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Neuronal 'Ensemble' Recording and the Search for the Cell Assembly: A Personal History

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ABSTRACT

This contribution is part of the special issue on the *Hippocampus* focused on personal histories of advances in knowledge on the hippocampus and related structures. An account is offered of the author's role in the development of neural ensemble recording: stereo recording (stereotrodes, tetrodes) and the use of this approach to search for evidence of Hebb's "cell assemblies" and "phase sequences", the holy grail of the neuroscience of learning and memory.

When I was a graduate student at Dalhousie University in the late 1970s I worked with my fellow graduate student Rob Douglas and our Ph.D. advisor Graham Goddard on the associative properties of LTP in the perforant pathway to the dentate gyrus. The story of our discovery of cooperativity/associativity (McNaughton, Douglas, and Goddard 1978) has been recounted already (McNaughton 2003). At that time, Donald Hebb was a professor emeritus at Dalhousie, and at the end of the 2003 paper I described Hebb's response to being shown that his ideas about synaptic associativity ("Hebb's Rule") were essentially correct: "His suggestion to me was that I would have a much more interesting career if I focused on his cell assembly and phase sequence concepts"... which he considered to have been his important contribution to the study of the neural basis of memory.

I understood that this was a tall order and that finding cell assemblies in the brain might be like looking for the proverbial needle in a haystack. At the very least, it would require being able to record simultaneously from multiple neurons engaged in memory tasks, and ideally to be able to quantify their synaptic interactions, since assemblies imply some degree of reciprocal connections. At the time, this was very wishful thinking at best, since the technology of the day was limited to recording from one or two neurons at a time, and even that was a fraught endeavor due to uncertainties in isolating the activity of single cells using extracellular microelectrodes, particularly in behaving animals (Figure 1).

Therefore, I considered that a wiser choice for my postdoctoral studies would be to continue to study LTP and synaptic physiology using the rapidly emerging technology of in vitro brain slices and sharp electrode, intracellular, recording. "Patch clamping" hadn't been invented yet but experiments in the Andersen laboratory in Oslo (and elsewhere) were making rapid progress in characterizing hippocampal synaptic transmission and pyramidal cell biophysics with sharp intracellular electrodes. So, I went to Oslo in early 1979 to do intracellular recording studies in the dentate gyrus (McNaughton, Barnes, and Andersen 1981). Towards the end of my second year there, I had a faculty position lined up at McMaster University and was preparing to return to Canada, when there was a lab visit from John O'Keefe, who a few years earlier had reported what they (O'Keefe and Dostrovsky 1971) called "place cells" in the hippocampus. O'Keefe suggested that instead of taking a secure faculty position I should come to London and study place cells—he thought he "might" be able to find some money to support me. I had a

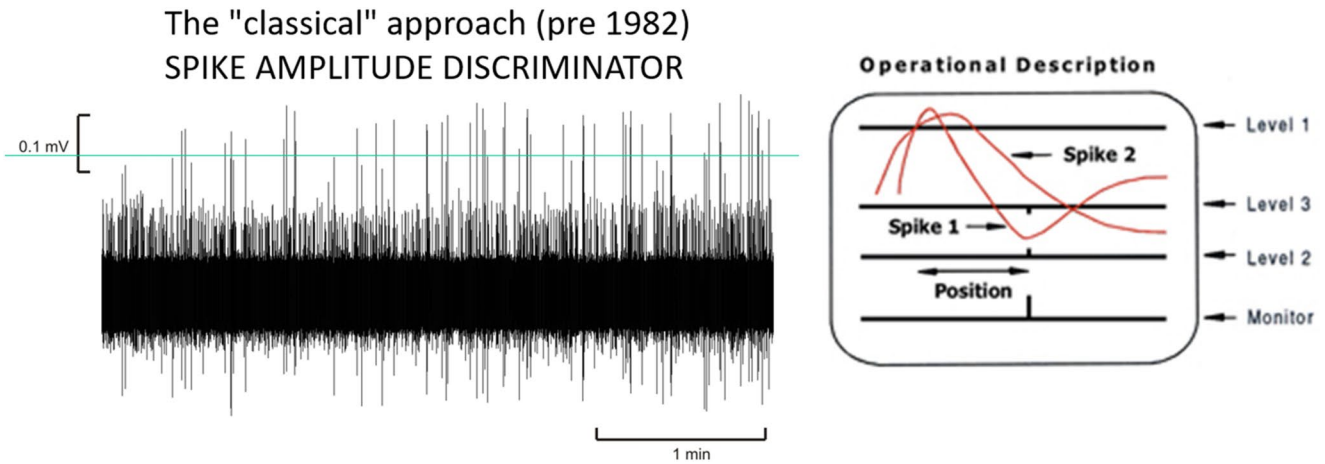

FIGURE 1 | Single unit isolation pre-1982. Left: Typical record of multi-neuron activity recorded from a single microelectrode in rat hippocampus. Originally "units" were "isolated" by setting a level detector called a "Schmitt trigger". Today, we would be very dubious that the signals crossing the threshold shown were contributed by a single neuron. More advanced versions (right) had two thresholds and could be set to trigger an output pulse only if the signal fell below the lower threshold within a certain time window. To obtain spike signals large enough that only a single neuron contributed to the output signal required a very fine-tipped, high-impedance (and hence noisy) microelectrode. Recordings with such electrodes were typically very unstable, due to minute brain movements which resulted in large shifts in extracellular spike amplitude.

few months of support left on my postdoc award so I decided to take a chance in the hope that this would be a step towards finding 'cell assemblies'.

