Characteristic Neural Firing Profiles in Different Hippocampal Subfields for Successful and Unsuccessful Memory

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Characteristic Neural Firing Profiles in Different Hippocampal Subfields for Successful and Unsuccessful Memory

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Abstract

Memory is our ability to encode, store, retrain, and subsequently recall information and past experiences. Different areas of the brain are responsible for different aspects of memory, including the hippocampus which enables us to form, organize, and store new memories. Numerous research studies show that the hippocampal subfields are affected by memory related diseases such as Alzheimer's Disease and Schizophrenia in different ways. Understanding what the different hippocampal subfields do is important for basic science, but also for understanding neurodegenerative disorders which are associated with structural and functional abnormalities of hippocampal neurons. In order to examine the effects of memory success and failure of the firing patterns of the hippocampal neurons in the different subfields, I used a unique dataset, published by Faraut et al (2018), of a large sample of intracranial neural spiking data from humans.) and ran a hierarchical clustering algorithm on the neural firing patterns. Results suggest that the neurons in the different hippocampus subfields (CA1, CA2, CA3, and DG) have certain firing profiles which as a result causes them to group together according to these specific subfields. These firing patters were different in some degree depending on weather on successful and unsuccessful memory – and thus suggest each subfield processes memories in a different way.

Introduction

Memory can be a complex phenomenon, especially in humans. The Multiple Memory Systems theory states that different brain systems support memory in a different way (Squire 2004). Moreover, in this Multiple Memory Systems theory, different kinds of information are processed and stored in different areas of the brain. These areas include the amygdala, neostriatum, cerebellum, and hippocampus. The hippocampus, located in the medial temporal lobe (MTL), is one of the most critical components for declarative memory, which is our ability to recollect factual knowledge and everyday events (Eichenbaum 2000).

The hippocampus is comprised of different subfields, including the Dentate Gyrus (DG) and a series of *Cornu Ammonis* (CA) areas (CA1, CA2, CA3; Lingford-Hughes et al 2012). Each of these distinct subfields contributes to memory in different ways. Previous rodent research shows different patterns of deficits between rodents with selective CA1 and CA3 lesions. Memory was severely affected by damage to dorsal CA3 subfield when temporal processing demands were minimal. Temporal processing refers to the processing of acoustic stimuli over time (Staff 2014). However, damage to the dorsal CA1 subfield only produced memory impairment when the temporal processing demands (audible or acoustic event) were increased. (Favorik et al 2009). This suggests that there is a difference in memory processing in different experimental conditions, supporting the idea that different hippocampal subfields support memory in different ways.

Similarly, research in humans suggests that it is the dynamic information transfer between the DG and CA3 that helps distinguish between two similar memories (Yassa 2011). The back-projections from CA3 to the DG are the prime candidates for this modulation

(Scharfman 2007). Importantly, neurocognitive aging and dementia tend to lead to deficits in pattern separation, such as confusing two alike memories (Yassa 2011) and therefore, it is beneficial to study how single neurons in these distinct subfields process memories in humans, because this understanding may in turn help understand the causes of memory failures in aging related disease.

One issue with cognitive and clinical neuroscience research in humans is that the macroscopic correlations between hippocampal regions and disease progression and symptoms, such as activity level measured with fMRI, do not tell much about how neuronal firing in these subfields contribute to the disease, if they do at all. Much of the evidence we have about neuronal firing properties in these subfields comes from the rodent experiments cited above. There is, therefore, a big need for studies that are able to get data at the level of single recording units from the human brain. In this thesis, I used a unique dataset, published by Faraut et al (2018), of a large sample of intracranial neural spiking data from humans. By quantifying the different firing patterns in neurons from the different hippocampal subfields and using a hierarchical clustering algorithm, I was able to report different neural firing dynamics in different subfields of the human hippocampus. In addition to finding different neural dynamics, another aim of my thesis was to show how these firing dynamics contribute to memory successes and failures (e.g., failing to discriminate a new stimulus from old ones as a form of pattern separation failure) and to determine whether these firing dynamics relate more to an abnormal CA3 than CA1 firing pattern. Gaining a better understanding of how the neurons in these subfields behave when an individual has successful memory versus when they fail to remember can give us a better understanding of their contribution to memory and can help provide a better understanding of deficits in memory related diseases.

Literature review

Our memory is what makes us who we are. It is memory that helps us function in everyday life and form long lasting relationships. Human memory is the result of complex system interactions, being composed of many separate parts each of which may contribute differently to the whole memory system. This is known as the Multiple Memory System theory, which states that the different kinds of information are processed and stored in different area of the brain, such as the amygdala, neostriatum cerebellum and the hippocampus (Squire 2004). Most of what we know about human memory comes from both amnesic and healthy patients, as well as experimental animal models (Squire 2004). The hippocampus, located in the medial temporal lobe (MTL), is one of the most critical components for memory, specifically declarative memory, which is our ability to recollect factual knowledge and everyday events (Eichenbaum 2000). We know this in part thanks to Henry Molaison (H.M.), who suffered from severe epileptic seizures for roughly ten years. To try to alleviate the seizures, H.M had a bilateral resection of an area including the hippocampal gyrus, and as a result was left with anterograde amnesia or a loss of ability to create new memories (Corkin 1997). Studies on H.M show that the hippocampus is a core brain structure supporting memory along with permanent consolidation of memories (Eichenbaum 2013).

The significance of the hippocampus in relation to memory is also observed in individuals who suffer from MTL amnesia. These individuals suffer loss of episodic memory which is known as failure to remember autobiographical events such as times, places, associated emotions, and other contextual memories. Episodic memories are ones related to past personal experiences that occurred at a specific time and place. We now know, following cases like H.M.,

that the hippocampus is an area in the MTL that is responsible for forming these types of memories (Brown et al 2001). Importantly, the hippocampus includes different subfields, namely CA1, CA2, CA3 and the Dentate Gyrus (DG; Lingford-Hughes et al 2012). These distinct subfields may contribute to memory in different ways.

