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iDISCO Inferno: Mapping Connectivity Changes Following TBI

Brain-Wide Reconstruction of Inhibitory Circuits After Traumatic Brain Injury

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Despite the fundamental importance of understanding the brain's wiring diagram, our knowledge of how neuronal connectivity is rewired by traumatic brain injury remains remarkably incomplete. Here we use cellular resolution whole-brain imaging to generate brain-wide maps of the input to inhibitory neurons in a mouse model of traumatic brain injury. We find that somatostatin interneurons are converted into hyperconnected hubs in multiple brain regions, with rich local network connections but diminished long-range inputs, even at areas not directly damaged. The loss of long-range input does not correlate with cell loss in distant brain regions. Interneurons transplanted into the injury site receive orthotopic local and long-range input, suggesting the machinery for establishing distant connections remains intact even after a severe injury. Our results uncover a potential strategy to sustain and optimize inhibition after traumatic brain injury that involves spatial reorganization of the direct inputs to inhibitory neurons across the brain.

Commentary

Traumatic brain injury (TBI) induces a cascade of cell death and circuit reorganization. Consequences of TBI include cognitive impairments in memory and executive functioning, and post-traumatic epilepsy. Interneurons have been shown to be particularly vulnerable to cell death after injury[1,2] and to have altered patterns of local connectivity.[3,4] Interneurons play an important role in shaping learning and memory,[5] which has been particularly well-studied in local circuits. However, comparatively less is known about changes in long-range connectivity following injury.

The study by Frankowski et al[6] uses cutting edge techniques to elegantly map the changes in long-range connectivity to somatostatin (SST) interneurons of the dentate gyrus in the controlled cortical impact (CCI) model of TBI. Somatostatin interneurons are one of the largest classes of interneurons in the cortex and influence pyramidal cell spiking via dendritic inhibition.[7] To label monosynaptic inputs to the SST interneurons, they used a viral strategy combining a Cre-mediated "helper" virus and a retrograde monosynaptic rabies tracer virus to label both the SST starter cells and input cells. In order to visualize the long-range connections, they performed whole-brain imaging after brain clearing, with optimization to allow for deep tissue immunolabeling. The resulting image volumes simultaneously contain salient information about injury-induced changes in neural circuitry and are aesthetically pleasing (e.g., see Supplemental Movie 3 in their paper).[6]

After TBI, SST neurons receive proportionally more local connections, particularly from CA1 pyramidal neurons. Long-range connections are lost, including from the medial entorhinal cortex (ENTm) and the nucleus of the diagonal band (NDB), 2 major sites of distant input cells. Cholinergic neurons were identified as the major input type lost from the NDB in the basal forebrain. Despite the loss of synaptic inputs and likely axonal damage, there was no reduction in the number of cholinergic neurons in the NDB or in cholinergic projections in the hippocampus. Similarly, no difference was observed in the number of Reelin + excitatory neurons of the ENTm, which project to the dentate gyrus. Surprisingly, the authors also observed a difference in SST + interneuron connectivity in the prefrontal cortex (PFC) far from the site of injury. In this region, they also find overall greater local and reduced long-range connectivity. These findings are perhaps reflective of the effect of damage to the fibers of passage projecting to the PFC. Alternatively, these changes in PFC connectivity may occur secondarily from the altered local activity of the hippocampus or from generalized seizure activity.

The authors next sought to map the connectivity of transplanted interneurons, which have shown therapeutic potential after TBI.[8] Amazingly, transplanted interneurons were shown to receive both local and long-range inputs. Notably, although transplanted interneurons receive orthotopic inputs, which suggests that the mechanisms for forming long-range connections are intact, the spatial distribution of their inputs is quantitively similar

to native, injured SST interneurons, with higher local connectivity and reduced long-range inputs. These circuit changes may be compensatory, perhaps increasing the local inhibitory circuits to minimize hyperexcitability and seizures. The transplanted interneurons may supplement the pool of available local inhibition for quelling seizures. Since the transplants adopt the same abnormal connectivity pattern of the native neurons, one might expect some deficit to remain. However, prior work has shown that such transplants reduce seizures and restore behavioral and memory deficits after TBI,[8] suggesting that either the transplants establish a "novel alternative solution" to hippocampal inhibitory circuitry or perhaps there are subtle, uncharacterized cognitive deficits. As not all animals develop epilepsy after CCI,[9,10] it would be interesting to determine if there are differences in the connectivity patterns observed between seizing and nonseizing TBI animals. Similarly, it would be interesting to see whether transplanted interneurons integrate differently in seizing versus nonseizing TBI animals.

Mapping cellular resolution brain-wide connectivity is a herculean task. This study offers several new insights into how patterns of connectivity change after injury. In both the injured and uninjured condition, the authors highlight that these neurons exhibit small-world connectivity, characterized by high numbers of local connections and few long-range ones.[11] However, small-world networks have predominantly been defined in terms of excitatory connections of varying weights. Interpreting the functional impact of changes to *inhibitory* circuitry in a highly heterogenous network is not straightforward. For example, the observed TBI-induced loss of long-range excitatory projections onto SST interneurons represents a loss of feedforward inhibition, which presumably functionally unmasks remaining long-range excitatory connections. The net effect of such changes may actually be to increase long-distance excitation to the hippocampus. Pairing the anatomical characterization introduced in the highlighted work with in vivo physiological measurements of connectivity will further elucidate the functional impact of observed changes in interneuron connectivity.

Although the authors indicate that the injured SST neurons are converted to "hyperconnected hubs," it is interesting to note that the number of input neurons per mapped SST cell was approximately constant in the uninjured and TBI groups. What changes is not the number of input cells, but the spatial distributions of the inputs to SST neurons (i.e., they become hyper-*locally* connected). Understanding the factors driving these changes will be key to discovering mechanisms of posttraumatic epileptogenesis. Increased network activity has been shown to drive a homeostatic increase in the excitatory drive onto interneurons.[12] One possible explanation for the changes observed in the highlighted work is that acute posttraumatic seizures (or nonconvulsive network hyperactivity) similarly drive the formation of new excitatory inputs, primarily from nearby CA1 pyramidal cells. This increased inhibitory drive would likely decrease the acute hyperactivity, but may, in turn, produce pathological rhythms that alter connectivity in distant, noninjured brain regions, such as the changes observed in the PFC. While prior literature identified SST interneurons as a logical place to start for this study, it is likely that there are TBI-induced changes to the circuitry of many cell types. Applying similar mapping strategies to different cell types will reveal the specificity of the observed changes to SST interneurons and inform a line of research aimed at distinguishing network changes that are compensatory and purely restorative from those that are epileptogenic. Thus, in addition to characterizing a novel TBI-induced rewiring of SST interneurons, the highlighted work by Frankowski et al provides a framework for mapping brain-wide anatomical connectivity changes associated with TBI. Ultimately, combining this approach with physiology and seizure semiology will continue to build our understanding of how network connectivity is changed after injury and how such changes relate to epileptogenesis.

Lauren A. Lau, PhD & Kyle P. Lillis, PhD
Department of Neurology, Massachusetts General Hospital, Harvard Medical School

ORCID iD

Kyle P. Lillis. https://orcid.org/0000-0003-0219-8113

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