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Sergio Canavero, MD; XiaoPing Ren, MD
HEAVEN/GEMINI International Collaborative Group, Turin, Italy

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Editorial

Could an old brain be made young again?

Jean M. Hébert

Department of Neuroscience and Genetics, Albert Einstein College of Medicine, New York City, New York, United States.

E-mail: *Jean M. Hébert - jean.hebert@einsteinmed.edu

***Corresponding author:**
Jean M. Hébert,
Department of Neuroscience and Genetics, Albert Einstein College of Medicine, New York City, New York, United States.

jean.hebert@einsteinmed.edu

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As we age, so do our brains. Brain aging, like aging of the rest of the body, is accompanied by complex forms of stochastic damage that occurs to all our classes of macromolecules. Damage occurs to DNA in various forms including base pair mutations, causing loss of information content. DNA damage alone can drive many other features of aging. Damage also accumulates in lipids, including those associated with myelin in the brain, as well as myelin itself, which is very long-lived. And perhaps most damningly, age-associated damage to the extracellular matrix (ECM) alone, like DNA damage, drives many or most of the features of aging.[7] The ECM is largely made of proteins and carbohydrates, some of which once made, do not turnover for life. Extracellular carbohydrates remain poorly studied (we still have not come close to cataloguing all their species) and the damage they accumulate is largely understudied due to a lack of tools, but this damage is nevertheless likely to be daunting in its complexity. The types of age-associated damage found in extracellular scaffolding proteins are somewhat better studied. These include forms of glycation, carbamylation, carbonylation, lipoxidation, mineralization, deamidation, racemization, fragmentation, denaturation, and aggregation. Most of these stochastic nonenzymatic forms of damage are problematic because there exists no repair machinery to reverse them, and in many cases, proteins that accumulate damage, such as collagens, become resistant to extracellular proteases.

This paints a grim picture for our chances of ever reversing aging of the brain (or of any organ for that matter). In fact, if our principle approach is to continue characterizing these complex forms of macromolecular damage so that one day we might address all of them with a dizzying battery of drugs or gene therapies, then brain age reversal has little hope of becoming a reality in the foreseeable future. Drugs are not smart enough to recognize stochastic modifications without disrupting biologically encoded and useful ones. Moreover, the sets of genes that would be required to recognize and reverse the forms of age-related stochastic damage have yet-to-be invented, not to mention the means of somehow delivering these new sets of genes to most cells of the body and brain without causing more harm than good.

PROGRESSIVE BRAIN REPLACEMENT: FOUNDATIONS

The solution, both for the body and the brain, is replacing old tissues and organs with pristine damage-free new ones. Although many groups are developing lab-made organs and body parts (many of which are being used in people already), an obvious question is whether the idea of replacement can apply to the brain. The answer appears to be yes. Two established principles in neurobiology suggest that progressively replacing brain tissue over time will be possible without a discontinuity of function or self.

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Plasticity

The first principle is plasticity, which is particularly evident for the neocortex, the part of the brain that encodes our highest cognitive functions, long-term declarative memories, self-identity, and consciousness. Plasticity evolved so that we can learn new things all the time, allowing us to adapt to our ever changing world. Although cortical plasticity has been documented across mammalian species for most or all cortical functions, the most illustrative examples for our discussion can be found in humans of advanced age. If, for example, the eloquent area of the neocortex is destroyed over the course of a few years due to a benign glioma (in contrast to being suddenly destroyed due to a stroke for example), then the individual never loses the ability to speak as language is seamlessly and progressively re-encoded in other areas of the neocortex while the tumor grows.[3,4] Another example can be found in a man in his 70s with an astrocytoma the size of an orange spanning his two frontal lobes, which was surgically resected, but without the man showing any change in personality, decision-making, planning, or attention (i.e., the functions encoded in these frontal lobes relocated to new cortical areas). Although these examples of plasticity in humans offer insights into how we might progressively silence and remove old tissue without disruption of function, the question remains: how do we go about introducing new pristine tissue?

Brain precursor cells

This brings up the second established principle in neurobiology that suggest brain age reversal might be possible. Brain precursor cells are programmed to generate a brain (without us knowing how they do it). Our brains develop from a simple sheet of embryonic neuroepithelial cells that are innately programmed to generate normal brain tissue – in all its complexity, with normal local and long-distance wiring. Even when transplanted into the adult neocortex, neocortical precursor cells differentiate and physiologically integrate remarkably well with the surrounding host brain tissue.[5,6,10,11,13,16] Yet, to date, these cells are unlikely to function normally and could not yet be considered for tissue replacements. Transplants thus far lack essential cell types and have disorganized cytoarchitectures. What remains to be done is to obtain tissue that is good enough to be used as replacement tissue for the old brain.

