helping minimize pain in spinal surgery. Though opoid use was not significantly reduced, further adjustments to perioperative cocktails of medicines and interventions may achieve even more dramatic improvements in pain scores and outcomes. Hopefully, this can lead to lower narcotic use, faster mobilization, improved patient perceived outcome and shorter hospital stays.

JOHN H. CHI

CLARITY—A Clearer View of the Brain

Ramon y Cajal revolutionized biology by making visible what was previously invisible: individual brain cells and their interconnections. A recent Nature paper imparted the same excitement as Cajal's illuminating treatise **(Chung K, et al. Structural and molecular interrogation of intact biological systems. *Nature.* 2013;497(7449):332-337)**.[1] Kai Deisseroth's group overcomes major limitations of current techniques by inventing CLARITY, a transformative method that enables 3-dimensional structural and molecular analyses of topologically intact brains and other biological tissues.

The authors postulated that non-destructive removal of lipid membrane bilayers that make tissues impermeable to macromolecules and photons would enable whole organ imaging. They hypothesized that maintaining the tissue's structural scaffolding and integrity would best preserve an organ's intrinsic biological information. CLARITY converts intact tissues and organs into optically transparent, macromolecule-permeable structures, thus providing a natural anatomical unit that is conserved for molecular and structural analysis.

To maintain tissue morphology, the authors infused hydrogel monomers, formaldehyde, and thermally triggered initiators into tissues at 4°C. Formaldehyde caused tissue crosslinking and provided covalent linkage of the hydrogel monomers to cellular proteins, nucleic acids and small molecules. Then a tissue-hydrogel hybrid construct was created via 37°C incubation of infused tissues. This hybridization provided tissue support and chemical incorporation of biomolecules into the hydrogel mesh. Unbound lipids and other biomolecules were then rinsed away with a novel ionic detergent extraction that does not quench fluorescence, a common problem of currently used organic lipid extraction solvents. The authors also created a technique called electrophoretic tissue clearing (ETC) that uses charged ionic micelles to create a tissue active-transport system accelerating detergent micelle diffusion into tissues.

To demonstrate CLARITY technology, the authors completely processed a whole mouse brain in 8 days. The intact transparent brain was imaged in a refractive-index-specific solution matching the CLARITY hybrid, to prevent scattering from macromolecular heterogeneity of the protein and nucleic acid complexes. Therefore, whole mouse brain imaging at cellular resolutions was achieved even with single-photon microscopy (Figure). Researchers viewed fluorescence-labeled neurons in areas as deep as the thalamus, limited only by the microscope objective's working distance.

The authors demonstrated CLARITY's ability to provide molecular phenotyping in addition to structural information. Native antigens are preserved in the hydrogel-hybridization process. CLARITY processing resulted in far less protein loss compared to traditional methods. Immunohistochemistry on intact mouse brains was successful, and only moderate signal attenuation was observed in the deepest brain regions even after 2 weeks of incubation. Furthermore, small biomolecules such as GABA and messenger RNAs were also preserved. CLARITY-processed brains were able to withstand multiple rounds of elution without antigen degradation, thus demonstrating another advantage over traditional elution methods that cause antibody loss and fluorescence quenching.

Chung et al further demonstrated that CLARITY technology is applicable to archived human tissues. They used CLARITY to process postmortem human brains, and successfully performed immunohistological visualization and identification of neuronal projections. Formalin-fixed frontal lobe tissues from autism patients (stored for more than 6 years) were successfully stained with neuro-filament protein and myelin basic protein to trace individual axonal fibers. They further visualized the distribution of parvalbumin-labeled neurons and processes in these autistic brains, and discovered that many parvalbumin$^+$ interneuron connections formed 'ladder-like' abnormalities previously observed with mutant Down-syndrome cell-adhesion molecule (Dscam) or protocadherin proteins. Previously unknown, this link between abnormal parvalbumin$^+$ neuron connections and genes associated with autism-related disorders revealed through CLARITY molecular/functional analysis exemplifies the potential of this technique.

CLARITY technology has broad clinical implications. For example, CLARITY will allow researchers and clinicians to view normal neuronal networks and trace detailed changes associated with various disease processes. Large clinical banks of rare disease-associated tissues can be examined after CLARITY processing. Due to the stable hydrogel-tissue hybrid, these precious, rare samples will also remain available and reusable for many tests. CLARITY provides a novel method to preserve and examine intact brains and other tissues for structural and molecular relationships, and better define normal and diseased states of the intricate human brain.

