
Recordings in the Human Temporal Lobe: Data Analysis and Spike Sorting

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Abstract

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In the present thesis we study the recording of neurons in the human medial temporal lobe (MTL). We take a dual approach to the topic concordant to the scientific nature of the problem. On the one hand, the MTL is one of the most studied structures on the brain, strongly correlated to the formation and retrieval of conscious memories. On the other hand, the direct recording of neurons is a challenging operation requiring advanced methods of signal processing.

We used recordings from electrodes implanted in epileptic patients to study the behaviour of MTL neurons with strong responses to visual stimuli. We studied how the repeated stimulus presentation modulated the firing of these neurons. The results showed decreased activity with each presentation and differences between areas in the line of the suggested roles in previous works.

We analysed the performance provided by the algorithms used for the extraction of single unit activity out of the signal recorded by electrodes in the brain: a process called spike sorting. The results quantified an inherent limitation to these algorithms, with a maximum number of detected units and significantly reduced performance for those neurons with low firing rate.

In summary, the work presented here contributes to the understanding of the ongoing processes in MTL neurons and the problematic of recording the activity of neurons, in especial the ones with low levels of activity.

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List of Publications

Journals

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Part I

Introduction

Chapter 1

Aims and Motivations

The brain is one of the most fascinating mysteries present in the world. It is the brain that distinguishes us most from the rest of the species on Earth (Gazzaniga, 2008). Our brain is the tool that has allowed us to develop a complete range of cultures, thousands of languages, arts, science and, even, trips to the moon. However, we are only starting to understand its functioning mechanisms, with countless challenges to face in a brilliant and exciting prospect for the field of neuroscience.

One of these challenges is the understanding of how memory works. Memory is one of the most important features of our brain and who we are. We take as natural the ability to learn, to store experiences and skills from our past and use them at some distant point in time or in our everyday life. Therefore, how can we distinguish ourselves from our memories? Who are we without our experiences? Without remembering and knowing about our friends, family and the experiences we have shared with them, we would not be able to build up the relationships constituting our social world. How could we live through our life without the knowledge accumulated throughout the years? Additionally, without a system providing us with the ability to store and recall in a ready-

to-use fashion we would need to learn to walk, to run, to speak, to read again and again.

For these reasons, scientists have spent decades studying the systems supporting memory in the brain (Squire and Kandel, 1999; McGaugh, 2000). A milestone in this study was the report of undesired secondary effects of therapeutic surgery performed on an epileptic patient: the bilateral removal of the **medial temporal lobe (MTL)**(Scoville and Milner, 1957). The patient, known as H.M., developed a profound amnesia affecting the events of his life close to the surgery and the ability to create new memories from then onwards (Scoville and Milner, 1957). It has been suggested that this case started the modern age in the study of memory (Squire, 2009). I review the characteristics of H.M's condition in chapter 2.

This case turned the attention of scientists toward the MTL and its influence in memory and other cognitive processes. While reviewing the literature (see chapter 2) we discover that the MTL is related to a complete range of processes (Squire et al., 2004). Complementary, further research unfolded a series of memory types associated to different brain areas (Gazzaniga et al., 1998; Squire and Kandel, 1999; Kandel et al., 2000).

The majority of the data available concerning the functions of the MTL in humans comes from lesion studies (see Rosenbaum et al. (2005) and Squire (2009) for examples) or non-invasive measurements of brain activity such as electroencephalography (EEG), positron emitting tomography (PET) or functional magnetic resonance imaging (fMRI). However, none of these methods presents the necessary time or space resolution to provide conclusive results about neural processes at cell level (Engel et al., 2005; Logothetis, 2008).

In recent years a new technique allowed direct observation of MTL neurons at work in awake humans (Fried et al., 1997). A series of studies analysing the

responses obtained in these recordings led to what was rated as one of the top 100 Science Stories of year 2005 by *Discover Magazine*: neurons expressing the conscious perception of abstract concepts (Quian Quiroga et al., 2005). The study of this type of cells constitutes a challenge for neuroscientists and offers a unique opportunity to understand how memories are formed at the neural level (Quian Quiroga et al., 2005, 2008a). The latest studies have presented promising results for uncovering the neural basis of memory and constitute a new perspective in the study of the MTL (Gelbard-Sagiv et al., 2008; Mormann et al., 2008; Quian Quiroga et al., 2008b, 2009; Viskontas et al., 2009).