One example is pattern separation, or the process of transforming memories or representation that are qualitatively similar into different non overlapping neural representations. This may be one of the key functions of the hippocampus, (Bakker et al 2008), and an abundance of evidence has shown that the DG is necessary for pattern separation (Yassa et al 2011). Rodent lesion and genetic knockout studies have also suggested that CA3 subfield may contribute to this process. It is hypothesized that the DG responds to small changes in input that potentially drives pattern separation signals in CA3, leading to an ability to discriminate between two alike stimuli (Yassa et al 2011). Selective damage to the DG/CA3 network leads to pattern separation deficits, which may be the reason for many of the episodic memory problems that are seen in older individuals (Yassa et al 2011). Comparing how single neurons behave in each of these subregions when one has successful memory versus when memory fails, as I do in this thesis, can help us further understand these processes and how they may affect clinical cases.

Beyond normal aging, diseases also give rise to hippocampal damage and memory deficits. Alzheimer's Disease (AD) is one example: a neurodegenerative disease which is the most common cause of dementia and is characterized by many pathological markers, one of which is neuronal decline (Hardy et al 1992). Older adults with AD suffer from selective hippocampal subfield atrophy. Hippocampal subfield CA1 was found to be associated with both visual and verbal episodic memories and therefore, smaller CA1 volumes are associated with poorer episodic memory and may be a marker for normative cognitive decline (Zammit et al

2017). Individuals suffering from AD have a significant reduction in neuronal density in hippocampal subfields CA1 and CA3 in comparison to healthy individuals, with the highest decrease in volume in CA1 (Padurariu et al 2012). Because not all subfields are affected equally, studying different neural firing profiles in these areas during successful and unsuccessful memory, as I do in this thesis, may therefore help us gain a better understanding of the potential causes of memory deficits in AD.

More broadly, understanding whether firing patterns of these different hippocampus subfields give rise to successful and unsuccessful memory is also beneficial for understanding myriad other neuropsychiatric disorders which are associated with structural and functional abnormalities of hippocampal neurons. Schizophrenia is a neuropsychiatric disorder which can affect the mental state of an individual such as effects on one's thoughts and feelings, as well as cognitive challenges including problems with memory and attention. It was found that there is a significant reduction in hippocampal neurons in individuals that suffer from schizophrenia. This damage is primarily in the CA1 subfield of the hippocampus. (Eggers et al 2013). Because diseases like Schizophrenia also do not affect the hippocampus subfields equally, understanding how neurons in each of the subfields behave during memory can help us better understand altered cognition and memory phenomenon.

In this thesis, I aimed to find data that help us better understand the relationship between neuronal behavior in the different hippocampal subfields and memory performance. If we can understand how the neurons in these subfields behave when an individual remembers versus when they fail to remember, we can have a better understanding of their contribution to memory and how we can apply this knowledge to memory related diseases, such as AD and other forms of dementia, that affect these different hippocampal subregions.

Methods

Data Set

Human neural activity data are traditionally collected via the utilization of volunteer subjects. However, data that require surgery, as is necessary for single neuron activity measures, are not something that can be done voluntarily. Therefore, it is rare to obtain direct recordings from neurons in human hippocampal subfields. We have to obtain these data from individuals who are undergoing a medical procedure and need the electrodes surgically implanted as a result their medical conditions. In order to address the question of subfield differentiation in firing patterns, I used a public dataset published by Faraut et al that provide the rare opportunity to investigate human single neuron spiking on a large scale, which could be used to investigate the variety of firing patterns of neurons in the different hippocampal subfields.

The dataset included 42 patients, male and female ages 16-70, with intractable epilepsy that underwent intracranial monitoring for localization of their epileptic seizures (see Table 1). Electrodes were implanted intracranially (electrodes placed directly on the exposed surface the brain) in the hippocampus and the amygdala (see Image 1). During the time they were being monitored, the patients participated in a memory task. As a result, we get single neuron recordings from 1,576 neurons in the human hippocampus and amygdala from 42 individuals whilst completing a recognition memory task

Memory task

During the time they were being monitored subjects completed two trials of a recognition memory task.

The first trial included **encoding**, where subjects were shown 100 images. The encoding trial was followed by a **retrieval** trial where the subjects were shown another set of 100 images, 50 of those were from the previous encoding trial and 50 were novel images. Stimuli were displayed for either one or two seconds. (see Image 2).

Subjects were asked to recall whether they had previously seen the image or if it was new and rate their confidence in their answer on a scale of one to six. Subjects' neural activity was recorded from the amygdala and hippocampus. The data that was collected included 1576 single neurons from the 42 participants.

Data Retrieval

These data are stored in a MATLAB file. To retrieve the data, we used information from the original paper (Faraut et al 2018) which gave directions and information on which MATLAB files included which data. The location coordinates of the intracranial electrodes in a standardized brain template space are given in MNI coordinates, which allows for identification of the brain region in which an electrode was implanted, and single neuron spiking data were retrieved in this way. It should be noted that the location coordinates were in a standardized template brain space, therefore, they indicate to which brain area a specific neuron most likely belongs, but not the precise localization that would be done from examining a single patient's anatomy. This allowed us the ability to estimate the localization of a specific firing pattern to a hippocampal subfield. in addition, some neurons were not included in the analysis because they did not have MNI coordinates or neural firing patterns that changed over the course of time.

Location Coordinates

Statistical Parametric Mapping (SPM) on MATLAB was used to input the MNI coordinates and quantify the probability of the location of each electrode in the area of interest,

which included four areas in the hippocampus (CA1, CA2, CA3, and the DG) and 2 areas in the amygdala (see Image 1).

Data Analysis

Neural firing patterns for all 1576 neurons in the 65 sessions were combined together for data analysis. There were four separate condition groups (each including the firing pattern of the 1576 neurons in that group's condition), showing the neural firing pattern when the subject recognized an image either correctly as old (Old) , correctly as new (New), incorrectly as new when it was old (Old_i), or incorrectly as old when it was new (New_i). The firing pattern for each of the neurons was temporal, showing its spiking pattern across seven time bins of 500 ms each. The mean firing pattern for each neuron in each of the four conditions was calculated.