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Neural Stem Cell-Mediated Enzyme/Prodrug Therapy for Glioma

Glioblastoma (GBM), the most common primary brain tumor in adults, portends a grim prognosis with median survival rates typically less than a year.[1] The aggressive, infiltrative nature of GBM precludes a surgical cure, and the blood-brain barrier has limited the efficacy of systemic chemotherapeutic interventions. Previous research has demonstrated the tumor-tropic behavior of neuronal stem cells (NSC), whereby these cells preferentially aggregate to glioma foci when implanted in the brain.[2] Thus, stem cells have become an attractive tumor-specific delivery device surmounting the limitations of surgery or chemotherapyin the treatment of high grade glioma.[3]

Recent research published in this month's issue of *Science Translational Medicine* by Aboodyet al, has exploited the tumor localizing characteristics of NSC and tested a novel enzyme/prodrug approach for the treatment of gliomas.[4] Utilizing a cytosine deaminase (CD) expressing-human NSC line, HB1.F3.CD, the group hypothesized that the stem cells would co-localize to tumor cells and convert a systemically administered prodrug, 5-fluorocytosine (5-FC) to the active, nucleic acid analog 5-fluorouracil (5-FU), thereby leading to cell death. Their results demonstrate a potential breakthrough in the treatment of this most challenging pathology.

To begin, the team created a stable human NSC line from HB1.F3 using a transgene for

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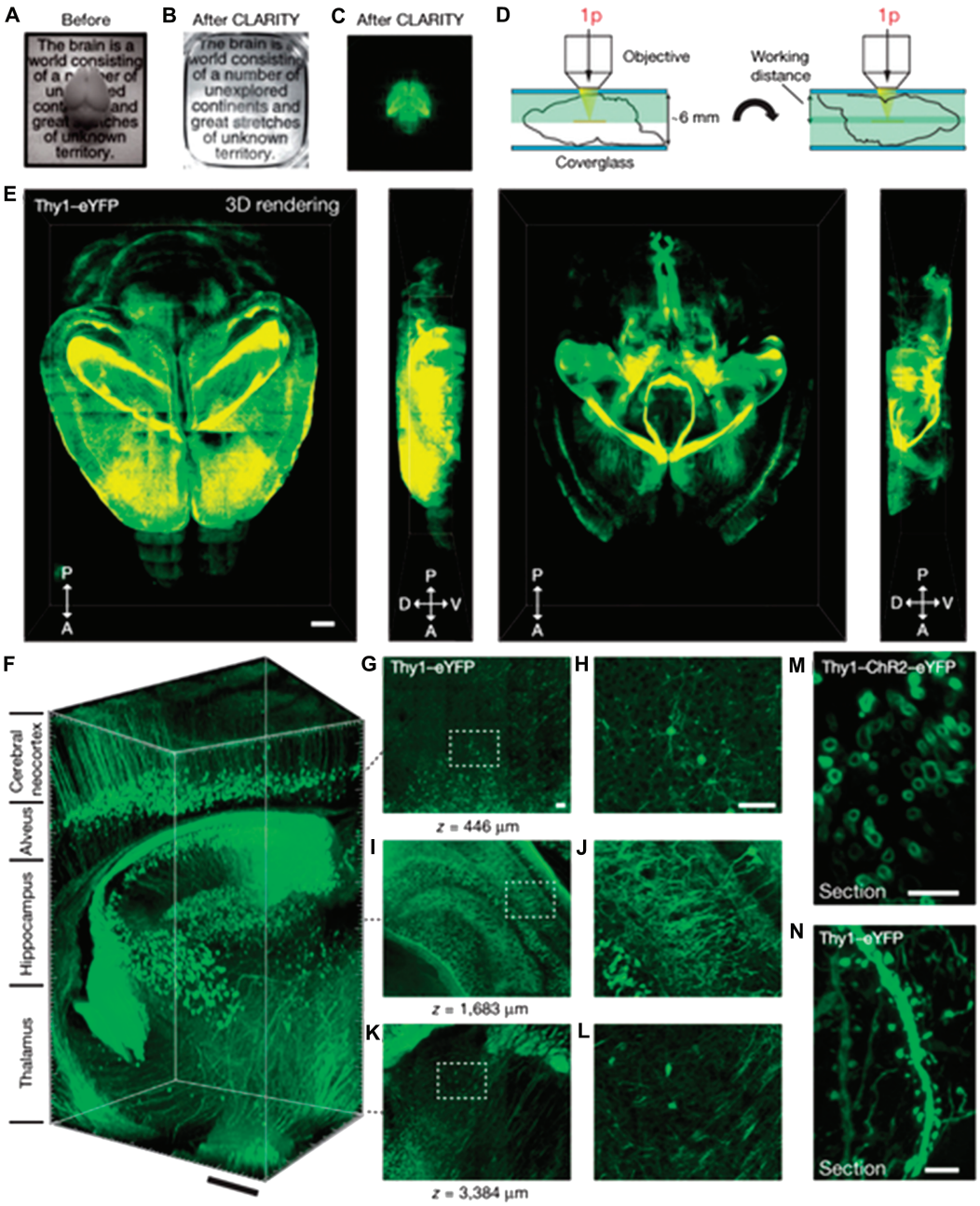

