PRIZE ESSAY

CATEGORY WINNER: CELL AND MOLECULAR BIOLOGY

William E. Allen

William E. Allen received his undergraduate degree from Brown University in 2012, M.Phil. in Computational Biology from the University of Cambridge in 2013, and Ph.D. in Neurosciences from Stanford University in 2019. At Stanford, he worked to develop new tools for the large-scale characterization of neural circuit structure and function, which he applied to understand the neural basis of thirst. After completing his Ph.D., William started as an independent Junior Fellow in the Society of Fellows at Harvard University, where he is developing and applying new approaches to map mammalian brain function and dysfunction over an animal's life span.

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CELL AND MOLECULAR BIOLOGY

Brain mapping, from molecules to networks

Bridging multiple levels of brain function reveals the neural basis of thirst motivation

By **William E. Allen**

Charting what the pioneering neuroanatomist Santiago Ramón y Cajal called the "impenetrable jungle" of the brain (*1*) presents one of biology's greatest challenges. How do billions of neurons, wired through trillions of connections, work together to produce cognition and behavior?

Like an orchestra, wherein many instruments played simultaneously produce a sound greater than the sum of its parts, thought and behavior emerge from communication between ensembles of molecularly distinct neurons distributed throughout vast neural circuits. Although we know much about the properties of individual genes, cells, and circuits (see the figure, panel A), a vast gap lies between the function of each brain component and an animal's behavior. Bridging this gap has proven technically and conceptually difficult.

Inspired by the fact that the development of high-throughput DNA sequencing led geneticists to shift focus from individual genes to the entire genome, I wanted to develop approaches that could simultaneously link multiple levels of the brain, from molecules to neurons to brain-wide neural networks. My goal was to capture a global perspective while maintaining the high resolution and specificity necessary to understand the function of individual components at each level. This new viewpoint, I hoped, would reveal how the collective properties of the brain's building blocks give rise to behavior.

MAPPING MOTIVATED BEHAVIOR FROM GENES TO CIRCUITS

During my doctoral studies at Stanford University with Karl Deisseroth and Liqun Luo, I developed new methods to map the architecture and activity of mammalian neural circuits. I applied these approaches to understand the neural basis of thirst, a fundamental regulator of behavior (*2*).

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Need-based motivational drives, such as hunger and thirst, direct animals to satisfy specific physiological imperatives important for survival (*3*). Despite decades of research, at the beginning of my studies it was unclear how the activity of neurons that sense these needs causes an animal to engage in specific motivated behaviors (e.g., eating or drinking) to maintain homeostasis (*3*). Thirst, a relatively simple yet important drive, thus seemed the perfect model system for investigating multiple levels in the brain.

I first traced thirst motivational drive from cellular gene expression to a circuit mechanism. Using a new version of targeted recombination in active populations (TRAP2), a tool to genetically label neurons according to their activity, I found that neurons in the median preoptic nucleus (MnPO) of the hypothalamus became activated in thirsty mice (*4*) (see the figure, panel C). Single-cell RNA sequencing revealed that these neurons formed a single molecularly defined cell type.

Artificial activation of these neurons caused mice to drink water within seconds, whereas their inhibition prevented mice from drinking, which suggested that these MnPO neurons were master regulators of thirst. Drinking water also gradually reduced the activity of these neurons. Finally, activation of these neurons was aversive. Together, these results suggested a surprising "drive reduction" model of thirst motivation: Genetically hard-wired thirst neurons become active when mice need hydration, which causes mice to drink water.

This ability to ascribe specific functional relevance to genetically defined neurons inspired me to develop new techniques to map cells within their native tissue architecture in even greater molecular detail. To this end, I co-developed STARmap, an approach for highly multiplexed in situ RNA sequencing to measure the expression of hundreds of genes simultaneously within a brain section at the level of single mRNA molecules (*5*) (see the figure, panel B). In combination with genetic markers of activity, this technique powerfully describes the molecular identity of behaviorally activated neurons and their neighbors at single-cell resolution.