Analysis was done using a hierarchical clustering algorithm. This algorithm clustered the neurons according to how similar (or dissimilar) their firing patterns were. Clusters at one level join with clusters in the next level up (i.e., an agglomerative algorithm), using an increasing degree of dissimilarity until the clusters all join together at the top. I was interested in investigating the first two levels to see if the groupings were based on a specific neural firing profile characteristic of a certain hippocampal subfield. Two separate analyses were done. The first analysis was at the first level of clustering, where the dendrogram first splits into two clusters (representing the two most dissimilar patterns of firing) and the second was at the breakdown of the aforementioned two clusters (representing dissimilarities within those first two primary clusters). The goal of these analyses was to investigate if there is a unique firing pattern causing these neurons to get clustered together at these two levels according to their subfield.

A chi-square test of goodness-of-fit was performed to determine whether neurons in each of the five brain areas were equally distributed between the clusters formed (that is, the Null,

indicating that neurons of a given brain area do NOT have a dominant firing pattern at the first or second level of clustering for a given memory condition), or if the clustering was based at least in part on subregion identity. This analysis was done for each of the brain areas (CA1), CA2, CA3, DG, and amygdala) in each neuron condition (Old, Old_i, New, and New_i). (See images: 3-6 and tables 6-7).

Results

First Level Analysis

Condition: New

These results include firing patterns of each neuron when the subject correctly recognized an image as a novel one during the recognition trial in the memory task. There were no significant difference in CA1, CA2, and CA3 neuron distribution when compared to an even distribution (see Table 2a). There were, however, a significant difference in DG neuron distribution when compared to an even distribution, X^2 (4, N = 92) = 6.2, p = .01. Significant distribution was also found with neurons in the amygdala, X^2 (4, N = 846) = 81.01, p < .01 (see Image 2). For the purposes of this thesis, this result suggests that DG has a characteristic firing pattern for correctly identified/discriminated new stimuli.

Condition: New_i

These results include firing patterns of each neuron when the subject incorrectly recognized an image as old when it was novel, during the recognition trial in the memory task. There were no significant differencse in CA1, CA2, and DG neuron distribution when compared to an even distribution (see Table 3a). There was a significant difference in CA3 neuron

distribution when compared to an even distribution, X^2 (4, N = 116) = 4.14, p =.04. Significant results were also found in neurons in the amygdala, X^2 (4, N = 784) = 92.87, p < .01. For the purposes of this thesis, this result suggests that CA3 has a characteristic firing profile for incorrectly identified new stimuli.

Condition: Old

These results include firing patterns of each neuron when the subject correctly recognized an image as one they had previously seen during the encoding trial in the memory task. There were no significant difference in CA1, CA2, and DG neuron distribution when compared to an even distribution (see Table 4a). These results suggest that there is not a unique firing pattern grouping these neurons. Results showed a significant difference in CA3 neuron distribution when compared to an even distribution, X^2 (4, N =116) = 4.12, p = .04. These results suggest that these neurons have a similar firing pattern grouping them together. Similar significant results were also found in the amygdala, X^2 (4, N = 842) = 28.9, p < .01. For the purposes of this thesis, this result suggests CA3 has a characteristic firing pattern for correctly identified/discriminated new stimuli.

Condition: Old_i

These results include firing patterns of each neuron when the subject erroneously called an image new (i.e. they failed to recognize it).. There was a significant difference in the distribution of CA1 neurons when compared to an even distribution, X^2 (4, N =226) = 44.05, p < .01. Similar results were found with the remaining brain areas in this condition: CA2, X^2 (4, N =50) = 15.68, p < .01, CA3, X^2 (4, N =116) = 28.75, p < .01, DG, X^2 (4, N =78) = 16.61, p < .01, and the amygdala, X^2 (4, N =822) = 159.41, p < .01 (see Table 5a). These results suggest

that all hippocampal subfields had a characteristic firing pattern for when subject incorrectly recognized stimuli as new when it was old.

Second Level Analysis.

The purpose of the first Level analysis was to identify whether subregion identity contributed to the most prominent organizational principle in the data for each of the four memory conditions. The Second Level analysis asked whether, in cases where that was not true, if the subregion identity was a "secondary" organizing principle in how neurons fired. (See Images: 3-6 and Tables 6-7).

Condition: New

There we no significant difference in DG neuron distribution when compared to an even distribution (see Table 2b). There was, however, a significant difference in CA1 neuron distribution when compared to an even distribution, X^2 (4, N = 227) = 13.9, p = .003. Significant distributions were also found in CA2, X^2 (4, N = 74) = 15.14, p = .001, CA3, X^2 (4, N = 118) = 8.72, p = .033, and the amygdala, X^2 (4, N = 847) = 92.40, $p < 01$. This result suggests that CA1, CA2, and CA3 region identities were secondary factors that influence spiking patterns for New stimuli.

Condition: New_i

There were no significant differences in CA1, CA2, and DG neural distribution when compared to an even distribution (see Table 3b). A significant difference was found in CA3 neuron distribution when compared to an even distribution, X^2 (4, N = 117) = 10.96, p =.011. Significant distributions were also found in the amygdala, X^2 (4, N = 785) = 95.38, $p < 01$. This result suggests that CA3, region identities were secondary factors that influence spiking patterns

for when the subject incorrectly identifies a stimulus as one they had previously seen, when it was a novel stimuli.

Condition: Old

No significant differences were found in CA3 neural distribution when compared to an even distribution (see Table 4b). However, there was a significant distribution found in CA1 neurons, X^2 (4, N = 227) = 19.83, p < 01. Additionally, a significant distribution was also found in CA2 neurons X^2 (4, N = 74) = 15.13, p = 001, neurons in DG, X^2 (4, N = 92) = 11.91, p = 007, and in the amygdala, X^2 (4, N = 842) = 196.08, p < 01. This result suggests that CA1, CA2, and DG region identities were secondary factors that influence spiking patterns for New stimuli.

