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Converging assemblies: a putative building block for brain function and for interfacing with the brain

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The organization of biological neuronal networks into functional modules has intrigued scientists and inspired engineers to develop artificial systems. These networks are characterized by two key properties. First, they exhibit dense interconnectivity (Braitenburg and Schüz, 1998; Campagnola et al., 2022). The strength and probability of connectivity depend on cell type, inter-neuronal distance, and species. Still, every cortical neuron receives input from thousands of other neurons while transmitting output to a similar number of neurons. Second, communication between neurons occurs primarily via chemical or electrical synapses. The transmission of information is mediated mainly during presynaptic spiking events that generate postsynaptic inward currents and intracellular depolarization, which in turn induce postsynaptic spikes. However, these two properties alone cannot explain the complex mechanisms of information processing in neuronal networks.

The connection probability of neurons in biological networks can be estimated from high-density extracellular recordings of multiple neurons (**Figure 1A** and **B**). Using recordings from a sub-millimeter portion of hippocampal region CA1 (Spivak et al., 2024), we found that the connection probability between excitatory neurons (typically pyramidal cells, PYRs) and inhibitory interneurons (INTs) asymptotes to about 0.2 (**Figure 1A**). Because circuit topology is not fully characterized by connection probability, a network of interconnected neurons might be structured in several different manners. At one extreme, the neurons in the network may be randomly connected to all other neurons, whereas at another extreme, neurons may form largely non-overlapping local sub-networks (**Figure 1C**–**E**). However, even perfect knowledge of the functional connectivity graph falls short of explaining how information is processed within a network. In the context of information transmission, presynaptic spikes may contribute to postsynaptic spike generation in various manners: individually, linearly, or supra-linearly. Therefore, the ability of a presynaptic spike to trigger a postsynaptic spike may also depend on the activity of other presynaptic neurons. In the context of information storage, long-term connectivity changes in a network may depend on various aspects of the experienced spiking activity, including the firing rates and the relative timing of pre- and postsynaptic spikes.

We approached the problem of information processing in neuronal networks by working with freely-moving subjects, as opposed to *in silico* or *in vitro* experiments. First, we developed a mathematical framework to accurately infer the functional connectivity in a network while also recording the simultaneous spiking activity of dozens to hundreds of neurons (Spivak et al., 2022). Second, instead of merely observing neuronal activity during natural behavior, we imposed activity patterns on the network using multi-site optogenetic manipulations (Stark et al., 2012) so as to test how information is processed in the network and how the experienced spiking changes functional connectivity. Third, to avoid long-distance interactions and excitatory feedback loops, we focused on the interface between excitatory PYRs and inhibitory INTs in local cortical circuits (Levi et al., 2022; Spivak et al., 2024).

Below, we review our recent findings, which suggest that PYR-INT networks are organized in modules called "converging assemblies" (CAs) that support information transmission and storage. We present a hypothesis regarding information transmission via CAs and outline its relevance to brain-machine interfaces. We outline some directions for future research.

Converging assemblies as building blocks for brain function–experimental evidence: To study information transmission as defined by the propagation of informative spiking in the intact brain, we imposed random patterns of optogenetic input (Gaussian white noise filtered with a 3 ms alpha function; µW-scale light power; Levi et al., 2022) on PYRs in the neocortex of freely-moving mice. We monitored the spiking of local circuit PYRs and INTs using high-density extracellular recordings, and we quantified the information carried by each spike train and the temporal precision of that train concerning the white noise input (Levi et al., 2022). We found that individual trains of directly activated PYRs (DA PYRs) exhibited a precision of about 3.2 ms. However, postsynaptic INTs indirectly activated (IDA INT) by the white noise exhibited improved precision, with a median of 2.3 ms. Furthermore, more information was carried out by the IDA INT spike trains compared with the DA PYR spike trains, referred to as error correction. Improved precision and error correction were accentuated when larger numbers of PYRs participating in the same CA were activated. Thus, the convergence onto IDA postsynaptic neurons improves both information transmission and precision.

In the framework of DA PYRs converging onto IDA INTs, it is possible to assess which aspect of spiking is responsible for the improvements. Using data-driven modeling, we found that an individual PYR spike train cannot drive spike generation in an IDA INT. A simple summation of the short-term postsynaptic effects of multiple DA PYR spike trains could drive INT spiking only in the absence of any other inputs, but the resulting INT trains did not exhibit error correction or improved precision. In contrast, coincidence detection, modeled as a non-linear combination of the postsynaptic effects of spikes from multiple same-CA PYRs converging on the same INT, was sufficient to replicate the observed improvements. Thus, CAs may use coincidence detection for error correction and improved precision along the PYR-to-INT interface. Similar results were observed in a cortical area that has different architecture, the hippocampal CA1 region, suggesting the universality of CAs as a building block for robust and informative spike transmission.