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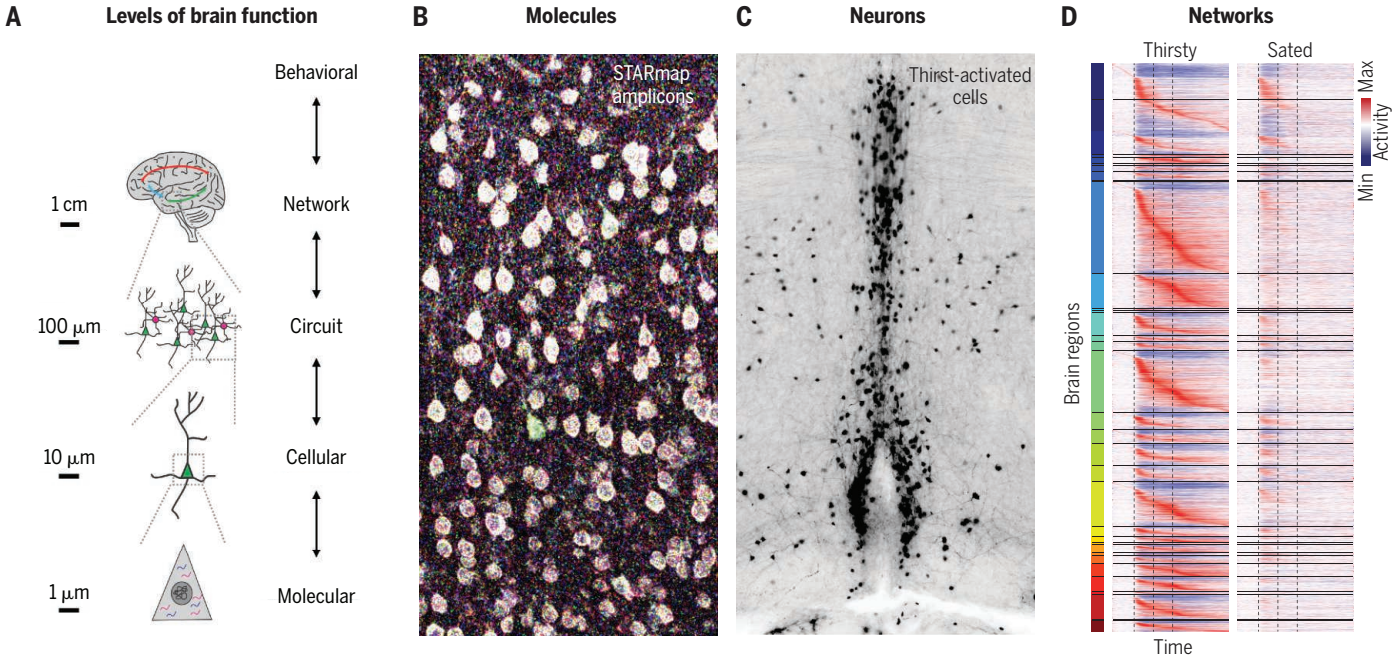
New large-scale, high-resolution approaches to bridging multiple levels of brain function



A new approach to brain function mapping. (A) An illustration of the levels of brain function and how they are interlinked. (B to D) New approaches to bridging levels: (B) STARmap amplicons barcoding 1020 RNA species simultaneously with single-molecule resolution in the mouse visual cortex. (C) Genetic labeling of neurons according to activity reveals thirst neurons in the median preoptic nucleus of the hypothalamus, used to identify the motivational mechanism of thirst drive. (D) Brain-wide activity map of the response of thousands of neurons across dozens of brain regions to a water-predicting sensory cue, in thirsty or sated mice, reveals widespread broadcasting of thirst state.

LINKING INDIVIDUAL NEURONS TO BRAIN-WIDE NETWORKS

Despite these insights, a question remained: How do thirst-sensitive neurons deep in the brain coordinate activity in distributed circuits spanning sensory perception, cognition, and motor output to produce motivated behavior? I found that MnPO thirst neurons projected to many brain regions potentially serving different behavioral roles (*4*), but the gap between individual neurons and brain-wide networks was daunting.

Earlier in graduate school, I had developed several new microscopy techniques to characterize brain-wide (*6*) or neocortex-wide (*7*) activity, which revealed that global neural activity was present during even simple motivated behaviors. However, because of the mammalian brain's opacity, these approaches were limited in their ability to record fast neural activity throughout the brain at the scale required to understand thirst motivation.

Fortunately, however, developments in microelectronics enabled me to construct global maps of neuronal activity with microsecond-level temporal resolution. Using advanced "Neuropixels" probes (*8*), thin silicon needles that can be acutely inserted into the brain to record the electrical signals of hundreds of neurons simultaneously, I developed an experimental approach to record the activity of huge neuronal ensembles across the brain and reconstruct the anatomical location of each recorded cell (*9*).

Applying this technique, I mapped the brain-wide flow of activity through ~24,000 single neurons during thirst-motivated behavior (*9*) (see the figure, panel D). My experiments revealed that this simple behavior produced an unexpectedly global coordination of activity throughout the brain. By observing how activity changed as mice drank water, as well as directly stimulating hypothalamic thirst neurons, I showed that this activity wave was dependent on the animal's motivational state.

Surprisingly, the activity of a few hundred thirst neurons instantly modulated the state of the entire brain. Even more surprisingly, I found many neurons, distributed throughout the brain, that directly encoded thirst. These results suggest that even simple behaviors, such as thirst, are emergent properties of the entire brain.

I hope these new approaches will at last enable us to comprehend the rules that transform distributed patterns of electrical activity in neural circuits into thoughts, emotions, and perceptions. Understanding how molecules, neurons, and networks interact to shape these rules will have a sweeping impact on our understanding of brain function in health and disease. ■

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GRAPHIC: N. DESAI/*SCIENCE* FROM W. ALLEN, WANG *ET AL.* (*5*), ALLEN *ET AL.* (*4*), ALLEN *ET AL.* (*9*)

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