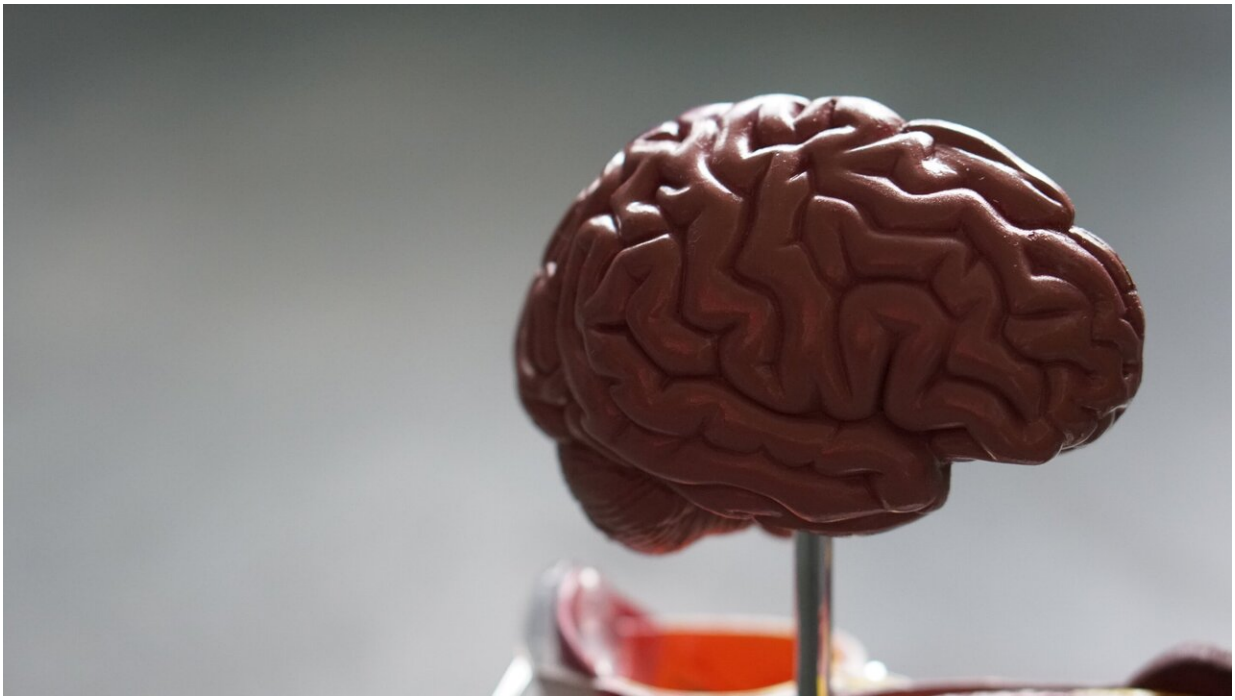

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Sclerosis, Parkinson's disease, Alzheimer's and epilepsy are central nervous system disorders. They are also very difficult to treat, since the brain is protected by the blood-brain barrier.

The blood-brain barrier works as a border wall between the blood and the brain, allowing only certain molecules to enter the brain. Water and

oxygen can get through, as can other substances such as alcohol and coffee. But it does block more than 99 percent of potentially neuroprotective compounds from reaching their targets in the brain.

Now, in a study conducted in vivo, including conscious mice, a team of researchers from the University of Copenhagen found direct insights into how to trick the blood-brain barrier's impermeable walls to allow drug delivery to the brain.

They investigated so-called nanoparticle liposome drug carriers and delivered them past the blood-brain barrier while tracking and monitoring them all the way through the system.

"Before this study, the community had no insight what was happening in the blood-brain barrier in the living brain, and why some nanoparticles crossed and others wouldn't. In this regard, the blood-brain barrier was a black box where the events between drug administration and detection in the brain remained obscure. It was even doubted whether nanoparticle entry to the brain was possible at all. With our paper, we now provide a direct proof of nanoparticle entry to the brain and describe why, when and where it happens," says Assistant Professor Krzysztof Kucharz from the Department of Neuroscience.

The researchers, aided by colleagues at the Technical University of Denmark and Aalborg University, used two-photon imaging to deconstruct the blood-brain barrier in order to understand how the nanoparticle drug carriers travel past the blood-brain barrier in a living organism.

"We monitored the nanoparticles entry to the brain at each step of the process, providing valuable knowledge for future drug design. Specifically, we show which vascular segments are the most efficient to target with nanoparticles to allow their entry to the brain. And because

we were able to monitor the drug carriers at the level of a single nanoparticles, we now provide a novel platform to develop more efficient and safer therapeutic approaches," says Kucharz.

The study, released in *Nature Communications*, shows that nanoparticles targeted to the brain are picked up in the capillaries and venules by endothelial cells, which are the cells in the blood-brain barrier that allow or reject access of molecules to our brain tissue.

"Analogically to the mythical Trojan horse they are recognized by endothelium and transported across the blood-brain barrier to the brain. These nanoparticles have a cargo space that can be loaded with neuroprotective drugs to treat many neurodegenerative diseases. This approach is currently being tested in many clinical and preclinical trials in brain cancer, stroke, Alzheimer's and Parkinson's disease. However, the levels of nanoparticle transport into the brain are still low and need to improve to reach clinical significance. Therefore, there is a great need to optimize nanoparticle drug delivery and to do so, it is crucial to understand how nanoparticles interact with the blood-brain barrier. This is where we came into play," says Kucharz.

The researchers used a two-photon imaging approach to study nanoparticles, allowing them to open the blood-brain barrier black box and get a full picture of nanoparticles route across the blood-brain barrier. They tagged the particles with fluorescent molecules, which allowed the microscopy of nanocarriers in the living, intact brain at the resolution level of a single nanoparticle.

They observed how nanoparticles circulate in the bloodstream, how they associate over time to the endothelium, how many were taken up by the endothelium, how many are left behind, what happens to them once inside the blood-brain barrier and where the nanoparticles exit to the brain. Then, they observed that brain vessels handle the nanoparticles

differently, allowing or rejecting access of nanoparticles to the brain tissue depending on the vessel type.

"Although the anatomy and function of the endothelium differ between different vessel types, this principal feature of the brain had so far been overlooked in drug delivery studies, and whether or how it impacted drug delivery had been unknown," says Kucharz.

They show that nanoparticles can enter the brain mainly at large vessels, i.e., venules, which are surrounded by so-called perivascular space, and not, as previously believed, small and numerous capillaries. The perivascular space surrounds venules, making it easier for nanoparticles to exit the endothelium and progress further into the brain; this space is absent in capillaries.

"Our results challenge the assumed view that capillaries constitute the main locus for nanoparticle transport to the brain. Instead, venules should be targeted for efficient nanoparticle drug delivery to the brain," says Kucharz.

The methodological platform developed by authors may constitute an excellent platform to fine-tune nanoparticle formulations for increased transport to the brain and provide valuable information for the future design of novel drug delivery systems. This will hopefully provide a great leap forward to efficiently treat brain disorders.

More information: Krzysztof Kucharz et al, Post-capillary venules are the key locus for transcytosis-mediated brain delivery of therapeutic nanoparticles, *Nature Communications* (2021). DOI: 10.1038/s41467-021-24323-1

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