We assessed information storage in a separate study (Spivak et al., 2024). In reduced preparations, the relative timing of spikes emitted by pre- and postsynaptic neurons influences changes in functional connectivity, a process called spike-timing dependent plasticity (Song et al., 2000; Aljadeff et al., 2021). We hypothesized that a similar process might occur in the PYR-to-INT model system in the hippocampal CA1 region of intact subjects. To study information storage causally, we detected the occurrence of PYR spikes in real time during an "Experience" epoch, and we triggered optogenetic activation of parvalbumin-immunoreactive (PV) INTs 3 ms later. We quantified the spiking patterns actually experienced using the difference between deconvolved PYR-to-PV cross-correlation histograms (Spivak et al., 2022) during light stimuli and between light stimuli.

We found that the spiking patterns experienced by a single presynaptic-postsynaptic PYR-PV pair could be used to predict changes in functional connectivity between a pre-experience epoch and a duration-matched post-experience epoch. The effect size of the prediction was small, exhibiting an area under the curve of 0.51 where the chance is 0.5. In contrast, connectivity changes within an entire CA were easier to predict, with an area under the curve of 0.63. Focusing on sub-millisecond timing of spikes within a short 21 ms window around the presynaptic spike increased the area under the curve to 0.75. Initial conditions and postsynaptic excitability changes were insufficient to explain the observed changes in functional connectivity. Thus, the precise spike timing experienced by an entire CA predicts long-term changes in functional connectivity between PYRs and the postsynaptic PV in that assembly.

Converging assemblies as modules for interacting with the brain–a hypothesis: The abovementioned studies revealed that in local circuits, error correction, improved precision, and functional connectivity changes all depend on the precise timing of spiking within a PYR-to-INT CA. Related work showed that PYRs in the same CA have a common developmental origin (Huszár et al., 2022). We hypothesize that beyond serving as natural building blocks for information transmission and storage, CAs may be useful for neuro-engineering purposes. Interfacing with the brain at the level of CAs might simplify information transmission in both directions: from the brain to an external device ("reading"), and from an external device to the brain ("writing"). A CA is defined by functional connectivity and not by physical distance between the constituent neurons. However, connectivity and inter-neuronal distances are closely related. In most parts of the mammalian archicortex and neocortex, a large fraction of the inter-neuronal connections are local, that is, to other neurons within a few hundred micrometers (Braitenburg and Schüz, 1998; Campagnola et al., 2022).

Evidence for improved "reading," namely information transmission from a pool of neurons to an external device, comes from the pre/motor neocortex of monkeys that participated in reaching, grasping, and drawing tasks. In that model system, we found that upcoming motor actions could be predicted most accurately using the agglomerated spiking activity (multi-unit activity; MUA) within a sphere of about 300 micrometers (Stark and Abeles, 2007). Technically, MUA was defined as the root-mean-square (low-pass filtering) of the spiking band (300–6 000 Hz) to which spikes of PYRs and INTs (but not local field potentials) contribute, thereby including the same building blocks as a CA. Similar findings were observed in other brain regions (posterior parietal cortex), model systems, and species, including humans (Haghi et al., 2024).

At the present, we do not have direct evidence of the usefulness of CAs for the other ("writing") direction. However, we hypothesize that if CAs are properly targeted, stimulating the brain at that level might be superior to interfacing with a random set of local circuit neurons: from the perspective of minimizing resources (fewer channels, less energy), and also from the perspective of information content (fewer errors, higher channel capacity). Because a given PYR typically takes part in multiple CAs via divergence (**Figure 1**), the key term here is "properly targeted." To provide a concrete clinical example, consider a neuro-prosthetic device designed to supplement a lost peripheral function, such as vision. We expect that the joint activation of a set of cortical neurons (for example, in V1) that have a common postsynaptic target (in the same brain region or another brain region) will be considerably more efficient compared with the activation of a random same-size set of cells.

Reservations and open questions: The estimates of connectivity and convergence shown in **Figure 1A** and **B** are based on empirical recordings. Each session included dozens to hundreds of simultaneously recorded PYRs and INTs, and the

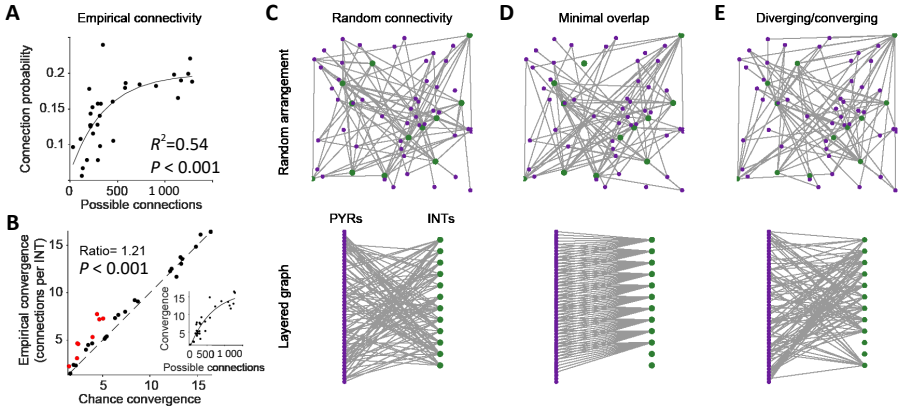

Figure 1 | **Converging assemblies (CAs) of excitatory pyramidal cells (PYRs) and inhibitory interneurons INTs in cortical circuits.**