There are three options for a source of this tissue:

a. Fetal tissue from pregnancy terminations (as was used for Parkinson's patients). Although fetal tissue is useful for research and proof-of-concept experiments, it is difficult to scale and would require continued immune suppression of the recipient patient after transplantation. Until therapeutic cloning materializes, which would obfuscate the need for immune suppression, we can put this option aside for now.
b. Laboratory grown or "synthetic" fetuses are under development that could be more easily scaled for widespread use and could be patient derived to avoid immune rejection.[1,2] However, this technology does not yet allow these synthetic fetuses to reach a stage when immature human brain areas such as the neocortex begin to form. Perhaps in combination with synthetic wombs, which are also being developed.[9,14] fetal brain tissue from synthetic fetuses could eventually be a source of tissue for replacement.
c. Reverse engineering of fetal-like brain tissue from human patient-derived iPS cells, which would avoid immune suppression and be scalable. Therefore, we will focus on this option from here on out. Although all brain structures would need to be replaced, let's first focus on the neocortex, the largest and arguably most important part of the human brain.

PROGRESSIVE BRAIN REPLACEMENT: PROPOSED TECHNIQUE

Step 1: Determine which fetal tissue stage would be best to reverse engineer

There are at least two considerations in identifying an optimal fetal stage for engraftments. The tissue must be young enough for the neurons that it generates to integrate with the host, but the tissue must also have enough structural integrity to maintain a normal layered cytoarchitecture (even the fetal neocortex is comprised of layers, for example, the ventricular zone, subventricular zones, subplate, nascent cortical plate, marginal zone, and pia, all of which play essential developmental roles in generating normal mature tissue). Initial testing can be performed using real fetal neocortical tissue obtained from pregnancy terminations because such tissue has all the precursor cell types with normal ratios, relative differentiation states, and cytoarchitecture. The tissues can be tested by transplanting into aspiration lesions to control the shape and size of the space needed for engraftment. Output measures of graft performance are those routinely used by laboratories, including ours.[5-8,10,11,13,15,16] These include measuring cell survival and cytoarchitecture, mapping of axonal and dendritic connections to and from the graft, and initial functional measures such as recording responses to sensory input and determining the appropriateness of these responses for the location of the graft (e.g., response to visual but not auditory stimuli when the graft is in the visual cortex). Most of these preclinical tests can be performed using adult immune-compromised mice as the hosts for human or primate fetal tissue grafts, although due to the size limitations of the mouse brain, some testing may require larger preclinical models.

Step 2: Once the optimal fetal tissue stage is determined, identify in detail the components of that tissue

A high-resolution picture of what comprises the stage selected fetal tissue can be obtained using single cell sequencing, which reveals the cell types present and their maturity states,[12] as well as proteomic and glycomic analyses of the different layers to identify the major extracellular scaffolding proteins and growth factors present in each precursor layer. Exact ratios of cell types and their laminar positions in the tissue can then be determined by immunohistology using native fetal tissue samples.

The gelling properties of layer-specific scaffolds can be optimized *ex vivo*, followed by *in vivo* testing for their ability with the identified growth factors to support vascularization and neuronal differentiation and integration with the host. Our laboratory has recently developed a platform for testing layered, vascularized, multicell type neocortical tissue prototypes in the adult mouse neocortex, which we have initially validated with transplanted mouse rather than human cells and using a commercially available, nonclinically relevant scaffold.[15] Nevertheless, functional blood vessels readily form, as do neuronal connections with the host that lead to graft-derived neurons in the visual cortex acquiring responsiveness to visual stimuli.

Step 3: Rebuild neocortical tissue at the site of aspiration lesions in the adult cortex of preclinical animal models

What facilitates the task of engineering human fetal neocortical tissue is that protocols already exist for generating each major fetal neocortical cell type from human iPS cells. For example, precursor cells for excitatory and inhibitory neurons, astrocytes, oligodendrocytes, microglia, and vascular endothelial cells can all be generated and purified from human iPS cells. These precursors could then be reassembled in normal ratios, maturity states, and layered architecture embedded in layer appropriate extracellular niches [Figure 1].

Analyses of graft development and performance would be as in Step 1 and again include characterizing cell survival, mature cell type ratios, tissue organization, axonal and dendritic connections to and from the graft, and functional measures such as responsiveness to visual stimuli when in the visual cortex or ability to elicit movement when in the motor cortex. In addition, a possible experiment to demonstrate that the new tissue encodes part of a useful behavior to the host is to replace a functionally defined host area with new tissue designed so that it can be transiently silenced chemogenetically or optogenetically.

Step 4: Developing the means for progressive silencing and removal of old tissue

To reverse aging of the brain, progressively adding young tissue is not sufficient; the old tissue must also be progressively removed. As shown in humans of advanced age with slow-growing benign gliomas (described above), if cortical functions are being used while the tissue encoding them is progressively destroyed or silenced, these functions become seamlessly encoded elsewhere. The same idea would apply to intentional silencing of a functional area over time, but without the use

