PRIZE ESSAY

FINALIST

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Michael Skinnider received his undergraduate degree from McMaster University and an MD/PhD from the University of British Columbia, during which time he was also a visiting PhD student at the École Polytechnique Fédérale de Lausanne. He then started his laboratory in the Lewis-Sigler Institute for Integrative Genomics and the Ludwig Institute for Cancer Research at Princeton University. His research focuses on the application of machine learning to problems in biology, chemistry, and medicine.

www.science.org/doi/10.1126/science.adk3912

eppendorf & Science PRIZE FOR NEURO BIOLOGY

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NEUROSCIENCE

From single cells to neural circuits

Neural circuits are mapped in high throughput with single-cell genomics

By **Michael A. Skinnider**[1,2]

The human brain is composed of billions of neurons, wired together into neural circuits by trillions of synapses. Deciphering the organization of these neural circuits, and how they allow for the processing of information and the execution of complex behaviors, is a fundamental goal of neuroscience. Historically, however, dissecting neural circuits has been a low-throughput endeavor. Neuroscientists had to carefully synthesize existing knowledge to formulate hypotheses about the roles of specific neurons and then deploy painstaking experiments to test those hypotheses (*1*).

Over the past two decades, a series of technological advances has markedly accelerated the pace at which neural circuits can be dissected. Among the most exciting of these advances has been the advent of single-cell transcriptomics. Single-nucleus RNA sequencing (snRNA-seq) can measure the expression of thousands of genes across tens of thousands of neurons in a single experiment. This technology has opened an unprecedented window into neuronal diversity in the brain and has revealed hundreds of molecularly distinct neuronal subtypes (*2*, *3*). But snRNA-seq produces fundamentally static maps of gene expression. Linking neuronal subpopulations defined by single-cell transcriptomics to specific neural circuits—e.g., those activated by stimuli such as hunger or fear—has remained challenging.

I came face-to-face with this challenge in my efforts to understand the neural circuits engaged by epidural electrical stimulation (EES). EES, which involves delivering bursts of electricity to the lumbar spinal cord using an implantable device, has been shown to instantly restore walking in patients paralyzed by spinal cord injuries (*4*–*7*). Unexpectedly, EES was also found to mediate longer-term recovery, to the point that several patients eventually recovered the ability to walk even after the EES device was disabled. However, the neural circuits underlying this remarkable recovery remained enigmatic.

My colleagues at the École Polytechnique Fédérale de Lausanne and I sought to identify the neurons that could restore walking after paralysis (*8*). In the absence of a specific hypothesis about their identity, we surmised that we would need to simultaneously measure the molecular responses to EES of every neuronal subpopulation in the spinal cord. Single-cell genomics provided the ideal platform to achieve this goal. Using snRNA-seq, we measured gene expression in 20,990 spinal cord neurons from mice subjected to various modes of EES. However, leveraging these data to identify the specific neuronal subpopulations activated by EES proved more challenging than anticipated. Classical markers of neuronal activation, such as the immediate early gene c-*fos*, were expressed so lowly that they were almost indistinguishable from noise.

Clearly, a new approach was needed. I reasoned that neuronal responses to a given stimulus are not limited to the activation of immediate early genes and sought instead to develop a method that could take the entire neuronal transcriptome into account. My key hypothesis was that neurons that undergo a profound transcriptional response to EES should become separated from their unstimulated counterparts within the multidimensional space of gene expression. Aware of the limitations of widely used measures of separability in single-cell data (*9*), I saw an opportunity to overcome these limitations using machine learning. I posited that a machine-learning model could be trained to predict whether a given cell came from the spinal cord of a mouse that received EES or from that of a control mouse. Then, knowing which mouse each cell came from, we could calculate the accuracy of these predictions. For neurons that were strongly activated by EES, I thought that the model should readily learn to predict whether a particular cell came from a

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stimulated or unstimulated spinal cord. By contrast, for neurons not engaged by the therapy, the model's guesses should be no better than random chance.

I implemented these ideas in a method that I named Augur (*10*). Initially, I tested Augur with simulated single-cell data and was excited to find that Augur correctly prioritized the activated cell types in every simulation. Moreover, in published single-cell data, I found that Augur was able to correctly recapitulate well-studied neural circuits. In the visual cortex, for example, Augur accurately identified the neural circuits activated in response to light.

The ultimate test of Augur, however, would be its ability to help identify the neurons activated by EES. Remarkably, when Augur was applied to our snRNA-seq dataset, it prioritized the same subpopulation of visual system homeobox 2 (*Vsx2*)–expressing ventral excitatory interneurons in response to every mode of EES. This observation was further strengthened by spatial transcriptomics data from the same mice. I extended the logic underlying Augur into the setting of spatial transcriptomics and captured this new spatial logic in a second method that I named Magellan. I found that Magellan circumscribed the response to EES within the intermediate laminas of the spinal cord—exactly where the interneurons that Augur prioritized reside.

To elucidate the functional and anatomical properties of the prioritized neurons, we leveraged whole brain–spinal cord imaging of injured tissues, population-specific neuronal tracing, neuron-specific cellular recordings, cell ablation, chemogenetics, and ultimately optogenetics. These experiments unequivocally demonstrated that a single neuron subpopulation is both necessary and sufficient for walking after paralysis. We had indeed found the neurons that restore walking after paralysis.

In our hands, Augur and Magellan have been transformative tools to dissect neural circuits in the spinal cord and understand how they can be therapeutically targeted after spinal cord injuries. For example, we have now applied Augur and Magellan to discover a subpopulation of neurons that leads to loss of blood pressure control after spinal cord injury and another subpopulation that can be regrown through an anatomically complete injury to reestablish communication between the brain and spinal cord (*11*). Augur and Magellan have also helped in the dissection of cellular responses to perturbations across the central nervous system more broadly, including those of glial, vascular, and peripheral immune cells (*12*). Together, these methods provide a framework to tackle a fundamental challenge in neuroscience using unbiased single-cell technologies, and my hope is that they will accelerate the pace at which the neural circuits underlying any arbitrary behavior can be mapped. ■

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10.1126/science.adk3912

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Science **382** (6670), . DOI: 10.1126/science.adk3912