The recording of neurons from epileptic patients is, however, a technical challenge that requires advanced methods of signal processing and data clustering (Lewicki, 1998). The correct classification of the recorded signals is a crucial step for posterior analysis of brain functions and possible applications (Brown et al., 2004; Nicolelis and Lebedev, 2009; Quian Quiroga and Panzeri, 2009). For this reason, advances on the methods for the analysis and processing of data are parallel to the understanding of anatomical structures and neural functions.

The aim of this thesis is, therefore, to take a dual approach to the study of recordings in the brain:

- On the one hand, we perform an analysis of data recorded from neurons in the MTL from epileptic patients and evaluate the effects of stimulus repetition on the abstract neurons described by Quian Quiroga et al. (2005).
- On the other hand, we study the performance reached by the algorithms used for spike sorting. We also consider the possible consequences of such limitations on the study of neural populations, including the possible bias introduced in the results derived from experimental conditions where the

algorithms do not reach the desired performance.

1.1 Organization of this thesis

In Part I I deal with the introduction of the work of the thesis. In Chapter 2 I review the literature comprising the MTL and the recording of extracellular activity. The research work carried out in this thesis is detailed in Part II. There, in corresponding chapters, I present the findings covering the two points mentioned above in the fields of MTL data analysis and algorithm development. I conclude the thesis with Part III, where I cover the conclusions of the work, including an overall discussion of them and presenting suggestions for future work.

Chapter 2

Literature Review

In this chapter I introduce the research on the Medial Temporal Lobe (MTL) and the structures conforming it. The literature on the topic is extensive, including a vast amount of studies conducted in both animals (rats and monkeys principally) and humans. Despite more than 50 years in the spotlight of neuroscience research, the understanding of the MTL – its anatomy, connectivity and, specially, its function – is one of the hottest topics in the field, with vivid discussions and confrontational approaches far from a consensual meeting point.

In the last few years a new technique allowed direct recording of neurons in the MTL of epileptic patients (Fried et al., 1997). A series of studies making use of this opportunity have reported neurons with striking characteristics related to perception of visual stimuli (Quian Quiroga et al., 2005, 2008a; Quian Quiroga and Panzeri, 2009). This offered a new window to the understanding of how our brain perceives and processes the information on the world around us.

The structure of this chapter is as follows: first, I introduce the anatomy of the MTL, describing its forming structures, mutual projections and connectivity to the majority of the cortical areas of the brain. Then, I describe the exceptional case of patient H.M., first reported more than 50 years ago (Scoville

and Milner, 1957), that converted the MTL in one of the most studied structures of the brain. Next, I introduce the main theories describing the major functions of the MTL, outlining the points and counterpoints for each one. Following, I review evidences collected from work in animals and humans of the role of the MTL in spatial navigation, perception and, principally, in the acquisition, storage and retrieval of memories, including the astonishing discoveries from the latest studies in human MTL. To conclude I review the methods used in the recording of epileptic patients, their present state and the challenges to be faced in a near future.

2.1 Recognition of Visual Stimuli

The MTL is located beyond the hierarchy formed by the ventral visual pathway: the collection of structures participating in the perception and recognition of visual objects (Logothetis and Sheinberg, 1996; Riesenhuber and Poggio, 2002). Figure 2.1 shows a visual representation of all the cortical connections activated after the presentation of a visual stimulus in the macaque monkey (from Felleman and Essen (1991)). The right hand side of the figure represents the areas participating in object recognition, commonly referred as the ventral visual pathway (see below). At the top of the hierarchy lays the hippocampus and, just below, the entorhinal cortex, with inputs from the perirhinal cortex (Brodmann Area 36, see Figure 2.2 for the location of Brodmann areas in the brain and section 2.2 for the anatomy of MTL). Unless stated explicitly otherwise, the following anatomical description is based on the electrophysiological data available from studies in monkeys. In general, it can be extrapolated to the human vision system matching dual areas from both species and corroborated by lesion studies and non-invasive image techniques (Grill-Spector and Malach,

2004).

When an object is presented in front of us the light hits the back of our eyes forming an inverted image in the retina. Photoreceptors located in its deepest layer are activated depending on the brightness of the part of the stimulus (or pixel) reaching each of them. The light information from the retina goes, through the lateral geniculate nucleus (LGN) to the back of the head, the occipital lobe, reaching neurons in the primary visual cortex (V1), located in Brodmann area 17 (Kandel et al., 2000).

