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Neuronal recording methods

Introduction: Why measure single-neuron activity in humans?

If we are to fully understand how the brain supports cognition, in addition to understanding network oscillations, it is vital to characterize how the activity of individual neurons across widespread regions relate to behavior. A range of laboratory neuroscience studies had examined single-neuron firing during a range of behaviors and showed that neuronal spiking activity contains a wealth of behaviorally relevant information. These studies have shown that neurons across the brain vary their firing rates in relation to behavioral events, including signals related to perception, motor, and memory processes. However, most existent studies on the brain's single-neuron firing patterns come from laboratory studies of animals. This leaves a substantial gap in our understanding because many cognitive processes are difficult or perhaps impossible to study without humans.

Fortunately, in recent years it has been possible to address this gap, with the development of microelectrodes that are deployable in neurosurgical patients (Fried et al., 1999). These electrodes, which extend from the tips of standard clinical depth electrodes, have a small conductive recording surface ($\sim$40 μm diameter) that allows them to record the spiking activity of individual neurons or sometimes small neuron groups (Quiroga et al., 2004). The activity from these cells is thus much more spatially precise compared to the larger-scale signals that are obtained from the macroelectrodes used for conventional intracranial EEG studies, which measure activity from $\sim 5 \times 10^6$ cells (?, ?).

Cluster cutting: Distinguishing individual neurons from microwire recordings

Once a microwire electrode is implanted and connected to a recording amplifier, it immediately begins recording electrical activity from the surrounding tissue. Given their high impedance, these electrodes often sample signals from a radius of roughly 100–200μm. Depending on the anatomical organization and neuropil density in the precise location where each electrode is implanted, this means that a microelectrode may measure the activity of multiple neurons. Fortunately, in many cases the waveforms of individual neurons will appear with different shapes. This is a result of the complex

spatial 3-D distribution of the voltage and currents in the immediate area surrounding each neuron (?, ?). Through a procedure commonly referred to as "cluster cutting" it is possible to differentiate among these waveforms to estimate which specific waveforms belong to separate neurons.

The procedure of cluster cutting is illustrated in Figure **??** (?, ?). Beginning with a recording of the voltage time series from one microelectrode (typically with a sampling rate of at least $\sim$ 10kHz), the purpose of this procedure is to obtain the times when individual action potentials occurred and to label each spike according to the neuron, or "cluster," from which it came. The first step in this process is to take the raw voltage time series and to filter it in at frequencies at $\sim$4kHz and above, which is the band where action potential waveforms show up. Second, a thresholding procedure is then applied, which identifies the timepoints when the amplitude of the filtered signal exceeds a threshold (usually 3–5 standard deviations), which makes then suitable candidates for being an action potential.

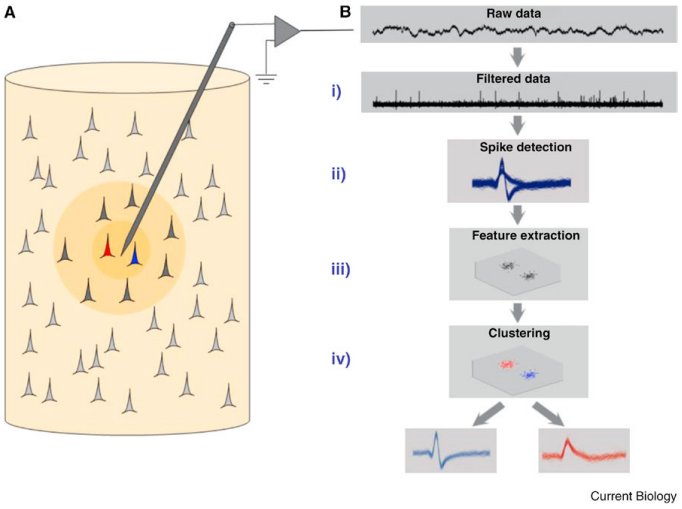

Figure 5.1: Figure from Quoriga 2012 spike sorting article

Following thresholding, the identified timepoints consist of large-amplitude fluctuations that have a duration roughly comparable to the 1–2-ms duration of an action potential. The following steps in cluster cutting are designed to distinguish whether these fluctuations reflect true neuronal action potentials or noise. If they are true action potentials, then this process tries to distinguish which spike waveforms came from different neurons on the basis of differences in wavefrom shape. To accomplish these goals, the next step of cluster cutting involves extracting various features from the shape of each neuron, such as identifying the height of its upward and downward peaks, as well as spectral measurses of its waveform such as its frequency and principal components. Together these measures provide a quantitative summary for the waveform shape from each action potential. This provides a visual guide so as to which neurons have similar shapes and thus are likely to come from the same underlying nueonr. This procedure is generally performed by creating a scatter plot of the features from individual spikes, which generally creates a series of visually discernible clusters—these plots are the cause of

the name "cluster cutting."