(A) Empirical connection probability in local circuits asymptotes to ~0.2. In extracellular recordings from the hippocampal CA1 region (31 sessions from four freely-moving mice; data from Spivak et al., 2024), the number of observed connections increases exponentially with the number of recorded PYR-INT pairs ("possible connections"). *P*-value, *F*-test. (B) Empirical convergence, defined as the number of PYRs per INT in actual recordings, is higher than chance. Chance convergence, mean convergence in 5 000 Monte Carlo trials in which the number of units and the connection probabilities are held constant. Red dots denote recording sessions with excess convergence. *P*-value, Wilcoxon's signed-rank test comparing to a ratio of 1. In an exponential fit of convergence *vs*. the number of possible connections (inset), asymptotic convergence is 16.6 PYRs/INT. (C) Top: Connectivity graph of an artificial network of PYRs and INTs (dots plotted at random 2D locations) with monosynaptic excitation (grey edges) from some PYRs to INTs. The net includes 60 units 80% of which are PYRs, with the connection probability derived from extracellular recordings as described in A. Connectivity is completely random. Bottom: Any network can be visualized as a multi-layered graph. In the feedforward PYR-INT network, plotting the PYRs on the left and the INTs on the right captures the entire structure and emphasizes the CA structure. (D) Top: The same number of connections (99/576 possible connections) between the same set of neurons as in C are chosen non-randomly, wiring 10 CAs with minimal overlap between the presynaptic PYRs. Bottom: The same network is visualized as a layered graph. (E) An artificial network with the same number of units and connection probability as in C and D, with the connections drawn at random, subject to the constraint of preserving the empirical convergence (and divergence) as quantified in B.

estimates were based on fitting curves and the asymptotic behavior of exponentials. However, the existing techniques do not allow recording all of the neurons in a local circuit deep within the intact brain, mainly because recording sites on penetrating planar probes are typically oriented in the same direction. We have recently developed dual-sided silicon probes (produced by Diagnostic BioChips, USA) that enable to monitor neuronal spikes from both sides of the probe (Levi et al., 2022; Someck et al., 2023; Spivak et al., 2024). The future development of multi-faceted ultra-thin probes is expected to allow recording of entire cohorts of local circuit neurons. Collection and connectivity analysis of larger fractions of neurons from the same local circuit is expected to facilitate fine tuning of the estimates, and possibly reveal features of network topology beyond those derived from pair-wise interactions.

Reading information from a proxy to CAs, the MUA, yielded superior predictions concerning hand kinematics in motor, premotor, and parietal brain regions of monkeys and humans. However, the neurons in these areas exhibit non-random organization concerning the variables of interest, namely position, velocity, and acceleration. In other brain regions, for instance, the hippocampus, it is unclear whether the encoded variables (for example, place coding) exhibit random or non-random organization. The hypothesis that CAs are useful for reading information using simple signal processing can be generalized and tested by comparing predictions of rodent kinematics based on spiking vs. MUA from hippocampal region CA1.

The hypothesis that CAs could be useful also for writing information into the brain should be tested. A putative approach involves writing information into random sets of neurons vs. CAs and comparing the behavior of the subject contingent on the injected neuronal activity. Experiments of this type could initially be performed on animals, requiring the subjects to be able to respond to focal stimulation (Houweling and Brecht, 2008) and a methodology to stimulate individual neurons with a local network deep within the intact brain.

In the abovementioned works (Levi et al., 2022; Spivak et al., 2024), we focused on the PYR-to-INT interface in the neocortex and hippocampal region CA1 of freely-moving mice. However, in the neocortex and many other brain regions of rodents and primates including humans, information processing is supported by recurrent excitation. Future studies should repeat, adapt, and expand the work discussed here to the PYR-to-PYR interface. In such experiments, the main challenge would be to ensure that the analysis involves a single interface, that is, only two layers (**Figure 1**). This could be done by carrying out experiments between two distant brain regions, but then the circuits would not be local. Carrying out local circuit experiments on the PYR-to-PYR interface deep in the brain requires single-spike cellular-resolution targeting, a technology that is currently available (Tarnavsky Eitan et al., 2021).

Conclusions: To date, almost all invasive clinical work with human patients who suffer from neurological disorders employs much larger intracranial electrodes and poorer sampling of neurons, compared with the state of the art of animal studies. Technological developments are expected to change this situation (Paulk et al., 2022). Circuit-level interaction with the human nervous system for clinical applications is expected to gain from approaches that harness the natural building blocks of the system: CAs are one such building block.

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