My first experience with a single microelectrode, extracellular, recording in rat hippocampus was disappointing, to say the least. Having just come from doing intracellular recording, where the signals were in the 10s of millivolt range, and where there was no issue about whether one was recording from single cells, I was confronted with very noisy signals in the range of 50–150 microvolts in which spikes from numerous neurons were clearly present. One had only a thresholding method (the so-called Schmitt trigger, Figure 1) to filter out all but the largest of spikes. This did enrich the fraction of spikes from single cells, although clearly imperfectly, had the effect of losing spikes from the chosen 'unit' if there was any variance in the spike amplitude. Traditional neurophysiology doctrine was that spike amplitude was constant; however, it was already established (mostly through the seminal work of James Ranck) that this was not the case in hippocampal pyramidal cells, which often emitted 'complex spike' bursts of declining amplitude. So clearly there was a serious tradeoff between the clear isolation of one cell and losing many of its spikes. Moreover, although it was sometimes possible to record from two electrodes at once, this was only possible on days when O'Keefe wasn't using the other of the two available amplifiers for his own experiments. Finding assemblies seemed a remote dream.

UCL had a vibrant program of visiting speakers, and 1 day I heard a talk about auditory sound source localization based on detecting interaural time and level differences (ITD and ILD, respectively) at the two ears. The speaker, whose name I unfortunately forget, was indirectly responsible for the invention of stereo recording. I was enjoying a warm bath that evening when it occurred to me that, since extracellular potentials decreased as roughly the inverse square of the distance from the generating neuron, two microelectrodes at different distances from a target neuron should show spikes of different amplitudes. Indeed, there were demonstrations in the literature showing how spike amplitude changed over quite small distances as a microelectrode was advanced through neural tissue. I proposed this experiment to O'Keefe the next day, hoping that he would let me use the extra amplifier. At first, O'Keefe thought I was talking about differential recording, subtracting the signals on the two channels to reduce noise by "common-mode rejection", and he wasn't very enthusiastic. But once he understood the concept, he became more excited and suggested we try just twisting together two, 30-μm microwires. He had some appropriate wire in the lab and so we tried it. The results were immediate and striking. We displayed the two amplifier outputs on two different channels of an oscilloscope in XY mode (Figure 2C) and saw that spikes that looked the same on one channel often had completely different phase angles in the XY ("Lissajous") plot, meaning that there were clearly more than one unit generating them (Figure 2).

We knew that to fully resolve points in 3-D space, a *tetrahedral* array of recording points was theoretically required ("In theory, a closely spaced tetrahedral array of recording electrodes with tips sufficiently close together to record signals from overlapping populations of neurons should permit the unique identification of all neuronal spikes which exceed the noise level."; McNaughton, O'Keefe, and Barnes 1983); however, amplifiers in those days were expensive and O'Keefe was not willing to pillage the second recording rig in the lab so we went with two wires. Still, two electrodes were much superior to one and we showed that a lot of the earlier literature studies claiming to have recorded from single neurons were manifestly in error; this did not make us a lot of friends! It wasn't until about 10 years later that both we and O'Keefe's group finally got around to using four wires ("tetrodes"; O'Keefe and Recce 1993; Wilson and McNaughton 1993).

In the meantime, we developed a method for moving multiple microwire probes individually through the brain. We created