Condition: Old_i

There were no significant differences in CA2 and DG neural distribution when compared to an even distribution (Table 5b). However, CA1 neural distribution was found to be significant when compared to an even distribution X^2 (4, N = 215) = 945.62, p < 01. A significant distribution was also found in CA3 neurons, X^2 (4, N = 113) = 22.46, p < 01. And in amygdala neurons, X^2 (4, N = 809) = 224.96, p < 01. This result suggests that CA1 and CA3, region identities were secondary factors that influence spiking patterns for when the subject incorrectly identifies a stimulus as new.

Discussion

This present study investigated single neurons of subjects as they were performing a recognition memory task. Data included neurons from four hippocampal subfields (CA1, CA2, CA3, DG) and the amygdala. We know from previous research findings that deficits in these different brain regions have different effects on memory. Alzheimer's Disease and Schizophrenia

research shows that individuals suffering memory loss have a significant reduction in neuronal density in CA1 and CA3 hippocampal subfields (Padurariu et al 2012; Eggers et al 2013). Therefore, our goal was to investigate if such neurons have a unique firing pattern characterizing a specific brain area. By doing so we can gain a better understanding of how neurons in these subfields behave during memory success and memory failure. Taken together, the present findings provide evidence suggesting hippocampal neurons have firing profiles that organize according to specific hippocampal subfields. Which subregions had characteristic firing patterns differed to some degree according to memory success and failure, indicating that subregions may indeed process memories differently from one-another.

Analysis was first done at the first level (first split) to determine whether subfield was a factor that organized firing patterns at the highest level in the data. The next analysis was done at the second level (where the Level One clusters split further) to determine whether subfield was an organizing principle for neural firing patterns if, perhaps, it was not that primary factor (Level One). One interesting observation was that there were some subfields whose neurons clustered together at the first level of analysis, but not further at the second. This could have been a statistical power issue, due to the smaller sample size in each cluster, in comparison to the first level, for the Chi-Square test causing them to be non-significant. Another reason may have been that there were truly only two true groupings in the data according to region, which manifest at the highest level of the hierarchy, such that when we look at an additional split at the second level the relative number of bins in which there is a null difference in how a subregion is distributed is higher as a result of there only being two big differences but, four clusters were being tested.

Condition: New

We found that when the subjects correctly recognized the images as a new image there was a significant clustering of DG neurons at the first level, and also clustering of the rest of the subfields at Level 2. This suggests that these DG neurons had a similar firing profiles causing them to get grouped together. These results are consistent with research showing that the DG serves an important role in novelty detection (Lee et al 2015). Research has also shown the role of the DG along with CA3 involving pattern, the ability to distinguish between two alike memories, and here we see a dominant unique firing pattern in the DG neurons when an individual successfully recognized a new image as being novel.

Condition: New_i

Additionally, we found that when subjects incorrectly identified a new image as one they had previously seen (replied Old), there was a significant clustering of CA3 neurons. Previous findings indicate that CA3 may be involved in both memory retrieval and pattern separation (Kesner 2007), alongside the DG (Yassa 2011). Failure of the dynamic between the two regions may have caused the subject to incorrectly recognize a new image as "old". In this condition, when memory was unsuccessful, it may be that the DG is failing to create this firing profile needed for successful memory, which may be the cause of memory failure. We see evidence for this in the clear null clustering in DG. It is possible that this failed discrimination, in turn, contributes to the clustering in CA3 downstream from DG (note that CA3 neurons do cluster for Old stimuli).

Condition: Old

We found that when subjects correctly recognized an image as ones they had previously seen, CA3 neurons clustered at the first level. By contrast, the rest of the region were found to

cluster at the second level of the hierarchy. Neurons located in CA1 had the highest significance in distribution (albeit at the second level of the hierarchy) which may be consistent with previous research findings stating that CA1 is essential for object recognition memory (Mello-Carpes & Izquierdo 2013).

Condition: Old_i

Of note, we found that when subjects failed to identify an image as ones they had previously seen and thought the image was novel, there was a significant clustering in all subfields in comparison to an equal distribution at the first level. These results seem to suggest that the hippocampus at large has a certain firing profile that's really characteristic for when you fail to recognize something.

Limitations

A potential limitation for the present study was that the MNI coordinates, which provide the location of the electrodes in the hippocampus in standardized space, are not an exact measurement. This standardization system does not precisely account for individual differences in the position and overall shape of the subfields. As such, it allowed me to estimate the probabilities of electrode (neuron) location in each subfield, but these labels are not exact. Additionally, due to the nature of the data with one electrode per hemisphere per person, we had to combine all subjects' neural data together rather than investigating in each of them individually. Every subject's brain is slightly different and may have different firing profiles, but my analysis therefore had to test for commonalities across people. Using the mean neural firing patterns and generalizing across all subjects was a limitation of this investigation since this was not sensitive to the individual differences between subjects' brains. Another potential limitation is the sample size of the neural data. Although this was a uniquely large intracranial recording

dataset, some individuals did not have MNI coordinates for their electrodes, so I was not able to estimate the location of the neurons in the brain for analysis. Additionally, some neurons did not have a measurable firing pattern that changed overtime, and therefor had to be taken out of the analysis. Finally, a large parentage (over 50%) of the neurons recorded were located in the amygdala, and while research does show that the amygdala contributes to memory, these data could not be used to test my questions about hippocampal subfield differences. Lastly, the subjects who participated in the study all suffer from epilepsy, while we cannot know if this affected their memory in any way, we do not have data from healthy individuals to act as a control group for these data.

Future Directions

In the future, we hope to investigate the what the unique firing pattern of these regions are. We found results that suggest that there are firing profiles that are characteristic of specific brain areas and that vary for different memory states. It would therefore be interesting to investigate what the firing profiles are. For example, when we fail to remember does a CA1 neuron have high early firing activity followed by low activity? What would the mechanism significance of such a firing profile be? Investigating the specific spiking behavior can help us better understand or predict memory deficits in individuals with memory loss such as Alzheimer's.