Figure. Intact Adult Mouse Brain. Imaging was performed in adult mouse brains (3 months old). **A**, *Cajal quote before CLARITY.* **B**, *Cajal quote after CLARITY: Thy1–eYFP line-H mouse brain after hydrogel–tissue hybridization, ETC and refractive-index matching (Methods).* **C**, *Fluorescence image of brain depicted in* **B**. **D**, *Dorsal aspect is imaged (single-photon (1p) microscopy), then brain is inverted and ventral aspect imaged.* **E**, *3-dimensional rendering of clarified brain imaged (×10 water-immersion objective; numerical aperture, 0.3; working distance, 3.6 mm). Left, dorsal half (stack size, 3,100 µm; step size, 20 µm). Right, ventral half (stack size, 3,400 µm; step size, 20 µm). Scale bar, 1 mm.* **F**, *Non-sectioned mouse brain tissue showing cortex, hippocampus and thalamus (×10 objective; stack size, 3,400 µm; step size, 2 µm). Scale bar, 400 µm.* **G–L** *Optical sections from* **F** *showing negligible resolution loss even at ~3,400-µm deep: z = 446 µm (***G**, **H***), z = 1,683 µm (***I**, **J***) and z = 3,384 µm (***K**, **L***).* **H**, **J** *and* **L**, *boxed regions in* **G**, **I** *and* **K**, *respectively. Scale bars, 100 µm.* **M**, *Cross-section of axons in clarified Thy1–channelrhodopsin2 (ChR2)–eYFP striatum: membrane-localized ChR2–eYFP (1-mm-thick coronal block; ×63 glycerol-immersion objective; numerical aperture, 1.3; working distance, 280 µm). Scale bar, 5 µm.* **N**, *Dendrites and spines of neurons in clarified Thy1–eYFP line-H cortex (1-mm-thick coronal block; ×63 glycerol objective). Scale bar, 5 µm. Reprinted by permission from Macmillan Publishers Ltd: Nature (Chung K, Wallace J, Kim S-Y, et al. Structural and molecular interrogation of intact biological systems.* Nature. *2013;497[7449]:332-337), Copyright 2013.*

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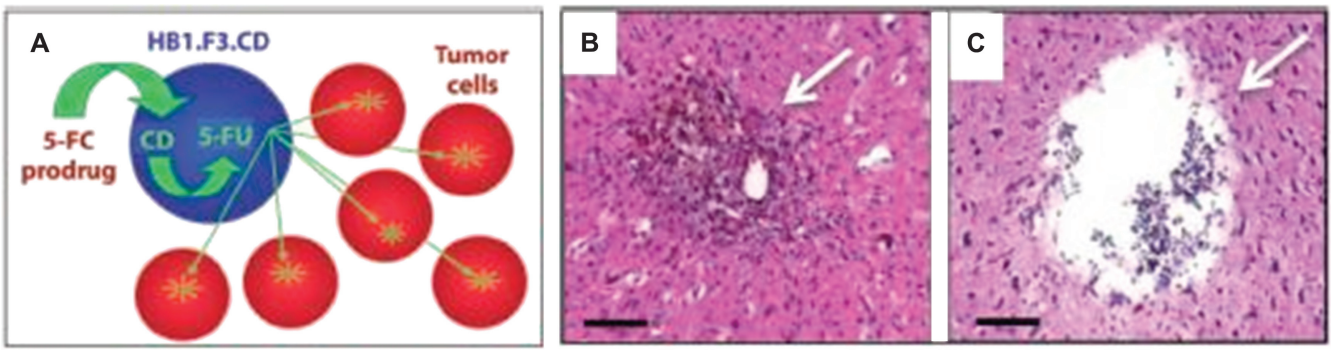