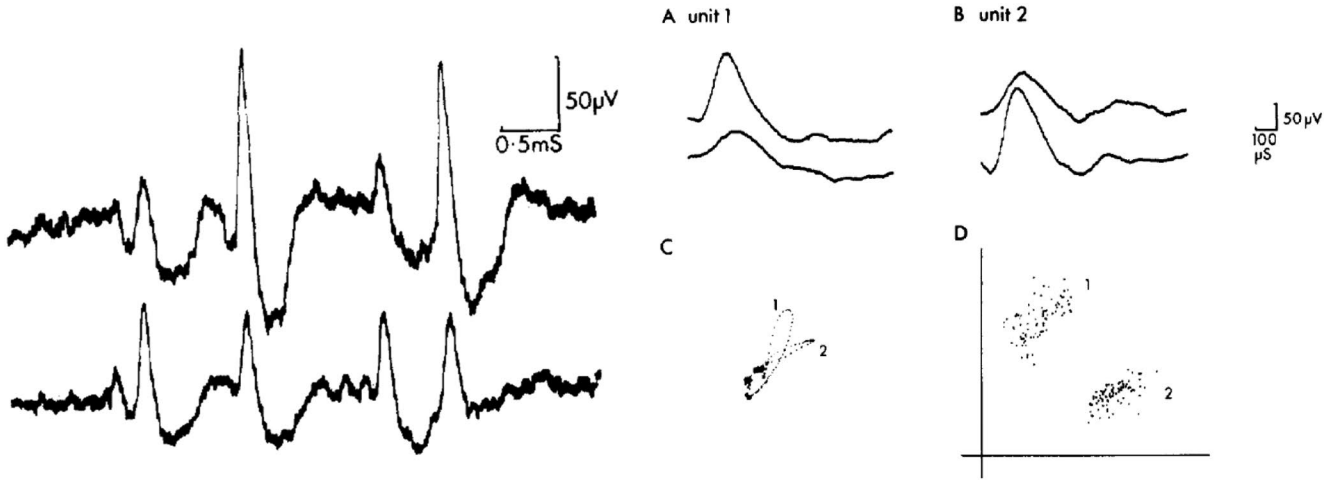

FIGURE 2 | Figures from McNaughton, O'Keefe, and Barnes 1983 illustrate the first demonstration of single-unit isolation based on relative signal height on two channels. The left panel illustrates two neurons that would have been confused as one based on single channel amplitude. The right panel illustrates the use of a 'Lissajous' display (using two signals to drive the horizontal and vertical axes of an oscilloscope, which, younger readers might not know, was a cathode ray tube display with a phosphorescent screen that glowed when struck by electron beams, allowing for visual representation of fast signals;) and spike amplitude scatter plot. One advantage of the Lissajous display is that it captures the phase shift (the change in angle in the x-y plane indicative of a shift in the ratio of the two signals) across the two channels in Unit 1 (probable pyramidal cell) and lack thereof in Unit 2 (probable interneuron). The phase shift likely reflects spike propagation in the apical dendrite of pyramidal cells.