Another interesting aspect to investigate in the future would be relating neural firing patterns to one's confidence in their task performance. We know from examining some of the 1576 individual neurons in the data that some appear to track a person's decision regardless of whether they are correct or not. Here I report characteristic firing patterns for memory successes and failures. Similarly, when one is confident that they are correct, whether they are right or

wrong, it is unknown whether there subfields with a similar pattern reflecting their confidence. These types of firing data could speak to memory errors, for example, if some subregions contribute to false memory when they fire a certain way.

Finally, future studies could also investigate what is going on in the other dimensions of the data to determine how the deeper layers of the clustering are organized. This organization may include different firing patterns for recognition of different types of stimuli, such as people vs objects, or different activity for encoding and recognition.

In conclusion, my thesis offers evidence that neurons in the different hippocampal subfields (CA1, CA2, CA3, and DG) have certain firing profiles casing them to group together according to specific subfields. These firing patters were different in some degree depending on whether memory was successful or unsuccessful. This suggests that each subfield processes memories in a different way.

Figures and Tables

Age	Sex	Epilepsy Diagnosis
55	M	Right Mesial Temporal
37	M	Left Frontal
16	M	Right Lateral Frontal
31	M	Bilateral Indep. Temporal
45	M	Right Mesial Temporal
34	F	Right Frontal
19	M	Left Inferior Frontal
40	M	Right Mesial Temporal
34	M	Left Frontal
20	M	Not Localized
40	M	Left Mesial Temporal
40	M	Bilateral Indep. Temporal
22	M	Right Mesial Temporal
17	F	Left Deep Insula
30	M	Right Mesial Temporal
29	M	Left Mesial Temporal
29	M	Not Localized
27	F	Left Mesial Temporal
57	F	Right Mesial Temporal
20	M	Right Mesial Temporal
54	M	Left Mesial Temporal
24	M	Bilateral Frontal and Temporal
47	F	Not localized
36	F	Bilateral Indep. Mesial Temporal
56	F	Left Mesial Temporal
44	M	Left Mesial Temporal
19	M	Left Neocortical Temporal
32	M	Left Neocortical Temporal
19	M	Not Localized (Generalized)
44	F	Right Mesial Temporal
70	M	Bilateral Mesial Temporal
33	F	Right Mesial Temporal
63	F	Right Mesial Temporal
26	M	Right Insula
25	M	Right Motor Cortex
25	F	Not Localized
42	F	Left Mesial Temporal
53	F	Right Mesial Temporal
32	M	Right Mesial Temporal
32	F	Left Mesial Temporal
24	F	Left Mesial Temporal
17	M	Not Localized (No Seizures)

Table 1: Shows the patients' demographics and pathology.

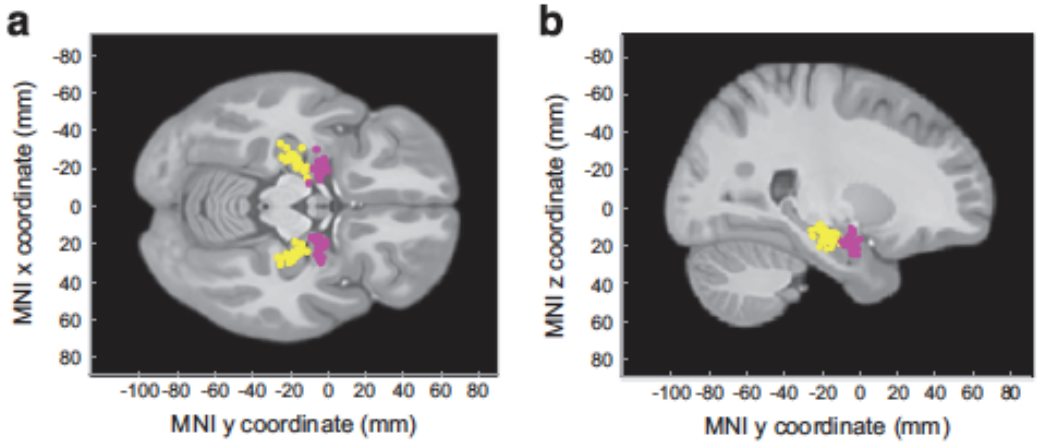

Image 1: Structural MRI showing the electrode placement for areas of patients in which the units were recorded. The yellow shows the hippocampus ad the amygdala is in purple.

