Single-cell Genomics: A Quantum Leap toward Understanding Complexity?

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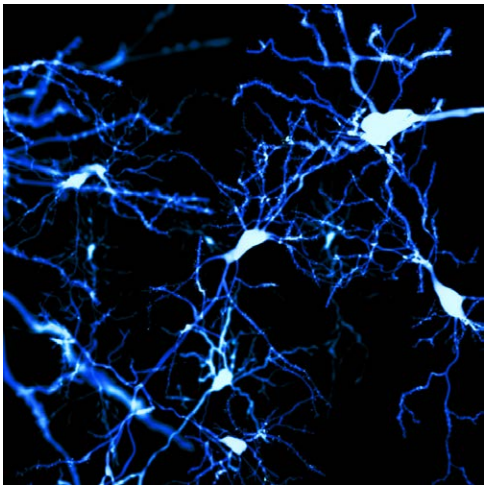
Complexity is an inherent property of biologic systems. Dynamic and highly orchestrated interactions between a myriad of intra- and intercellular pathways of multiple cell types determine context-dependent functional outcomes in body organs and, ultimately, in the whole organism. This well-recognized biologic complexity is in stark contrast to the understandably reductionist approach usually applied in experimental research. One unsurprising consequence of this paradox is the often-limited translational relevance of basic mechanistic experimental observations. Recent progress in single-cell genomics offers attractive alternatives to better approach complexity. Indeed, this technology opens the possibility for simultaneous genetic and molecular profiling of a large number of individual cells in a complex environment and can also give us some information on the mechanisms of interactions between various cell types.[1]



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In this issue of the Journal, Song *et al.* used a single-cell genomic approach to study the long-term effects of early-life sevoflurane exposure in the rodent hippocampus.[2] The authors used a well-established experimental paradigm in which repeated exposure of neonatal mice to 3% sevoflurane on postnatal days 6, 8, and 10 induces lasting memory impairment in these animals. One month later, on postnatal day 37, they performed single-nucleus RNA sequencing of the hippocampus to study exposure effects on transcriptional profiling *in vivo* at the single-cell level in tens of thousands of cells simultaneously. These investigations revealed sex-specific distribution of hippocampal cell types in control, nonanesthetized animals along with cell-type– and sex-specific effects of sevoflurane exposure on distinct neuronal and nonneuronal hippocampal cell populations and cell-signaling pathways. Despite the uncertain translational relevance of these observations, the enormous amount of data stemming from this work provides us with some new and important fundamental insights into the complexity of anesthesia effects on the developing brain. Most importantly, the analytic approach used by the authors provides an appealing alternative to investigate complexity in experimental research on anesthesia mechanisms of action and beyond. In fact, single-cell/nuclei transcriptomic experimentation is now considered the state-of-the-art approach to study cell-type compositions and functions within highly organized tissues.[3] The comprehensive genomic profiling at the single-cell level leads to a more precise definition of cell identity and function compared to traditional approaches based on predefined molecular markers.[1] A related advantage is that potential effects and mechanisms can be explored at the single-cell level within a complex environment. This is a huge qualitative leap forward from the so-called "bulk assays" in which biochemical or genetic analyses are usually conducted on tissue samples containing a heterogenous mixture of cells and, therefore, result only in "mixture averages" instead of functional cell-type–specific data.

What are the take-home messages of the manuscript by Song *et al.*? There are many since the manuscript contains a tremendous amount of interesting data extending over a set of eight highly complex main figures together with a

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This editorial accompanies the article on p. 477.

Accepted for publication February 22, 2023.

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 Anesthesiology 2023; 138:460–1. DOI: 10.1097/ALN.0000000000004547

very extensive supplemental content. Among them, probably most important concerns the role of sex as a biologic variable. Data provided by the authors in this context go well beyond the framework of developmental anesthesia neurotoxicity. Indeed, comparative cell cluster analysis of more than 20,000 cell nuclei from both male and female mice, based on canonical cell-type marker genes for major hippocampal neuronal and nonneuronal cell types, revealed sex-specific differences in hippocampal functional cell-type diversity in native, nonexposed animals. In addition, several-fold differences were noted in the proportion of some major cell types, including CA1 and dentate neurons as well as oligodendrocytes, between samples obtained from male and female mice. These provocative data on sex-specific hippocampal cell-type diversity are new and of potential fundamental importance. They should be confirmed by further investigations since they may provide functional explanations underlying sex-specific differences in hippocampal synaptic plasticity and functional connectivity under both physiologic and pathologic conditions.[4,5] By highlighting the cell-type– and sex-specific effects of early-life anesthesia exposure on the developing brain, the work from Song *et al.* also brings new knowledge and hypotheses to developmental anesthesia neurotoxicity research. Indeed, previous observations in this field suggest that, despite a comparable amount of apoptosis in both sexes, male animals appeared to be more vulnerable to anesthesia in terms of cognitive outcome.[6] The current study, showing that sevoflurane exposure mostly altered the differentiation patterns of neuronal subpopulations in male animals, may provide mechanistic explanations to these earlier findings. Exploring whether and how and which of the numerous sevoflurane-induced changes in cell differentiation and signaling relate to functional outcome would deepen our mechanistic understanding of anesthesia effects on the immature brain. Despite the uncertain clinical relevance of developmental anesthesia neurotoxicity, this fundamental knowledge would prove to be helpful in improving our consideration of general anesthesia as a tool to modulate neuronal plasticity.

Should there be an important place for single cell genomics in the armamentarium of anesthesia research and, from a broader perspective, of perioperative care? Through revealing tremendous complexity, the work from Song *et al.* strongly argues in favor of this possibility. Embracing complexity is a *sine qua non* to understand emergent properties that are critical attributes of biologic systems and stem from the interactions between the different parts of any given system. In fact, studying the individual parts of a complex biologic system without understanding the nature of interactions between these entities does not allow us to predict system behavior or function. For example, anesthesia-induced loss of consciousness and the related changes in brain homeostasis require a coordinated action of multiple cell types. Deciphering the molecular and cellular properties of this complex and simultaneous interplay between these highly heterogenous cell populations is essential to better understand the puzzle of anesthesia mechanisms of actions. While not the holy grail, single-cell genomics provide us with appealing tools to tackle complexity. These technologies are rapidly gaining importance in several areas of research extending from plant science to human biology.[3] The continuous refinement of sampling and analytic techniques in the field now enables accurate and rapid identification of millions of cells in tissues along with detailed cell-specific information about gene and protein expression as well as signaling pathways involved in cell-to-cell communication. This recent technological revolution in single-cell genomics triggered substantial advances in our understanding of physiology and disease in several fields of medicine including, although not exclusively, immunology, cancer, and neuroscience. Applying single-cell genomics to anesthesiology and perioperative medicine may also bring similar advantages to our own specialty and, ultimately, would lead to better and more individualized patient care.

Competing Interests

Dr. Vutskits is an Editor for Anesthesiology.

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