Hebb's Vision: The Structural Underpinnings of Hebbian Assemblies

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1 Abstract

In 1949, Donald Hebb proposed that groups of neurons that activate in a stereotypical manner form the organizational building blocks of perception, cognition, and behavior. He theorized that repeated activations induce the structural changes needed to group neurons in such assemblies. Despite Hebb's enduring influence, testing his predictions at relevant scales has been technically challenging. Here, we test the theory using a novel, large-scale dataset featuring in vivo calcium fluorescence imaging of neural activity with postmortem electron microscopy (EM) for detailed reconstruction of neurons from the same volume of mouse visual neocortex. A coregistration process matches EM-reconstructed neurons to their recorded fluorescence traces. From these traces, we extract cell assemblies from higher-order correlations in neural activity. We then show that these assemblies exhibit key Hebbian properties, including more reliable responses to repeated natural movie inputs than size-matched random ensembles and superior decoding of visual stimuli. Using co-registration to probe structural correlates, we find that neurons that participate in assemblies are significantly more integrated into the structural network than those that do not. Contrary to Hebb's original prediction, we do not observe a marked increase in the strength of monosynaptic excitatory connections between cells participating in the same assembly. However, we find significantly stronger indirect feed-forward inhibitory connections targeting cells in other assemblies. Intuitively, the delineation of assemblies can be realized either by internal excitation or external inhibition. Our findings support the latter mechanism. These results show the utility of assemblies in perception and provide a structural underpinning. They lay the foundation for future studies looking at the utility of assemblies in cognition and behavior, as well as the mechanisms for the formation and maintenance of such assemblies.

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2 Rationale

While Hebb's seminal postulation of activity-dependent strengthening of synaptic connections — often paraphrased as 'neurons that fire together, wire together' — gained widespread acceptance with the discovery of long-term potentiation, a critical aspect of his hypothesis remained untested. Specifically, Hebb proposed that cellular assemblies serve as building blocks of neural circuitry, linking brain structure to circuit computations that drive perception. This hypothesis has eluded direct validation for decades.

Rigorously testing Hebb's predictions at the level of both neuronal firing and synaptic circuit connectivity has posed significant technical challenges. Multiple studies[1 2 3] have examined the role of cell assembly activity in behavior and cognition, but extracellular electrophysiology and calcium fluorescence imaging alone lack the ability to elucidate connectivity patterns reliably. Other studies[4 5] have examined the details of synaptic connectivity, but in order to validate Hebb's theory, the synaptic scale must be bridged from structural to functional. Several studies[6 7 8] emerging from the lab of Thomas Mrsic-Flogel have bridged the structural and functional aspects of Hebb's theory. These combined in vivo calcium imaging and in vitro whole-cell recording. Unfortunately, traditional electrophysiological methods are difficult to apply at scale, so even when many neurons were recorded, the sets of simultaneously recorded cells remained very small. The largest of the above examples[7] features 283 total cells but can examine sets of connections only within groups of two to six cells. These small sets primarily reveal low-order (e.g., monosynaptic) interactions, leaving a 75-year gap at precisely the scope needed to test Hebb's theory.

The persistence of this gap in understanding is akin to the decades-long search for the Higgs boson, where only recently was theory met with direct evidence after analyzing petabytes of data at the Large Hadron Collider and confirming the proposed origin of mass of all fundamental particles in nature. We hereby present the first study ever to examine Hebb's assembly theory in a manner that bridges structural and functional aspects at scale to allow examination of higher-order connectivity and correlations. Our study leverages one of the largest multimodal datasets from the murine visual neocortex ever produced. We employ similarity graph clustering (SGC) - previously developed by other groups[9] but yet to be applied to large-scale cortical data - to extract assemblies through higher-order correlations in fluorescence activity, moving beyond traditional low-order pairwise correlation methods to capture complex interactions in the cell population. This is paired with higher-order graph-theoretic analysis of a connectomic network of 852 cells, of which 80 are coregistered to their fluorescence data, bridging one of the largest proofread connectomes ever produced to corresponding activity data.

Our findings uncover evidence of assemblies' role in the processing and decoding of complex stimuli, consistent with Hebb's functional postulates. However, the structural underpinnings of these assemblies diverge from his original prediction of strong excitatory connections within each group[10]. Instead, we observe significantly stronger inhibitory chains between assemblies. Such targeted inhibition has been hypothesized to play a crucial role in the delineation of assemblies[11 12]. Our findings offer compelling evidence in support of this hypothesis and mark the first demonstration of inhibition as a mechanism underlying the organization of Hebbian assemblies.

In addition to these insights, we observe a subset of neurons that do not belong to any assembly. These unaffiliated cells exhibit significantly lower betweenness centrality, a graph-theoretical measure of network integration, than their assembly-associated counterparts. The functional role of these unaligned neurons within cortical circuits remains an open question, but they potentially align well with Carandini's[13 14] proposed distinction between 'choristers' and 'soloists' in neurons of the primary visual neocortex.

Hebbian assemblies as a fundamental functional unit of information processing hold great scientific potential[15]. Beyond characterizing the relationship between connectivity and activity of these assemblies, we provide strong evidence of the reliability of their response to visual stimuli and their representation of stimulus-specific decodable information. Thus, these findings underscore the potential of assembly activations to be useful in generating perceptions and behavior and open the door for the rigorous application of cell assemblies in a broad range of neuroscientific, psychophysical, physiological, psychological, and even psychiatric inquiries into perception, cognition, and behavior.

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