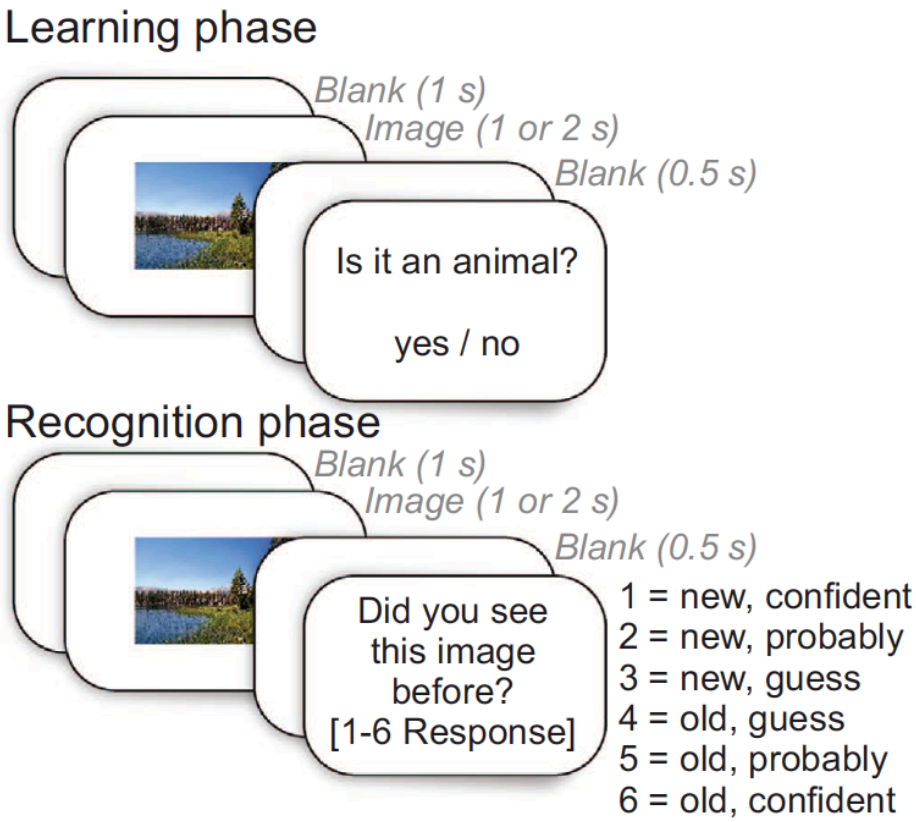

Image 2: Shows the memory task. The task was composed of a learning phase during which 100 new images are shown to subjects; and a recognition test phase showing both new and old images to subjects who indicate whether they had seen it or not in the learning phase by reporting their confidence level.

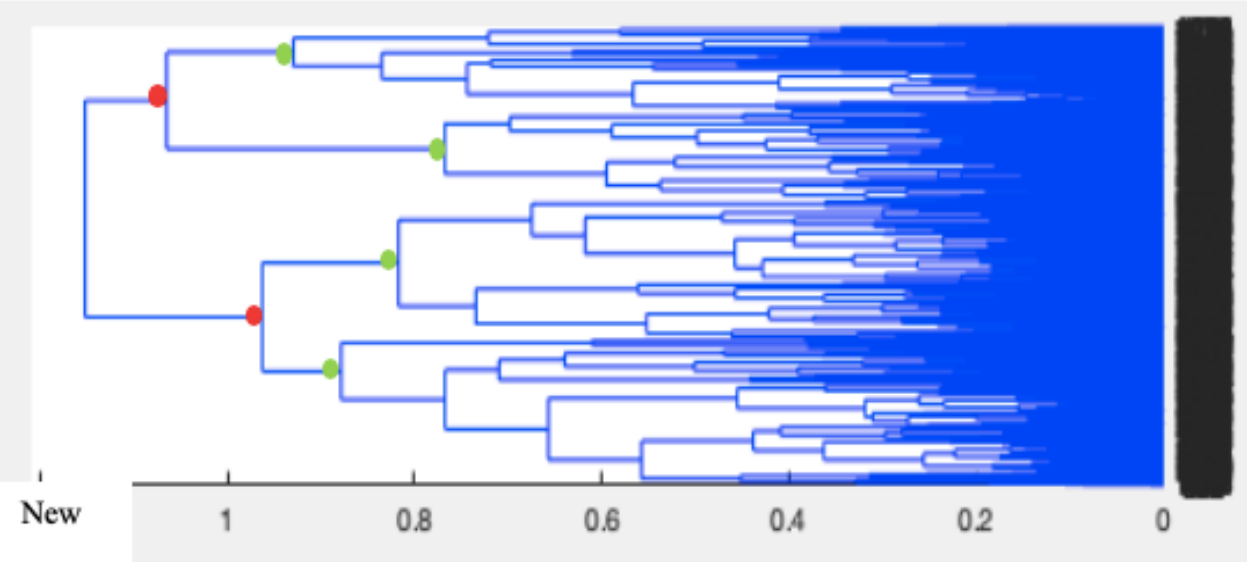

Image 3: Shows results of the hierarchical clustering algorithm for the "New" condition. Level one clusters are indicated by red dots, and level two clusters are indicated by green dots.

Condition:

Brain Area	Chi^2 Number	P-value
CA1	1.128	0.29
CA2	1.195	0.16
CA3	1.67	0.2
DG	6.20	0.01*
Amygdala	81.01	<.01*

Table 2a: Shows statistical values for the "**New**" condition in the **Level One** analysis "*" indicate significant values at the p = .05 level

Brain Area	Chi^2 Number	P-value
CA1	13.9	.003*
CA2	15.14	<.00 *
CA3	8.71	<.03*
DG	5.41	<.15
Amygdala	92.40	<.01*

Table 2b: Shows statistical values for the "**New"** condition in the **Level Two** analysis "*" indicate significant values at the p = .05 level

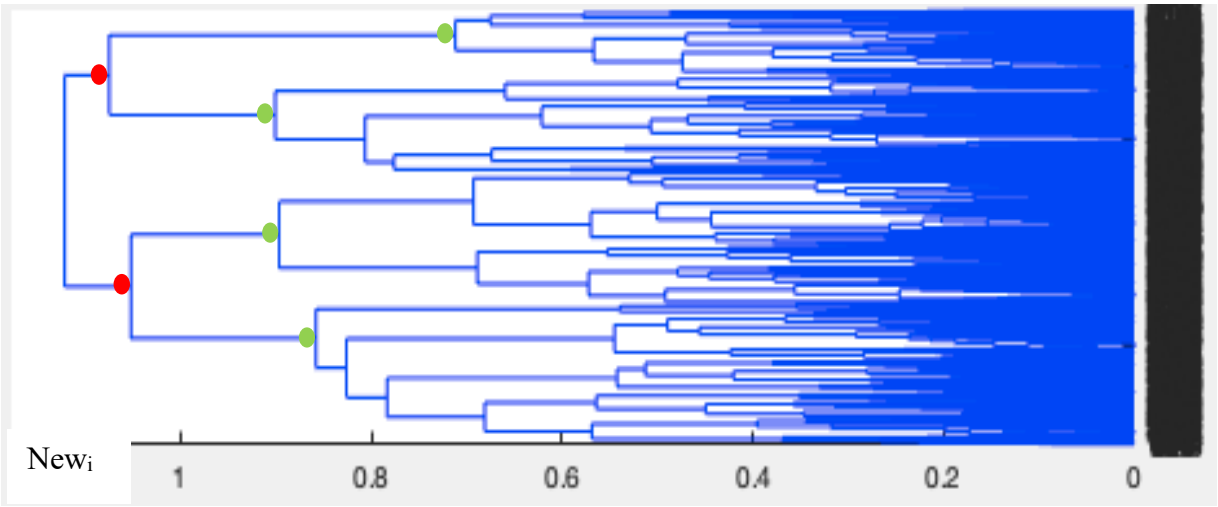

Image 4: Shows results of the hierarchical clustering algorithm for the "New$_i$" condition. Level one clusters are indicated by red dots, and level two clusters are indicated by green dots.