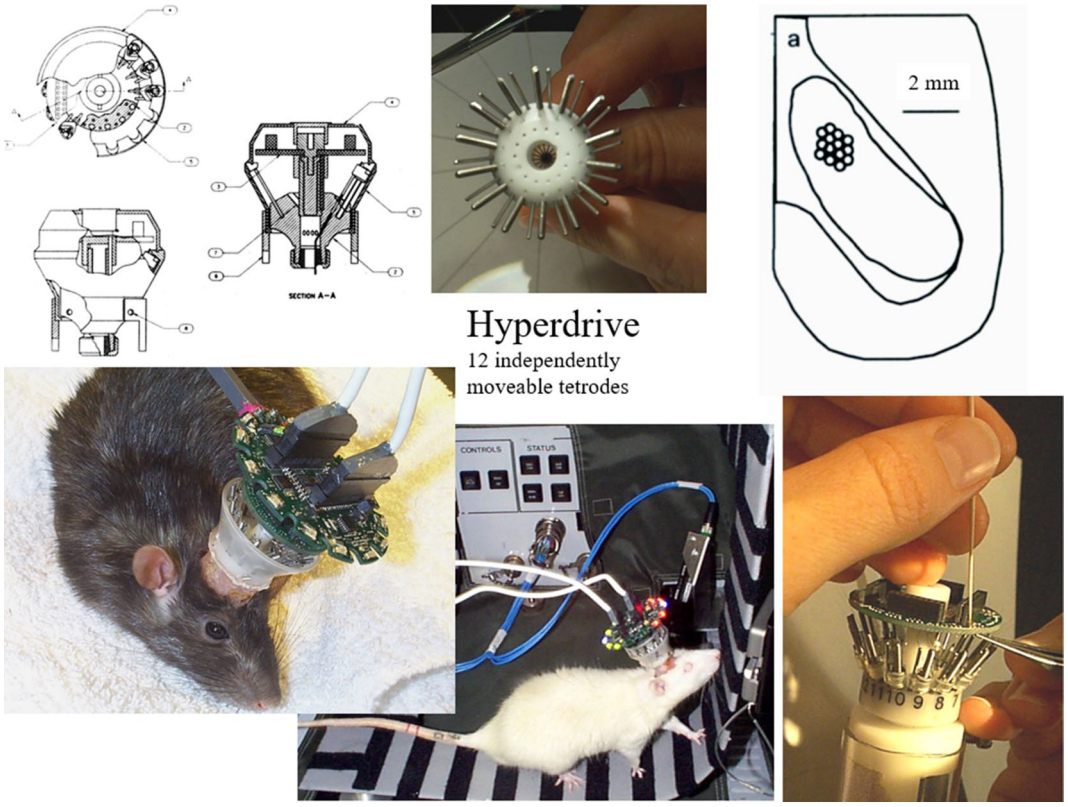

FIGURE 3 | Top row: Left) Engineering diagram of commercially produced hyperdrive (US Patent 5,928,143, 1999) which enables multiple fine wire bundles (tetrodes) to be advanced vertically through the brain by forcing them through curved tubes with a drive screw. The inspiration for this approach came from the mechanical shutter release cable used in photography and from how the proboscis of a mosquito works. Middle) An early prototype of the hyperdrive was personally machined by the author when he could still see clearly and had steady hands. Right) Layout of probe exits from hyperdrive bundle (14 probes—12 tetrodes + reference wires) over dorsal hippocampus. Bottom row: Left and middle) Implanted hyperdrives connected to Neuralynx HS-54 head stage and wire tethers, and rat exploring a 3-D track in microgravity from the STS-90 Neurolab mission investigating properties of hippocampal 'place' cells in zero-G. Right) Making fine wire connections to the Neuralynx Electrode Interface Board (EIB) using cactus (Cylindropuntia fulgida) needles pressed into the via on the EIB.

a microdrive (which we called a "hyperdrive") that could push 12 tetrodes through gradually bending cannulae, much like an external, mechanical, camera shutter release cable (Figure 3), which resulted in the first report of simultaneous recordings of over 100 neurons in vivo. We also switched to thinner wire (13 μm), which improved the spike amplitudes somewhat. Tetrodes became the *de rigueur* method for single unit recording, including in humans, for many years, used by many of the contributors to this issue (over 10 K published articles reporting tetrode use; see articles by Burwell 2024, Buzsaki 2024, Kubie 2024, Jeffery 2024, in this issue) and are still the method of choice when large numbers of units must be recorded from the thin cell layers of the hippocampus (e.g., Wu and Foster 2014). I guess it's a testament to the fact that the method is so standard that its origin is actually rarely cited, similar to patch clamping, fMRI, EEG, etc. It's interesting, however, that the potential advances made possible by tetrode, and neural ensemble recording in general, took quite a while to be recognized by the classical single-unit recording community. I once had an NIH grant rejected because the reviewers couldn't see the advantage of ensemble recording over recording one cell at a time, and (as mentioned above) I was in some cases sharply criticized for suggesting that many earlier 'single' unit recordings contained a lot of spurious spikes from other cells. I guess it's hard to teach old dogs new tricks and it was largely left to a new generation to exploit the power of ensemble recording.

A critical enabling factor in the widespread success of tetrode recording was the contribution of Keith (Casey) Stengel, who was our chief laboratory engineer and who subsequently started his own company Neuralynx, whose support for the systems neuroscience enterprise is legendary in the field. Casey developed the necessary programmable multichannel amplifiers and computer interfaces, and the circuit boards for connecting multiple tetrodes to the 54-channel headstage which he also designed. Our initial recording system consisted of six workstations with their clocks synchronized, each collecting signals from 2 tetrodes. Subsequently, under support from NASA and NIH, Casey developed an integrated system about the size of a large briefcase, capable of supporting 54 channels of 32 KHz acquisition. This system flew on the 1998 space shuttle 'Neurolab' mission (STS-90) where we explored the behavior of hippocampal 'place' cells in zero gravity (Knierim, McNaughton, and Poe 2000).

The tetrode array, however, while cheap, easily constructed, and effective, was extremely labor intensive. Construction of the tetrodes, loading the drive, and slowly advancing the probes into the target area over days typically took about a month's work per subject. One of the biggest problems was soldering all those microwires to the vias in the printed circuit board that connected to the 54 channel headstage. At the time we were based in Tucson Arizona, and as anyone who has walked in the Arizona desert knows, it's full of very nasty, sharp-needled cacti. One day, while extracting a bunch of needles from my dog's mouth I realized that they were nicely tapered, very hard, and could be rammed into the circuit board vias and then simply broken off, thus pushing the tetrode wire against the gold plating and making a robust, yet easily replaceable electrical contact (see Figure 3). Casey paid his young daughter $0.50 per needle to harvest them, until the demand from Neuralynx customers became too large (or his daughter found other interests), at which point he developed small gold pins that were cheaper, but a bit harder to work with and certainly less cool.

Constructing and using hyperdrives loaded with tetrodes was only the beginning of the problem. After recording, the spike signals from each tetrode had to be sorted into putative single-unit generators. The latter task was accomplished by the development of "cluster cutting" software that could rotate the parameterized spike data points through n-D parameter space and allowed manual drawing of boundaries around putative spike clusters (Mizumori, Barnes, and McNaughton 1989; X-Clust-Wilson and McNaughton 1993; MClust-Redish and McNaughton 1998; see Redish 2024, this issue); however, this also was extremely time-consuming and required considerable user sophistication. Over the years, many papers have been written on the science and art of spike sorting, and more automated software packages have been introduced (e.g., Lewicki 1998; Rossant et al. 2016); however, in almost all cases, experienced users still believe that manual curation of the spike generator clusters is an important step.