Finally, based on the clusters that appear in the scatter plot of spike waveforms, the final steps are to label the clusters that represent distinct neurons, as well as to distinguish the cells that seem to reflect noise clusters. In some cases it is easy to distinguish the clusters that reflect different neurons, if the clusters that reflect their waveform shapes are far from each other in feature space and highly distinct. However, in some cases there is overlap between these clusters and this lack of separation creates situations where the experimenter has to use their own judgement to estimate whether two point clouds reflect different neurons or the same one. A related issue concerns determining which identified waveforms reflect true neuron waveforms versus artifacts or background noise. One technique used to distinguish action potential waveforms that were caused by noise is by conducting spectral analysis of the timecourse of the action potentials from one cluster. This can identify, for example, potential spike waveforms that consistently appear at a freuency of 60 Hz or its harmonics, which would provide a strong indication an apparent cluster was actually a result of noise.

The above description of cluster cutting focuses on the mostly manual steps that must be performed as part of this process. In addition, there are several software packages that can be used to facilitate this process, such as Wave_Clus, Combinato (), or MountainSort, and others. However, even with the use of assistive software, Cluster cutting is an imperfect science that usually requires manual guidance. Nonetheless, the use of this procedure is vitally important step in single neuron physiology.

Following cluster cutting, the next step for most analyses is to compare the activity of each neuron to a person's behavior. Although there are many ways for assessing a neuron's activity from it's spiking, the most common method is to measure the frequency, or firing rate, of a neuron's action potentials in a given interval, and to measure how this quantity varies in proportion to a subject's simultaneous behavior following sections give several examples of how researchers have meausured changes in neuronal firing rates to characterize cells that encode activity related to memory or spatial processing. Together, the studies summarized below show that we now have evidence for neurons in the human hippocampal formation whose activities represents all the key types of features that comprise episodic memories: neural correlates of semantic item/object information, space, and time.

Single-neuron codes for concepts

Because the hippocampus is a common target region for microwire recordings, a focus of many studies is to examine how human single-neuron activity relates to high level cognitive processes including memory. The work in this area compared the firing rates of hippocampal cells as subjects recognized and processed individual stimuli, with a goal of identifying neurons whose firing rates distinguished the specific content of a viewed item. Early work in this area began by comparing neuronal firing rates as neurosurgical patients with implanted microwires viewed words on a computer screen. By comparing firing rates as differnet words were presented, ? (?) reported cells whose firing rates significantly changed according to the identity of a viewed word (Fig. **??**A). Neurons responded when subjects viewed some words but not others. This work raised the intriguing idea that human hippocampal

neurons show abstact representational patterns, and led to follow-up work exploring the specific nature of these representations.

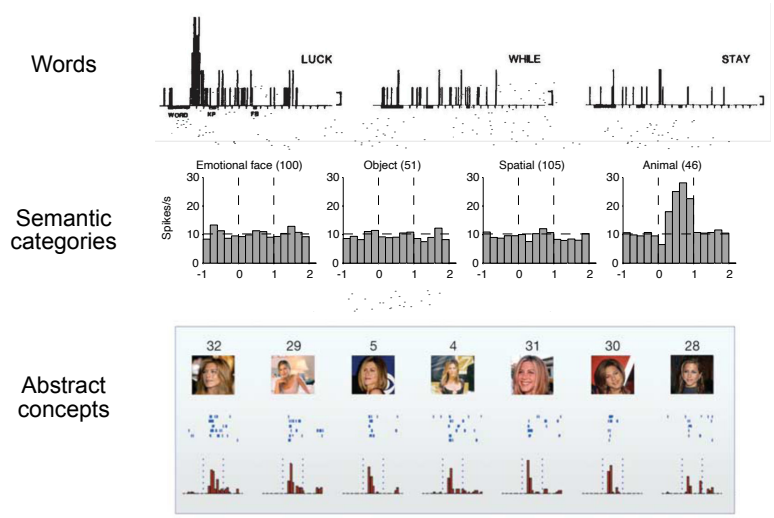

Figure 5.2: (a) word-specific neurons in memory Heit, Halgren, category specific neurons (kreiman 200), (c) jennifer aniston cells