Brain Area	Chi^2 Number	P-value
CA1	0.07	0.79
CA2	2.29	0.88
CA3	4.137	0.04*
DG	0.18	0.67
Amygdala	92.87	<.01*

Table 3b: Shows statistical values for the **"New_i"** condition in the **Level One** analysis "*" indicate significant values at the p = .05 level

Brain Area	Chi^2 number	P-value
CA1	4.9	.18
CA2	4.35	.34
CA3	10.96	.01*
DG	1.13	.76
Amygdala	95.38	<.01*

Table 3c: Shows statistical values for the "**New_i"** condition in the **Level Two** analysis "*" indicate significant values at the p = .05 level

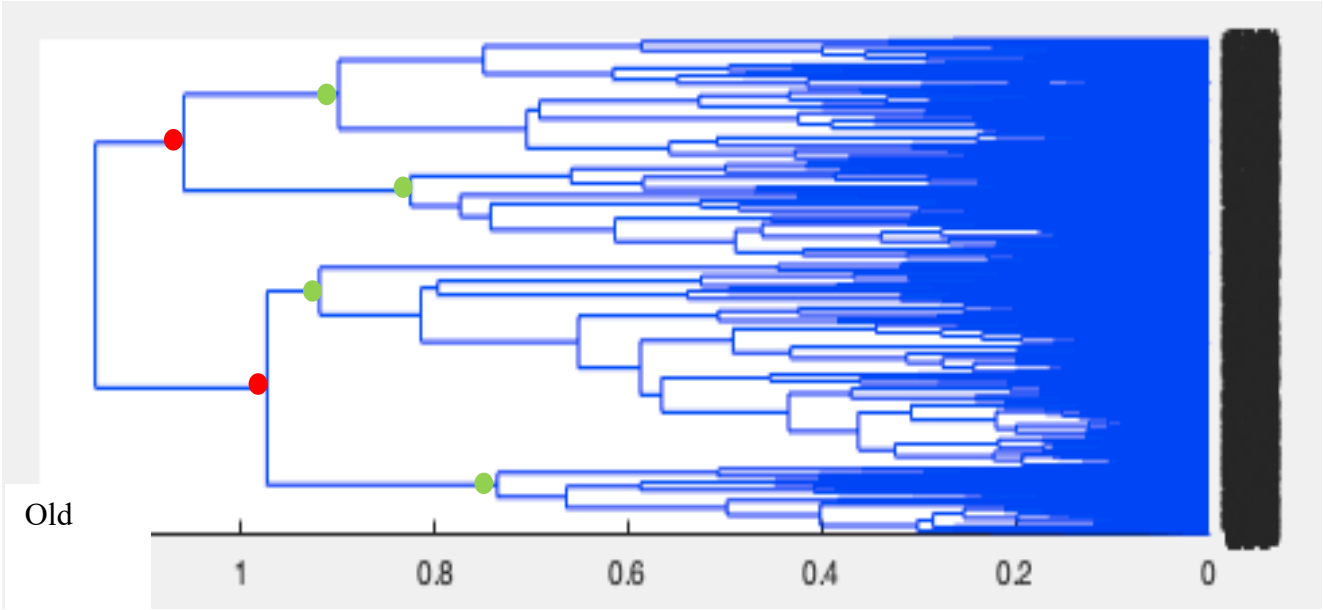

Image 5: Shows results of the hierarchical clustering algorithm for the "Old" condition. Level one clusters are indicated by red dots, and level two clusters are indicated by green dots.

Brain Area	Chi^2 Number	P-value
CA1	0.07	0.79
CA2	0.49	0.48
CA3	4.12	0.04*
DG	0.39	0.53
Amygdala	28.9	<.01*

Table 4a: Shows statistical values for the **"Old"** condition in the **Level One** analysis "*" indicate significant values at the p = .05 level

Condition: Old; Level 2

Brain Area	Chi^2 Number	P-value
CA1	19.83	<.01*
CA2	15.13	.001*
CA3	7.22	.065
DG	11.91	.007*
Amygdala	196.08	<.01*

Table 4b: Shows statistical values for the **"Old"** condition in the **Level Two** analysis "*" indicate significant values at the p = .05 level