In recent years, wire tetrodes are rapidly being replaced with high-density silicon-based neural probes with hundreds or thousands of recording sites (e.g., Blanche et al. 2005; Du et al. 2009; Fekete 2015), which were pioneered in the hippocampus by the Buzsaki group (Buzsaki 2024; this issue; Harris, Henze, Csicsvari, Hirase and Buzáki 2000). These probes considerably increase the yield of recorded units but suffer from several drawbacks compared to wire tetrodes: they are expensive, fragile, and usually cannot be moved once they have been implanted in the brain. The latter issue is of some importance because, for reasons incompletely understood (possibly gliosis), the recording quality of fixed, implanted probes typically degrades over several days. With wire tetrodes mounted in hyperdrives, it is usually possible to readjust the depth of the probe by a few tens of microns, thus encountering fresh, good-quality units. Around 2013, I was on sabbatical at Neuro-Electronic Research Flanders (NERF), which is a department within Imec, a leading nanoelectronics R&D center in Belgium. During a seminar, I presented the idea of making flexible silicon probes that could be mounted in hyperdrives and positioned by forcing them through the curved tubing, similar to what we were doing with wires. Some of the people in the room thought this was impossible because of the well-known fragility of silicon probes; however, there were some in the room who thought differently (Herwik, Paul, and Ruther 2011). Silicon probes are basically glass. Glass fibers can easily bend in a tight radius without breaking because glass is an amorphous solid. Why do silicon probes break so easily then? The answer is simple, we cut optical fibers, and glass in general, by scratching them; they break because a surface flaw allows a stress concentration which allows a crack to form. Most silicon probes are abrasively micromachined, leaving them full of surface flaws. The solution was to polish the machined fiber to get rid of those flaws. With the help of Arno Aerts (CEO, Atlas Engineering), we succeeded beyond anyone's wildest imagination (Figure 4). It remains a mystery to me why this method has not been exploited in the manufacture of modern Si probes such as NeuroPixels. Perhaps in the tradeoff between expense and fragility, fragility wins out, but it would have been a worthwhile investment.