*Figure. Diagramof CD-expressing NSCs localized to tumor cells, and CD conversion of 5-FC to 5-FU, which readily diffuses out of the NSCs to selectively kill surrounding, dividing tumor cells. (**B** and **C**) H&E-stained brain tumor sections from U251 glioma-bearing mice that received HB1.F3.CD NSCs only (**B**) or HB1.F3.CD NSCs in combinationwith 5-FC (**C**). White arrows indicate tumor region.NSCs-neural stem cells. CD-cytosine deaminase. Modified from Aboody KS, Najbauer J, Metz MZ, et al. Neural stem cell -mediated enzyme/prodrug therapy for glioma: preclinical studies.* Sci Transl Med. *2013;5(184):184ra59. Reprinted with permission from AAAS.*

CD, then expanded this colony for all future experiments. They subsequently assessed the immunogenicity of the HB1.F3.CD NSC line and determined that they expressed HLA class I antigens but not class II antigens. Therefore, their cell line was unlikely to induce an immunogenic response, a caveat that has plagued other attempts in utilizing this approach. The investigators then evaluated the tropism of their NSC in a murine model under different simulated conditions that human patients experience. Specifically, they determined that when implanted into mouse brains, their NSC co-localized to glioma cells, despite the mouse brains having been previously irradiated or treated with corticosteroids. Additionally, their NSC were able to migrate to the tumor sites when injected adjacent to the tumor or in the opposite hemisphere, regardless of prior radiation or corticosteroid therapy.

The safety profile and the tumorgenicity of their NSC line was then evaluated. Using a murine model injected intracranially with HB1.F3.CD, they wanted to determine if their NSC's were independently tumorogenic and whether there was a maximum tolerated dose of NSC HB1.F3.CD. At both 4 and 12 weeks post NSC implantation, the mice were euthanized and their organs harvested. All organ systems failed to demonstrate any evidence of human NSC, and no tumors were discovered regardless of the amount of NSC injected.

Having demonstrated tumor tropism and safety, the team analyzed the *in vivo* efficacy of their co-localizing NSC to preferentially kill the surrounding, dividing tumor cells. Mice were injected with glioma cells followed by the HB1.F3.CD NSC's 7 days later. Peritoneal injections of 5-FC were performed on days 11 through 17, and the brains were evaluated. Typical response to treatment demonstrated extensive areas of tumor necrosis surrounded by areas of residual viable tumor (Figure). The therapeutic efficacy was then determined by measuring the viable brain tumor after 1 round of treatment with 5-FC in 24 mice. Their volumetric analysis demonstrated that mice receiving the NSC/5-FC combination therapy had a significantly smaller tumor burden than those mice that did not receive combination therapy.

In conclusion, the researchers provide convincing evidence that an NSC-prodrug delivery system to combat glioblastoma is safe, effective and feasible in a murine model. These investigators have provided the necessary pilot data to translate these experimental results from the benchtop to the bedside in the first human clinical trial (NCT01172964) using a NSC-prodrug delivery system in patients with recurrent high-grade glioma. Future research in this area will hopefully offer new therapeutic options to treat this challenging disease.

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Pericytes as a Therapeutic Target in Scar Formation After Spinal Cord Injury

As is too often the case with neurological diseases, we have limited understanding of the pathophysiologic mechanisms of spinal cord injury (SCI). This paucity of knowledge has, in turn, greatly impeded the ability of physicians, surgeons, and scientists to develop novel treatment modalities aimed at restoration of neurologic function in patients suffering these life altering injuries. In fact, severe or complete SCI still carries a rather grim prognosis with respect to neurologic recovery due to the limited ability of neurons in the central nervous system (CNS) to regenerate.

The development of novel therapies for disease processes relies critically on the thorough understanding of the disease process itself. The characteristic "CNS scar" that forms at the location of neurologic injury is well-recognized as a key obstacle to neuronal plasticity and regeneration, and ultimately, recovery of neurologic function. However, the exact details of CNS scar formation are not thoroughly characterized. Many have postulated that astrocytes are the key cell-type involved in initiating fibroblast-induced CNS scar formation and serve as a potentially important therapeutic target. In a recent article, Goritz et al (**Goritz C, Dias D, Tomilin N, Barbacid M, Shupliakov O, Frisen J. A pericyte origin of spinal cord scar tissue. *Science*. 2011;333:238-242**)

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