A follow-up study by (?, ?) tested whether the activations of neurons in the human hippocampus were organized semantically, such as whether individual neurons specifically responded to sets of images or words from related categories, as opposed to activating for random stimuli. Figure **??**B shows one figure from this study, illustrating the activity of a neuron that had an increased firing rate when a subject viewed an image of an animal but not objects from other categories. These results emphasized the degree to which neurons in the human hippocampal formation can showed abstract coding properties, with their firing rates correlating with high-level features of a subject's cognitive state, in contrast to neurons from sensory regions that represented perceptual properties of viewed items (?, ?).

The hypothesis that neurons in the human hippocampal formation correspond to abstract representations of a person's cognitive state was boosted by a line of research beginning with ? (?), where subjects viewed different images of the same people. Strikingly, individual hippocampal neurons activated consistently when subjects viewed different images of the same person, such as the example in Figure **??**C, which illustrates the activity of a neuron that activates when the subject viewed images of the actress Jennifer Aniston. The images that caused this neuron to activate were strikingly different, and in fact the same cells activated even when hearing the actress's name. This pattern of results, which was present for many different individuals and sets of stimuli, supports the notion that human hippocampal neurons often show abstract responses that represent conceptual rather than perceptual information.

Across these studies, the pattern of results supports the view that individual cells in the hippocampal system activate to represent abstract features of a person's cognitive state, rather than those related to low-level percepts. Because the hippocampus is known to be vital for memory encoding (?, ?) and has widespread cortical projections (), these findings help explain the na-

ture of the brain-wide networks that support memory retrieval by indicating that the hippocampus is activated by highly abstract neuronal representations that then may reinstate brain-wide patterns that contain more detailed signals related to sensory information (?, ?).

Spatially modulated cells in virtual navigation

In addition to its known role in episodic memory encoding, the hippocampus and surrounding structures also play a role in spatial navigation. Recordings of hippocampal neurons in rats identiifed "place cells," each of which typically show low background firing rates but show high spiking activity when the rat is located at a particular locaiton in a spatial enviornment (O'Keefe & Dostrovsky, 1971). In the nearby entorhinal cortex, neurons behave as "grid cells", activating when a rodent occupies one of many spatial locations that are arrayed across a spatial enviornment as if occupying the vertices of a tesselating series of equilaterial triangle (?, ?). Studies in humans sought to confirm that this phenomenon existed in humans to confirm that this potentially important interspecies similarity related to spatial navigation and cognition (Ekstrom et al., 2003; Jacobs et al., 2013).

Although neurosurgical patients with implanted electrodes are normally confined to their hospital beds, it is possible to study neuronal responses related to spatial cognition using virtual reality. By performing a spatial memory task embedded within a computer-controlled 3D virtual environment, it revealed how neuronal firing rates varied as a function of a subject's virtual location and direction during navigation in virtual reality environments. These studies successfully identified neurons in the human hippocampus and entorhinal cortex that behaved as place and grid cells, by activating when the subject was located at one or many locations across an environment, respectively (Fig. **??**A,B). Furthermore, these studies also identified "head direction" cells, whose firing rate varied as a function of the direction that the subject was pointed in a virtual environment (Figure. **??**C), mirroring findings from rodents (?, ?).

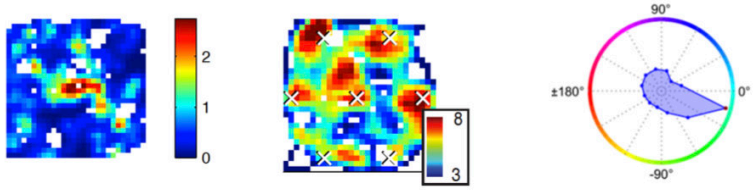

Figure 5.3: Examples of human place (A), grid (B), and direction cells (C). A and B from Jacobs et al., 2013; C from Kunz et al 2020 preprint.

More complex human spatial cell types

Beyond confirming that humans, like simpler animals, have neurons such as place and grid cells that faithfully activate according to a person's own location, more recent work has shown that during navigation humans also show novel spatial firing patterns that were not observed previously in other animals. A more recent study by ? (?) showed that humans also have

neurons whose firing rates correspond to remote locations, in contrast to place cells that represent an animal's own current location. These findings were made by having subjects perform a spatial task where they are directed to follow a specific path during navigation, as seen in Figure **??**A. During this task, the firing of ∼20% of hippocampal neurons significantly changed their firing rate as a function of the location where the subject was trying to go, which was marked in this task by a treasure chest. In contrast to how a standard place cell would be expected to behave, these cells did not respond to the subject's own position. These results suggested that the nature of a behavioral task and its demands are key factors in determining the responses of individual cells in the hippocampal formation, with neurons varying their firing characteristics to represent either a local or remote location according to task demands.

