Scientists find way to monitor progress of stem cells after transplantation into brain

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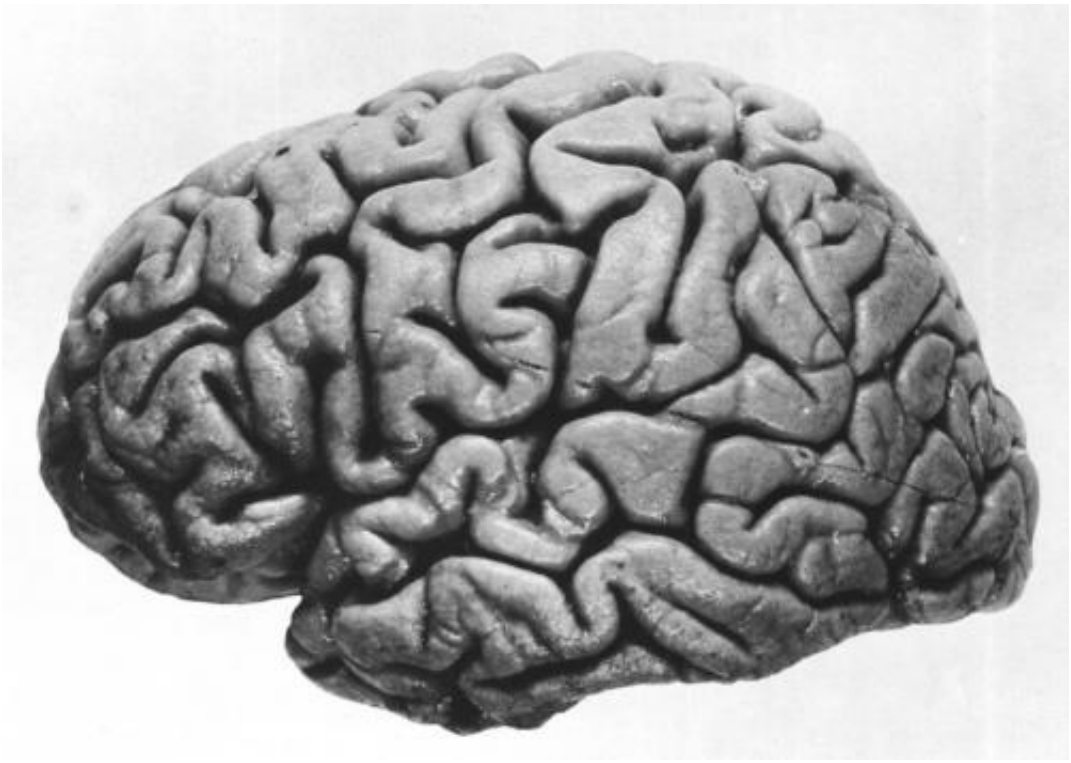

Left hemisphere of J. Piłsudski's brain, lateral view. Credit: public domain

Investigators at the Stanford University School of Medicine have devised a way to monitor neural stem cells after they've been transplanted into the brain.

The scientists were able to determine not only whether the stem cells

transplanted into living animals survived but whether they matured into nerve cells, integrated into targeted brain circuits and, most important, were firing on cue and igniting activity in downstream nerve circuits.

The new monitoring technique could in principle be used to determine the success of other kinds of stem cell transplantations. It promises in the near term to improve researchers' ability to optimize stem cell therapies in animal experiments and, in the intermediate term, to speed progress in human trials of stem cell replacement therapy, a promising but problem-plagued medical intervention.

Many disorders of the central nervous system, such as Parkinson's disease, are characterized by defective nerve cells in specific brain regions. This makes disorders such as Parkinson's excellent candidates for stem cell therapies, in which the defective nerve cells are replaced. But the experiments in which such procedures have been attempted have met with mixed results, and those conducting the experiments are hard put to explain them. There's been no good way to evaluate what the transplanted stems cells are doing. So optimizing the regimens becomes a matter of guesswork and luck.