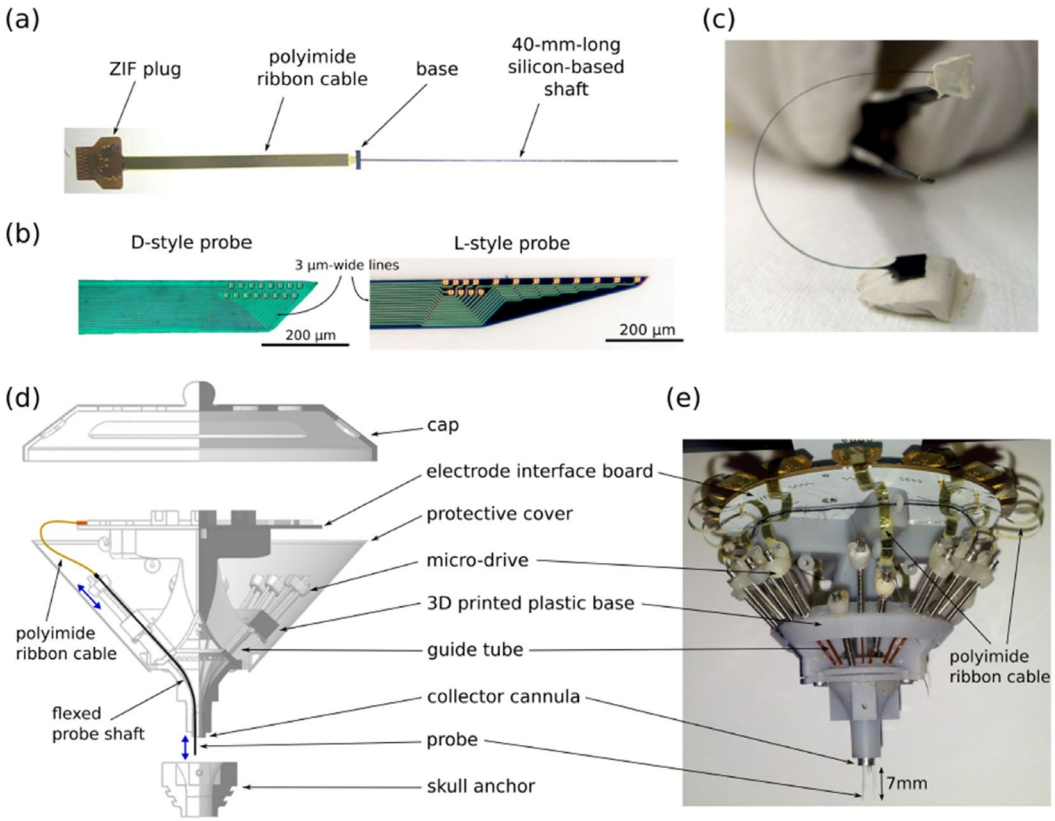

Figure 1. Integration of long, bendable silicon-based neural probes in a micro-drive array. (a) Photograph of fully assembled probe with silicon shaft, polyimide ribbon cable and zero-insertion force (ZIF) plug. (b) A close-up of the probe tip for the 'D'-style and 'L'-style electrode layouts. (c) Photograph demonstrating the flexibility of the silicon-based probe shank. (d) Cross-section (left: line drawing; right: shaded) of the 3D computer-aided design of the micro-drive array. The concept of a probe driven by an individually moveable micro-drive is shown on the left side. (e) Photograph of final array (without protective cover and cap) loaded with 16 probes. Two probes are fully extended from the bottom of the array. Note the polyimide ribbon cables that connect each probe to a ZIF connector on the electrode interface board.

FIGURE 4 | A flexible silicon-based neural probe that can be mounted in hyperdrives and adjusted after implantation to optimize the yield of recorded neurons. The flexibility enabled by polishing the silicon probe shanks is illustrated in C (Figure 1 and caption from Michon et al. 2016).

So, now, it is technically feasible to record from many neurons during memory tasks. Have we found cell assemblies and phase sequences? Well, not everything that glitters is gold. Many in the field have taken the low road and claim that any group of cells that fires together is an assembly, and so the term appears all over the ensemble recording literature, despite Hebb rolling over in his grave.

We next turned to the detection of cell assemblies and phase sequences. It was understood theoretically that a given cell may participate in many assemblies in an associative network, but if a given assembly is formed during behavior, and subsequently repeatedly reactivated, then the pattern of cross-correlations within the ensemble that appears during the formation of the assembly should reappear. With data sets of 150 recorded cells, we were able to study up to 11,000 cross-correlations from a single recording. Remarkably, almost every strong cross-correlation that appeared during behavior reappeared in subsequent sleep (Wilson and McNaughton 1993; Kudrimoti, Barnes, and McNaughton 1999). Because it is possible that the effects we were seeing were imposed from elsewhere in the brain, we could not claim to have found the cell assembly itself, but its footprints were everywhere! What about the phase sequence? We showed that not only were the relative magnitudes of the correlations preserved during sleep, but so were their temporal properties (Skaggs and McNaughton 1996). In other words, if cells A and B had sequentially overlapped place fields on the track, then their cross-correlograms were asymmetric. This asymmetry was preserved during sleep, indicating that there was a replay of what Hebb had called "phase sequences". Interestingly, the phase sequences actually appeared to playback as if the recorder were on fast-forward ('temporal compression'; Skaggs and McNaughton 1996). Subsequently, we found an experience-dependent expansion of place fields during route-following behavior that provides strong evidence for the asymmetric LTP that would be necessary to encode phase sequences (Mehta, Barnes, and McNaughton 1997). The need for asymmetric LTP to encode route information would also explain my earlier discovery (McNaughton, Barnes, and O'Keefe 1983; McNaughton, O'Keefe, and Barnes 1983), that when specific routes are used by the rat, the forward and return routes through the same space are encoded by different distributions of place cell firing. Otherwise, the asymmetry of the connections would cancel. We subsequently demonstrated that this directional orthogonalization of place cell sequences was indeed a consequence of network reorganization during the initial bidirectional traversals of a new route (Navratilova et al. 2012). Some of the further history of this "mental time travel" is reviewed by David Redish in this issue.

In the early days, it was assumed (McNaughton and Morris 1987; Treves and Rolls 1992; Tsodyks and Sejnowski 1995; Hasselmo,

Schnell, and Barkai 1995) that area CA3 would be the site of the reciprocal connectivity needed to encode cell assemblies (a.k.a. 'attractors'; Amit 1989; Samsonovich and McNaughton 1997; Colgin et al. 2010; see Rolls 2024, this issue). I should add, parenthetically, that the idea of CA3 being the site of cell assemblies really derived from David Marr's seminal, if somewhat opaque, work (Marr 1971), which I read as a graduate student but only vaguely understood. My own understanding of how assemblies might actually work only matured during the mid 1980′ s when Richard Morris invited me to write a paper with him, on the relation between Hebbian synaptic "enhancement" and distributed memory systems (McNaughton and Morris 1987). In our extended discussions, we essentially taught each other enough about this topic that we were able not only to use it as a conceptual framework for much of our subsequent careers but also, I think, to make it accessible to a general neuroscience audience (one of my students called it "Marr for the masses"). These ideas also played a major role in formulating the successful grant application for an international neuroscience research center in Trondheim, which Richard, May-Britt and Edvard Moser, and I wrote together around that period. The contribution of tetrodes to that enterprise goes without saying. I also owe a deep debt to Daniel Amit, one of the main originators of attractor theory, who, having read Richard's and my paper, invited me for a week of discussion in Rome. While giving me a guided tour and history lesson about Rome (his other passion), he tutored me on the finer points of the attractor theory and its extension to continuous attractors, which likely form the basis of much of the machinery of the spatial system in the medial entorhinal cortex and hippocampus (McNaughton et al. 2006).