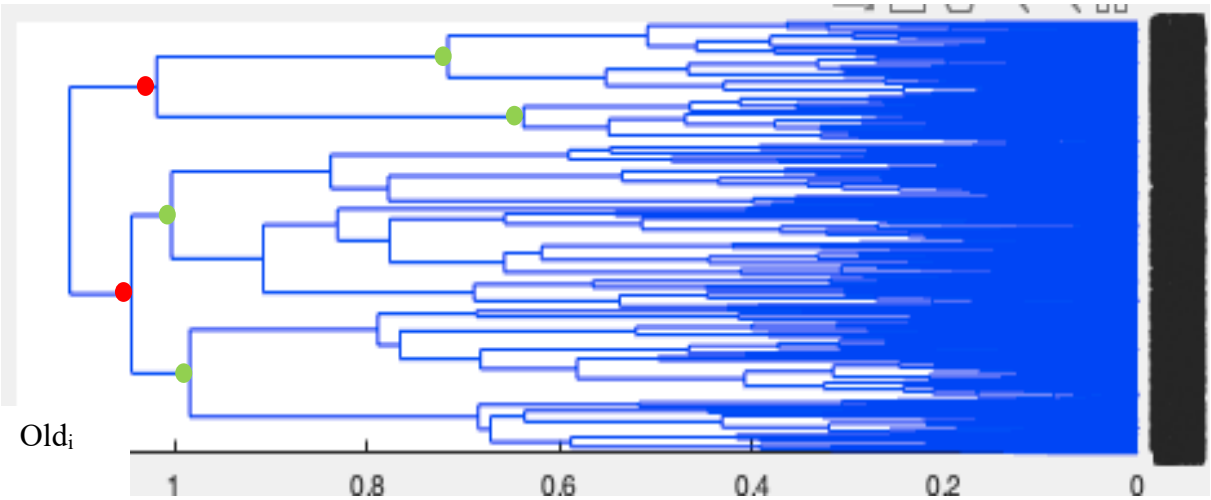

Image 6: Shows results of the hierarchical clustering algorithm for the "Old$_i$" condition. Level one clusters are indicated by red dots, and level two clusters are indicated by green dots.

Brain Area	Chi^2 Number	P-value
CA1	44.05	<.01*
CA2	15.68	<.01*
CA3	28.75	<.01*
DG	16.61	<.01*
Amygdala	159.41	<.01*

Table 5a: Shows statistical values for the **"Old$_i$"** condition in the **Level One** analysis "*" indicate significant values at the p = .05 level

Brain Area	Chi^2 Number	P-value
CA1	45.62	<.01*
CA2	1.44	.07
CA3	22.46	<.01*
DG	4.61	.20
Amygdala	224.96	<.01*

Table 5b: Shows statistical values for the **"Old$_i$"** condition in the **Level Two** analysis "*" indicate significant values at the p = .05 level

New			New_i		
Brain Area	# of Neurons	# of Neurons	Brain Area	# of Neurons	# of Neurons
	Cluster 1			**Cluster 1**	
	Observed	Expected		Observed	Expected
CA1	121	113	CA1	110	108
CA2	43	37	CA2	40	33
CA3	66	59	CA3	69	58
DG	58	46	DG	47	45
AMY	554	423	AMY	527	392
	Cluster 2			**Cluster 2**	
	Observed	Expected		Observed	Expected
CA1	106	113	CA1	106	108
CA2	31	37	CA2	27	33
CA3	52	59	CA3	48	58
DG	35	46	DG	44	45
AMY	293	423	AMY	258	392
Old			Old_i		
Brain Area	# of Neurons	# of Neurons	Brain Area	# of Neurons	# of Neurons
	Cluster 1			**Cluster 1**	
	Observed	Expected		Observed	Expected
CA1	115	113	CA1	63	113
CA2	34	37	CA2	11	25
CA3	47	58	CA3	29	58
DG	43	46	DG	21	39
AMY	499	421	AMY	230	411
	Cluster 2			**Cluster 2**	
	Observed	Expected		Observed	Expected
CA1	112	113	CA1	164	113
CA2	40	37	CA2	39	23
CA3	70	58	CA3	88	58
DG	49	46	DG	57	39
AMY	343	421	AMY	592	411

Table 6: Shows number of neurons that were observed in each cluster and the number of neurons that were expected (evenly distributed between each cluster) in level one analysis.

New			New_i		
Brain Area	# of Neurons	# of Neurons	Brain Area	# of Neurons	# of Neurons
	Cluster 1			**Cluster 1**	
	Observed	Expected		Observed	Expected
CA1	41	56	CA1	63	54
CA2	11	18	CA2	16	16
CA3	24	29	CA3	41	29
DG	28	23	DG	27	22
AMY	311	211	AMY	260	196
	Cluster 2			**Cluster 2**	
	Observed	Expected		Observed	Expected
CA1	80	56	CA1	48	4
CA2	32	18	CA2	24	16
CA3	42	29	CA3	28	29
DG	30	23	DG	20	22
AMY	243	211	AMY	267	196
	Cluster 3			**Cluster 3**	
	Observed	Expected		Observed	Expected
CA1	52	56	CA1	45	54
CA2	17	18	CA2	14	16
CA3	30	29	CA3	16	29
DG	19	23	DG	21	22
AMY	146	211	AMY	114	196
	Cluster 4			**Cluster 4**	
	Observed	Expected		Observed	Expected
CA1	54	56	CA1	61	54
CA2	14	18	CA2	13	16
CA3	22	29	CA3	32	29
DG	16	23	DG	23	22
AMY	147	211	AMY	144	196
Old			Old_i		
Brain Area	# of Neurons	# of Neurons	Brain Area	# of Neurons	# of Neurons
	Cluster 1			**Cluster 1**	
	Observed	Expected		Observed	Expected
CA1	34	56	CA1	25	53
CA2	5	18	CA2	15	18
CA3	17	28	CA3	21	28
DG	15	23	DG	21	23
AMY	120	210	AMY	63	202
	Cluster 2			**Cluster 2**	
	Observed	Expected		Observed	Expected

CA1	81	56	CA1	43	53
CA2	29	18	CA2	17	18
CA3	30	28	CA3	12	28
DG	28	23	DG	16	23
AMY	379	210	AMY	135	202
	Cluster 3			**Cluster 3**	
	Observed	Expected		Observed	Expected
CA1	59	56	CA1	54	53
CA2	22	18	CA2	22	18
CA3	34	28	CA3	35	28
DG	15	23	DG	25	23
AMY	146	210	AMY	302	202
	Cluster 4			**Cluster 4**	
	Observed	Expected		Observed	Expected
CA1	53	56	CA1	93	53
CA2	18	18	CA2	18	18
CA3	36	28	CA3	45	28
DG	24	23	DG	30	23
AMY	197	210	AMY	309	202

Table 7: Shows number of neurons that were observed in each cluster and the number of neurons that were expected (evenly distributed between each cluster) in level two analysis.

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