"That's the key missing step in stem cell therapy design: Once you've transplanted the cells, you can't tell exactly what they're doing afterwards," said Jin Hyung Lee, PhD, assistant professor of neurology, of neurosurgery and of bioengineering. In the case of brain-oriented therapies, you have to look for behavioral changes, she said. "And even when you see them, you still don't know whether the newly transplanted cells integrated into the right brain circuits and are now functioning correctly there."

Now there's a way to tell.

Transplanted stem cells did what they were supposed

to

Lee is the senior author of a paper, appearing online April 30 in *NeuroImage*, detailing a series of experiments in which she and her colleagues combined functional magnetic resonance imaging, or fMRI, with a relatively new but increasingly widespread technology known as optogenetics, which employs laser light to stimulate specific cells that have been rendered sensitive to particular frequencies of light. The combination let the scientists selectively stimulate only nerve cells derived from newly transplanted neural stem cells, while simultaneously assessing resulting nerve-cell activity at the site of the transplant and elsewhere in the brain.

The study showed that the transplanted neural stem cells had indeed matured into nerve cells that not only integrated into the brain's circuitry at the transplantation site but could be induced to fire electrical signals on command, and that this signaling triggered activity in other areas of the brain. Lead authorship of the study is shared by former graduate student Blake Byers, PhD, now a general partner with Google Ventures; postdoctoral scholar Hyun Joo Lee, PhD; and PhD students Jia Liu and Andrew Weitz.

The researchers first created induced pluripotent stem cells, or iPS cells, from the skin cells of a patient with Parkinson's disease. Like embryonic stem cells, iPS cells have the capacity to differentiate into every cell type in the human body. Next, they inserted a gene coding for a photosensitive protein into these iPS cells. The protein situates itself on the cell's surface and, in response to blue laser light, induces electrical activity in the cell.

Then, in a dish, the researchers differentiated the genetically altered iPS cells into neural stem cells. Unlike iPS cells, which can differentiate into every cell type in the body, neural stem cells can mature only into nerve

cells or a few other cell types that populate the brain.

The scientists transplanted these genetically altered human cells into the brains of rats that were normal except for the fact that their immune systems were compromised, reducing the chances of an immune attack on the foreign cells.

The particular region of the brain into which the cells were injected is called the striatum. In humans, deterioration of particular nerve cells in this area is a hallmark of Parkinson's disease, a progressive neurodegenerative disorder profoundly affecting movement and, frequently, mental function. Along with the new cells, the investigators implanted into each rat's brain a small cannula containing the end of a thin optical fiber whose far end could be connected to a laser light source.

From about three months to almost a full year after the procedure, Lee and her associates conducted experiments in which, using fMRI, they observed the rats' brains before, during and after stimulating the implanted cells with pulses of blue laser light or, as a control, yellow laser light. Blue-light stimulation triggered activity not only within the striatum but at several other areas in the brain. Yellow light had no effect—proof that electrical activity in these cells had been triggered by stimulating the genetically inserted protein, not merely by shining light on them.

Recording electrical activity

To explore activity in those areas, the researchers turned to a different observation method: electrophysiology. While fMRI has the advantage of imaging large portions of the brain simultaneously, it actually measures not electrical activity but blood flow in the small vessels permeating the entire brain. Active nerve cells require more nutrients,

and increased blood flow in a specific location in the brain is considered an excellent proxy of electrical activity at that location.

But, having now identified specific brain areas where fMRI scans indicated increased nerve-cell activity, Lee and her associates proceeded to directly record electrical activity in these areas by inserting electrodes there and watching what happened when they pulsed blue light into the striatum, where the neural stem cells had been transplanted. They saw, first, that the transplanted nerve cells had clearly integrated into striatal circuitry and were firing there when stimulated with blue light; and, second, that this triggered electrical follow-on activity in remote regions of the brain.

Anatomical inspections of the rats' brains confirmed that the new cells had integrated into the striatum and, in many cases, had grown long projections to the remote areas where follow-on activity had been observed.

"I'm hopeful that this monitoring approach could work for all kinds of stem cell-based therapies," Lee said. "If we can watch the new cells' behaviors for weeks and months after we've transplanted them, we can learn—much more quickly and in a guided way rather than a trial-and-error fashion—what kind of cells to put in, exactly where to put them, and how."

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