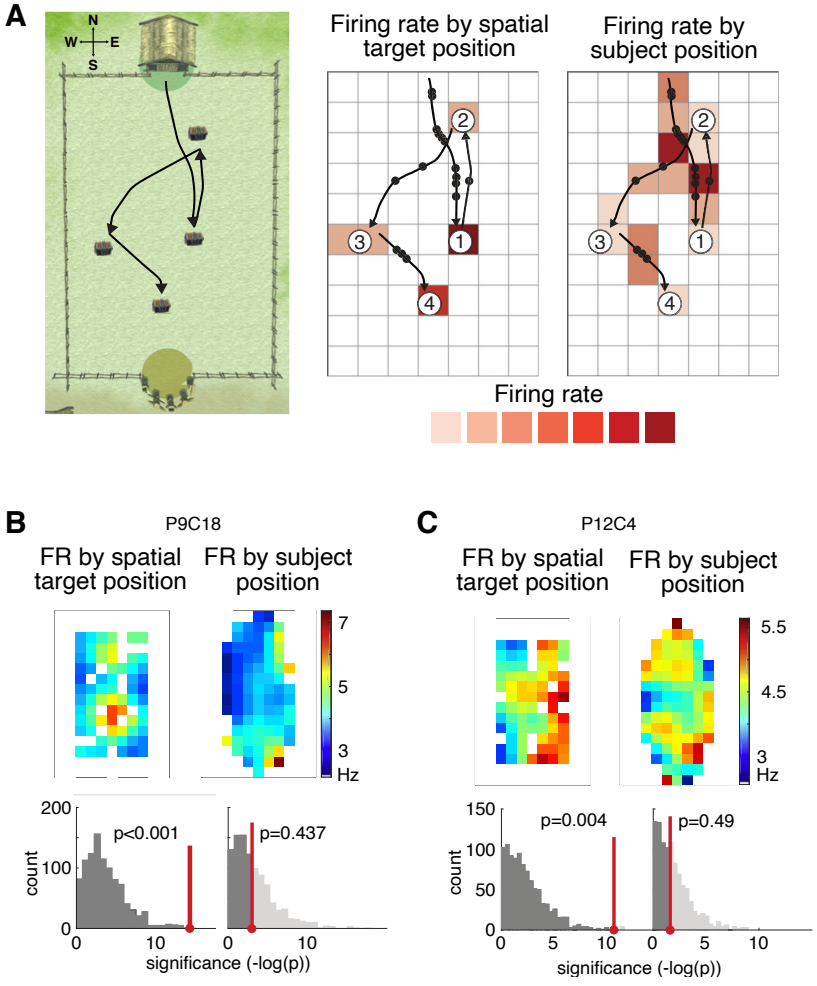

Figure 5.4: Neuron that represents remote spatial targets from Tsitsiklis et al. 2020

For a human neural representation of space to be relevant to support behavior, it might be useful for the particular locations that are represented by each cell to vary according to task demands. A recent study found evidence for exactly this kind of pattern in humans, by showing that spatially modu-

lated neurons in the human entorhinal cortex changed the spatial tuning of their firing according to a subject's memory state (?, ?). In this study, subjects performed a memory task where on each trial they were first given the name of a target object and then moved through a virtual envinment. The environment contained four invisible target objects, which were located at different hidden unmarked locations. Subjects were instructed to press a button when they were located at the position of the cue object.

Thus, on each trial of the task, the subject was focused on a different object and hidden location. To test whether this differing focus modulating neuronal firing patterns, the firing rates of individual neurons were then meausured as a function of the subject's location in the environment as before (Fig. **??**) with the exception that each cue condition was measured separately. By performing this procedure, in addition to identifying place cells, which responded at fixed spatial locations as in eaarlier studies, this study identified a new phenomenon, called "memory trace" cells (Fig. **??**). These cells shifted the locations of their firing fields between trials of the task where subjects were cued on differenet remembred locations. This result is important because it shows that the hippocampal representation of an environment is not a static representation but instead can be transformed according to memory or other behavioral demands. This study suggests that an important area of future research is to identify the factors that cause human neurons to change their spatial firing patterns and to distinguish these factors from signals seen in simpler animals like rodents.