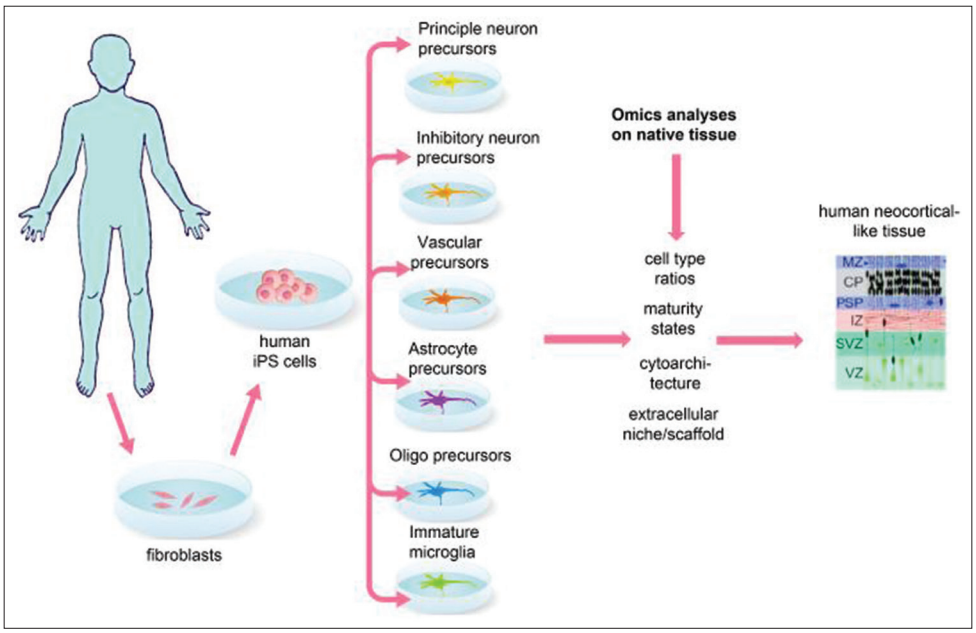

Figure 1: Broad outline of method that could be used for engineering fetal-like human neocortical tissue. MZ: Marginal zone; CP: Cortical plate; IZ: Intermediate zone; SVZ: Subventricular zone; VZ: Ventricular zone.

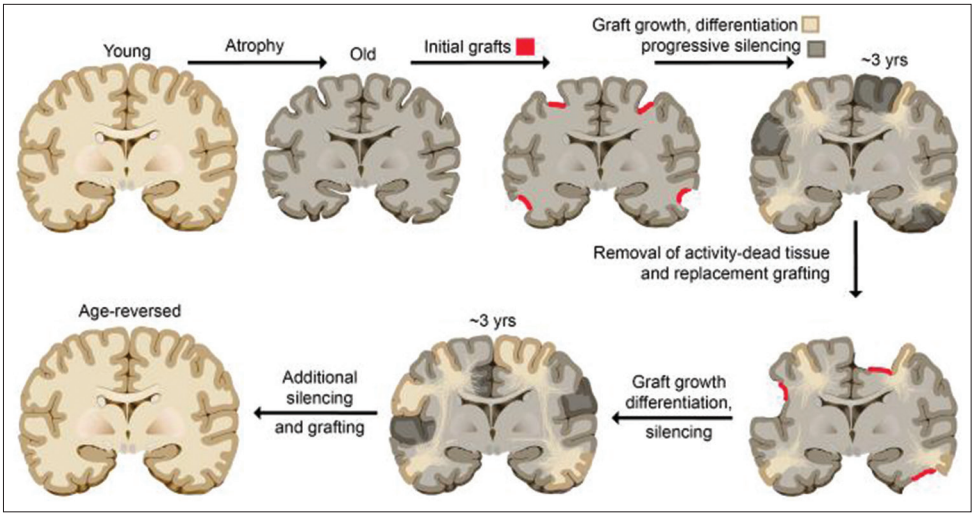

Figure 2: Cartoon of strategy used for replacing old brain tissue with new young brain tissue. Note that for reversing aging of the brain, similar approaches would need to be taken in conjunction for the one shown here for the neocortex.

of tumors. For example, progressive silencing from a pinpoint location outward could be achieved by infecting the tissue with one of several available red light-shifted opto-silencing channels and progressively increasing the diameter of the laser light used for silencing over time. Once an area of tissue is silenced, then it could be removed without loss of information or function to the individual (as with the resection of slow growing benign gliomas), creating space for new young tissue. These steps could in theory be repeated to revert the entire brain from old to young over the course of a couple of decades without interruption of function or discontinuity of self [Figure 2].

Step 5: Implementation in humans

Once engineered tissue is shown to encode useful information to its hosts in preclinical studies, then it will be ready to test in humans. The first clinical indication would not be brain aging, but instead insults such as stroke or trauma with local loss or degeneration of functional tissue. In this case, the information originally encoded in the tissue would be lost due to the suddenness of the injury, but new immature tissue would be expected by virtue of its extreme plasticity to help relearn the lost functions. Moving forward, damage to greater areas could be addressed, for instance in frontotemporal dementia, and finally, age-related degeneration using the more stepwise approach illustrated in Figure 2.

In sum, despite the massive amount of work needed to achieve engineered fetal-like brain tissue that is fit for progressive replacement in humans, the challenges, which are technical in nature, may not require as much innovation as empirical testing. Hence, brain age reversal could potentially be achieved sooner than we expect, depending on the effort allocated to the task at hand.

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