To test the attractor theory, while I was on sabbatical at Edvard and May-Britt's Ttondheim center, we (Colgin et al. 2010) developed a spatial 'morph' paradigm in which the animal was first exposed to two separate boxes, one circular, and one square while recording with tetrodes from CA3 and CA1. After the animals had developed orthogonal place cell maps for the two boxes, they were exposed to a morph series in which the boxes were gradually deformed from circle to square or *vice versa*. Cell assembly (attractor) theory predicted non-linear transitions between the two maps near the mid-point of the morph series. However, when the experiment was conducted by training on the two boxes separately, but with the boxes located *in the same place* in the laboratory, no non-linear transition was observed; the transformation was continuous and linear; however, if we trained the rats on the two boxes side by side, connected by a tunnel, so that they could walk freely between the two boxes, and then repeated the morph manipulation, a clear non-linear transition was seen. We concluded that fixed-point attractors (cell assemblies) were not a property of CA3 per se but were a property of the path-integration system, probably located in the medial entorhinal cortex. More likely, therefore, CA3 is principally involved in encoding phase sequences rather than fixed point or continuous attractors. I think this issue is still up in the air. Recent work from the Moser group provides conclusive evidence that the MEC attractor network is wired up in very early development (Matteo Guardamagna, personal communication, 2024) and is not a product of learning; thus, experience-dependent cell assemblies remain to be established.

In the last few decades, there has been quite a lot of progress towards understanding the Hebbian cell assembly concept and obtaining suggestive evidence for their existence (Miyashita 1988; Sakurai et al. 2018; Carrillo-Reid et al. 2021, 2019). This has been aided by previously almost unimaginable advances in the ability to record the activities of large numbers of neurons during behavior and rest and to manipulate the activity of sets of neurons optogenetically. In addition, by using long-duration electrophysiological recordings of neural ensembles in animals in the resting state, it is possible to infer synaptic connectivity among excitatory neurons ('functional' synaptic connectivity') using short latency (1–4 msec) spikes in the cross-correlations (Figure 5). The advantage of ensemble recordings is that they can greatly facilitate the study of spike-train interactions because the number of neuron pairs increases as the square of the number of units recorded. Long durations (large sample sizes) are required because cortical and hippocampal synapses are intrinsically weak (McNaughton, Barnes, and Andersen 1981; Mason, Nicoll, and Stratford 1991; Deuchars, West, and Thomson 1994; Markram et al. 1997; Thomson and Deuchars 1997; Reyes and Sakmann 1999), and so large samples are required for statistical validation (Schwindel et al. 2014).

In my view, however, no study to date has quite reached the goal. To justify this statement, I offer the following operational definition of a Hebbian cell assembly:

A Hebbian cell assembly is a set of neurons that are initially independent or have only sparse interdependence, but that, as a result of coactivation driven by experience, become functionally and synaptically reciprocally coupled such that activation of a subset enables pattern completion of the remaining members of the set.

The seminal work of Miyashita and colleagues on "pair coding" neurons in primate Perirhinal cortex (Miyashita 1988; Sakai and Miyashita 1991; Takeuchi et al. 2011) provides clear evidence for the formation of cell assemblies at the level of functional coupling. A contemporary approach (Carrillo-Reid et al. 2021, 2019) showed that optogenetic activation of some neurons in a visual stimulus-selective ensemble was able not only to complete the ensemble but to drive the corresponding behavioral choice. What is lacking; however, is the direct characterization of synaptic connectivity within ensembles of co-active neurons. It should be possible with current electrophysiological techniques to estimate 'functional synaptic coupling' within such ensembles using the short-latency correlation method (Figure 5); however, selective activation of subsets of such electrophysiologically recorded neurons is not currently possible. Such selective stimulation is possible with Calcium imaging; however, the low temporal resolution and slow nature of calcium dynamics do not allow examination of cross-correlations at the needed resolution. Hope is on the way, however, as the development of soma-localized voltage sensors (e.g., Aseyev et al. 2023; Piatkevich et al. 2018; Li et al. 2020; Kim and Schnitzer 2021) advances. In the future, we may see the combination of voltage imaging at single-cell resolution combined with holographic optical stimulation which, in principle, will allow us finally to find Hebb's golden needle (in the haystack).