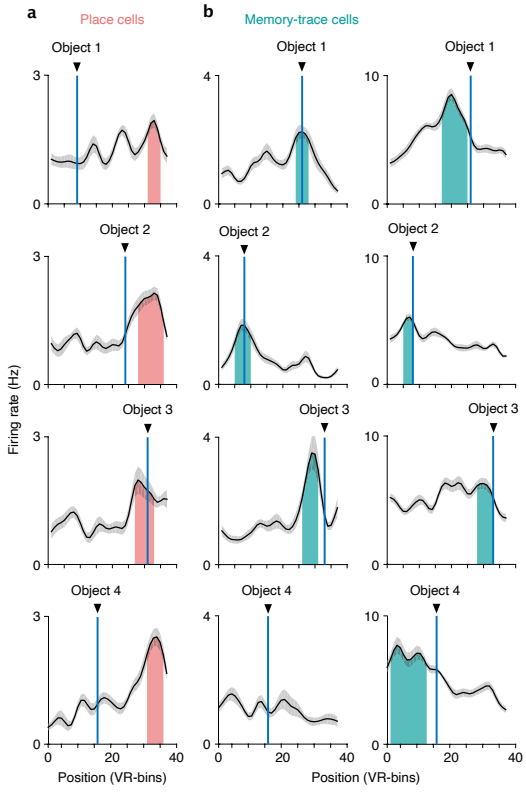

Figure 5.5: Neuron that represents remembered object locations from Qasim et al. 2019

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Time cells

In additional to single-neuron correlates of space and item information, as described above, a final aspect of episodic memory is the ability to associate events with particular moments in one's day or life. By examining recordings of single-neuron activity from subjects performing memory tasks, these studies tested for neural correlates of time by comparing how the firing rates of individual neurons changed throughout the course of memory encoding intervals. Using the spatial memory paradigm described above (Figure **??**), ? (?) identified neurons whose firing rates varied as a function of the order of the item in a list they were trying to learn. Individual hippocampal neurons showed increased firing rates when the subjects learned items at particular item positions (Fig. **??**a,b). Across cells, there was the greatest representation of items at the beginning of each list (Fig. **??**c), which may relate to behavioral findings concerning "primacy" items, by showing that they have stronger and more distinctive neuronal representations.

A related set of findings comes from a study of single neuron firing during the encoding phase of the free recall task (?, ?). Here, the firing rates of individual cells were measured as a function of time during encoding. The results demonstrated the existence of "time cells" in the human medial temporal lobe, each of which activated at a particular timepoint during the encoding interval (Fig. **??**d). Individual time cells activate at different moments during these encoding intervals (although, again, there is an overrepresentation of the beginning of the interval), which suggests that the neural representation from the population of time cells could provide a distinctive neural pattern that uniquely differentiates separate moments to support memory encoding and retrieval.

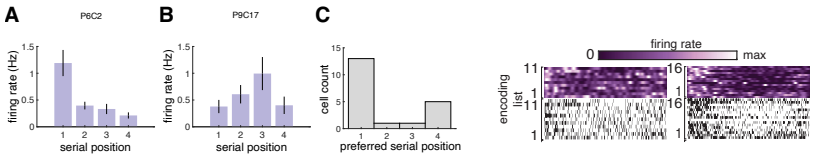

Figure 5.6: Neurons whose firing rates represnt time in the human MTL. (A) Neurons that represent specific moments during list lerning (Tsitsiklis et al 2020). (B) neurons that activate to represent times during liest learning (Umbach et al.)

Conclusion

In summary, single-neuron recordings from the human medial temporal lobe show that the firing rates of individual cells during behavior correspond to an array of different task-relevant variables. In many cases, these findings replicate findings in animals, as well as sometimes extending those results. Human single-neuron firing patterns often show highly abstract representations during complex behaviors, which is one way that the single-neuron firing patterns in the human hippocampus differ from those in other sensory- and motor-related regions. Notably, the neural representations in the hippocampus contain some of the key building blocks of episodic memories, including representations of the semantic content related to a new cognitive state, elapsed time, and location in space. Thus, these single-neuron firing patterns could be instrumental for allowing our brain to form memories.

Future research on neuronal firing patterns has the potential for identifying even further new types of neuronal activity related to cognition, by having human subjects perform richer and ever more complex behavioral tasks.

Applications