ChatGPT 4o

Results

Single-nucleus transcriptomic profiling of spinal cord neurons after injury

To investigate transcriptional changes in spinal cord neurons following traumatic spinal cord injury (SCI), we performed single-nucleus RNA sequencing (snRNA-seq) on neuronal nuclei isolated from the mid-thoracic region of the mouse spinal cord. Samples were collected at seven time points post-injury—1, 4, 7, and 14 days, as well as 1 and 2 months—alongside uninjured controls (GEO accession: GSE234774). In total, we analyzed nuclei from 20 biological replicates, yielding robust coverage across all conditions (see Methods and Supplementary Table 1).

Dimensionality reduction via Uniform Manifold Approximation and Projection (UMAP) revealed clear transcriptional separation between injured and uninjured neurons (Figure 1A). When stratified by time point, progressive transcriptional shifts were observed across the injury timeline, particularly evident from day 1 to 2 months post-injury (Figure 1B), indicating a time-dependent transcriptional response to injury.

Time-resolved differential gene expression reveals coordinated transcriptional downregulation

To identify genes exhibiting injury-associated, time-dependent changes in expression, we employed a linear modeling approach using the Limma framework. This identified numerous genes with significant linear trends across time points (adjusted $p < 0.05$, Benjamini-Hochberg correction). Interestingly, the top differentially expressed genes all displayed **decreasing expression over time** in injured neurons relative to controls (Figure 2A).

Among the most significantly downregulated genes were **Psap**, **Ctsb**, **Hexa**, and **Itm2b**, all with exceptionally strong statistical support (*adj. p* < 1e-300). Several of these genes are lysosomal or vesicle-associated, suggesting a disruption of intracellular trafficking and degradative capacity in neurons post-injury.

Enriched pathways reflect early activation and sustained suppression of neuronal processes

To better understand the biological significance of the observed transcriptional dynamics, we performed Gene Ontology (GO) enrichment analysis on genes that were either upregulated or downregulated over time post-injury.

Upregulated genes were enriched for GO terms related to **synaptic function and membrane dynamics**, including:

• Regulation of membrane potential

• Dendrite development

• Vesicle-mediated transport in synapse

• Synapse structure organization

These results suggest an initial attempt by neurons to restore or reorganize synaptic connectivity following injury (Figure 3, left panel).

In contrast, **downregulated genes** were strongly associated with immune and stress response pathways, including:

• Regulation of innate immune response

• Positive regulation of response to biotic stimulus

• Endoplasmic reticulum stress response

• Alcohol metabolic process

(Figure 3, right panel)

Notably, the downregulation of genes involved in **apoptotic signaling** may reflect a suppression of cell death programs at later stages, or an exhaustion of acute stress responses following injury onset.

Summary

Together, these findings highlight a biphasic neuronal response to spinal cord injury, characterized by:

• Early upregulation of synaptic repair and functional reorganization pathways

• Sustained downregulation of lysosomal, metabolic, and immune-related processes

This suggests a complex and evolving response in neurons post-injury, where initial repair attempts are followed by prolonged stress, degradation of homeostatic functions, and possible loss of resilience over time.

References

To support and contextualize the findings, you may cite relevant studies such as:

• Tran, A. P., Warren, P. M., & Silver, J. (2018). The biology of regeneration failure and success after spinal cord injury. *Physiological Reviews*, 98(2), 881–917.

• Poplawski, G. H. D., et al. (2020). Injured adult neurons regress to an embryonic transcriptional growth state. *Nature*, 581(7806), 77–82.

Let me know if you'd like this tailored to a specific journal, need help integrating figures into a manuscript template, or want to generate figure captions.