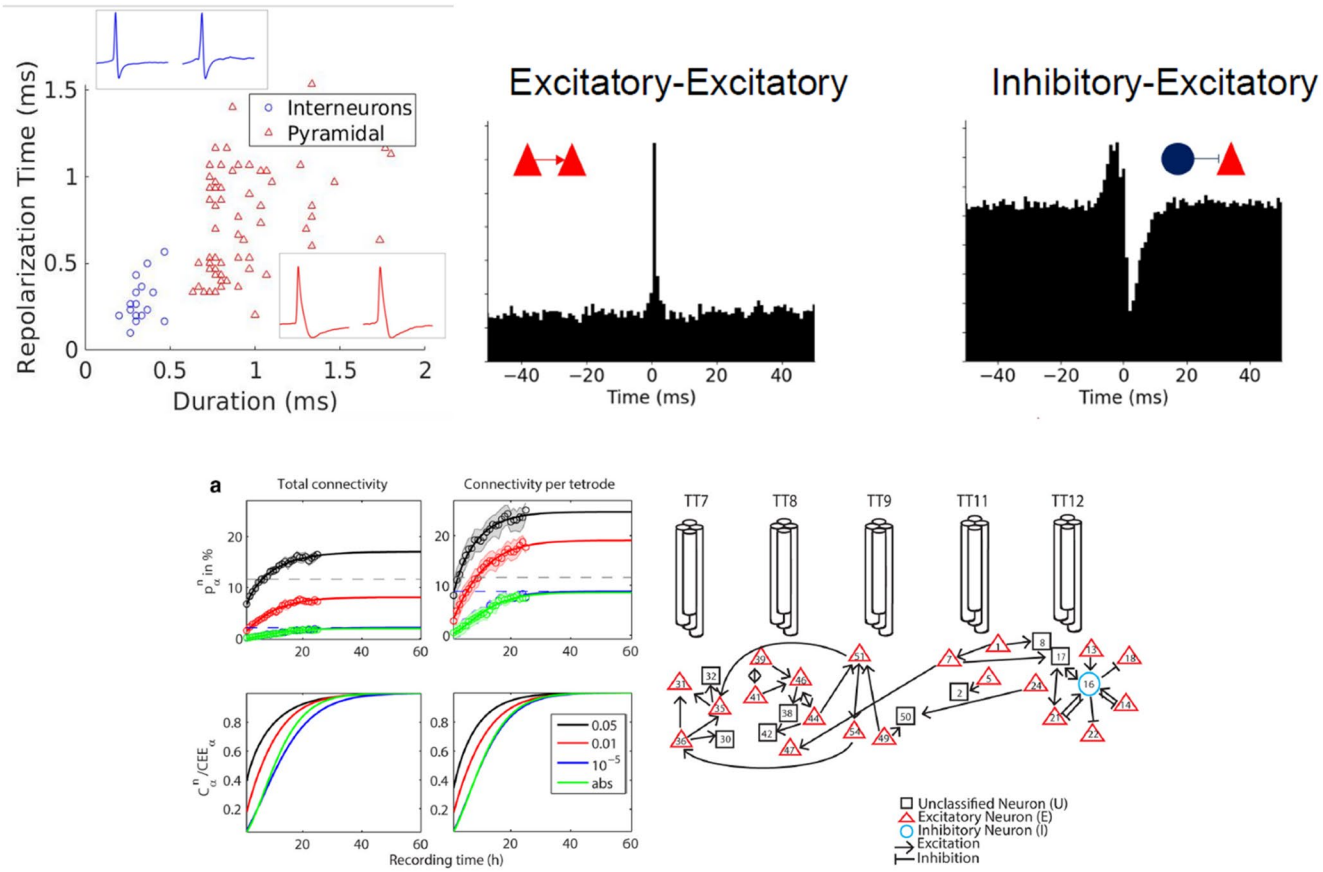

FIGURE 5 | Upper row: Unpublished data illustrating the use of short-latency cross-correlations to infer synaptic connectivity in a recorded neural ensemble (Saxena, R. PhD thesis 2024). Lower row: Detection of statistically significant excitatory functional synaptic connections in the hippocampus (and cortex) generally requires very long (~18–20 h) recording times due to the intrinsic weakness of central excitatory synapses. The connectivity map from one recording is shown at the right (Schwindel et al. 2014).

I might end with a 'no rose without a thorn' comment. In the old days of recording one neuron at a time, which I have denigrated in the foregoing, there was a very valuable factor that we have essentially lost. In those days, one ran the amplifier output through an audio monitor with a thresholdable clipping circuit (two back-to-back Zener diodes), that transformed the white noise of the actual recordings into a series of 'pops' when the largest spikes crossed the threshold. Investigators used actually to *watch* their animals and *listen* to their neural activity. This is how Hubel and Wiesel analyzed visual cortical responses, how O'Keefe discovered 'place' cells (11 units in the original paper), and how James Ranck discovered 'head-direction' cells. Now we only have masses of units that we subject to analyses based on our preconceived notions, but we almost certainly miss many of the truly remarkable behavioral correlates of neural discharge that might revolutionize our thinking. Something for something I guess.

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Data Availability Statement

The author has nothing to report.

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