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Dear Editor,

I am writing to submit our manuscript, "Hebb's Vision: The Structural Underpinnings of Hebbian Assemblies" to Nature Neuroscience, following the advice of Senior Editor Luis A Mejia, PhD in response to our presubmission inquiry (NN-PI90193).

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 from labs like those of György Buzsáki, Tanaka Keiji, Earl Miller, and Tomaso Poggio 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, including several from these same labs, have examined the details of synaptic connectivity. In order to validate Hebb's theory, however, the synaptic scale must be bridged to the scale of full functional assemblies. Several studies emerging from the lab of Thomas Mrsic-Flogel have bridged the structural and functional aspects of Hebb's theory by combining in vivo calcium imaging and in vitro whole-cell recording. Unfortunately, traditional electrophysiological methods are difficult to apply at scale, so the largest such studies to date examine sets of no more than six simultaneously recorded 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 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. 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. 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 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. 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 to the rigorous application of cell assemblies in a broad range of neuroscientific, psychophysical, physiological, psychological, and even psychiatric inquiries into perception, cognition, and behavior.

We respectfully suggest the following scientists as potential reviewers.

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We have no conflicts of interest to disclose. This manuscript has not previously been published and has not been submitted to nor is it under consideration for publication in any other journal. Its submission for publication has been approved by all authors.

Yours sincerely,

Julian Wagner-Carena, corresponding author.