The primary visual cortex is the input gate for image processing of the brain. Neurons in V1 respond to local orientations in a particular area of the visual space, which is called the receptive field of the neuron (Hubel and Wiesel, 1977). The activity of these neurons have a topographical organisation, i.e. two adjacent neurons possess receptive fields adjacent in space. From the activity of these neurons, a hierarchical structure participates in the perception of objects in the visual space until they are identified and their locations are determined (Felleman and Essen, 1991). These two processes (object recognition and localisation) are performed by two collection of neural aggregates: the **ventral visual pathway** and the **dorsal visual pathway**, respectively (Van Essen et al., 1992; Kandel et al., 2000). In Figure 2.3 a simpler version of Figure 2.1 is presented. On it, the right hand side of the figure represents structures of the ventral visual pathway, while the left hand side represents the ones in the dorsal pathway.

The Dorsal Visual Pathway

The dorsal visual pathway processes locations and movements of objects in the visual field. This pathway sometimes is also called the “where” or “action” pathway (the later for its detection of movements and relation with the planning

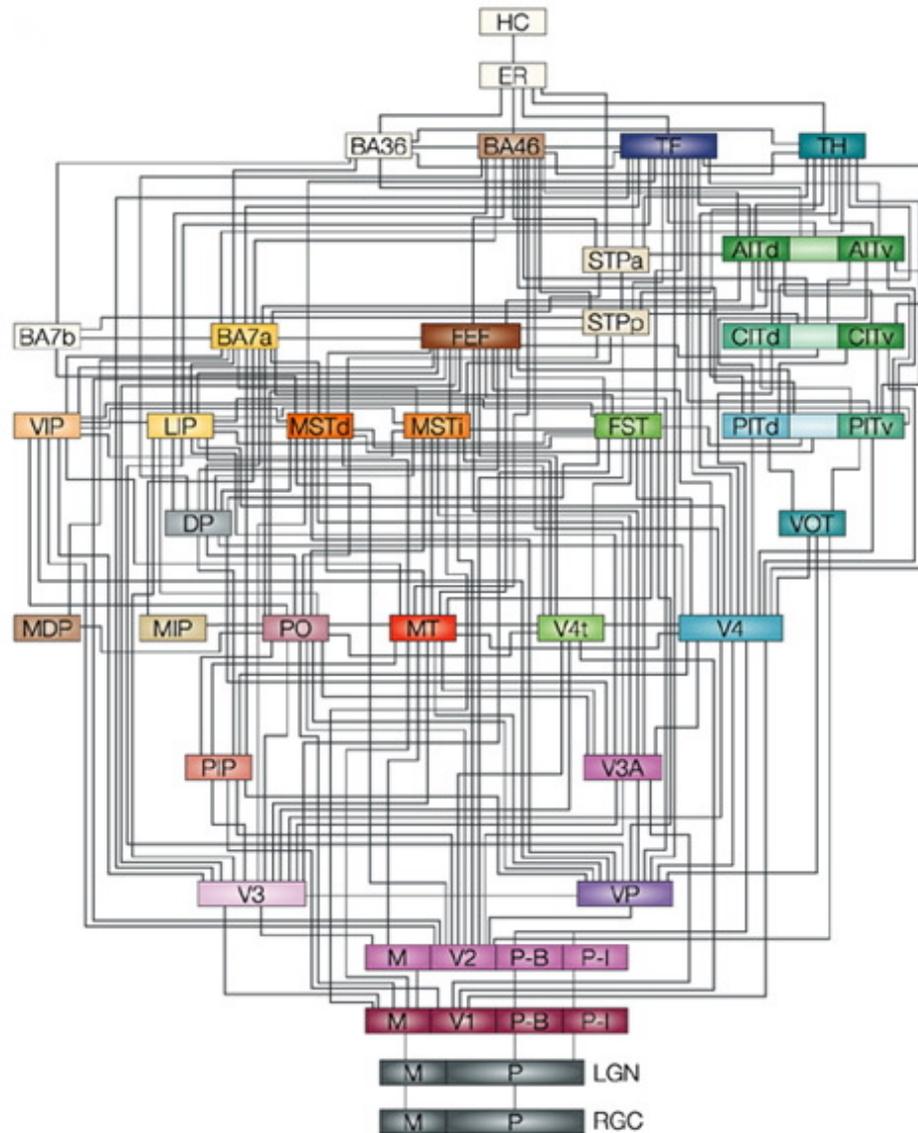
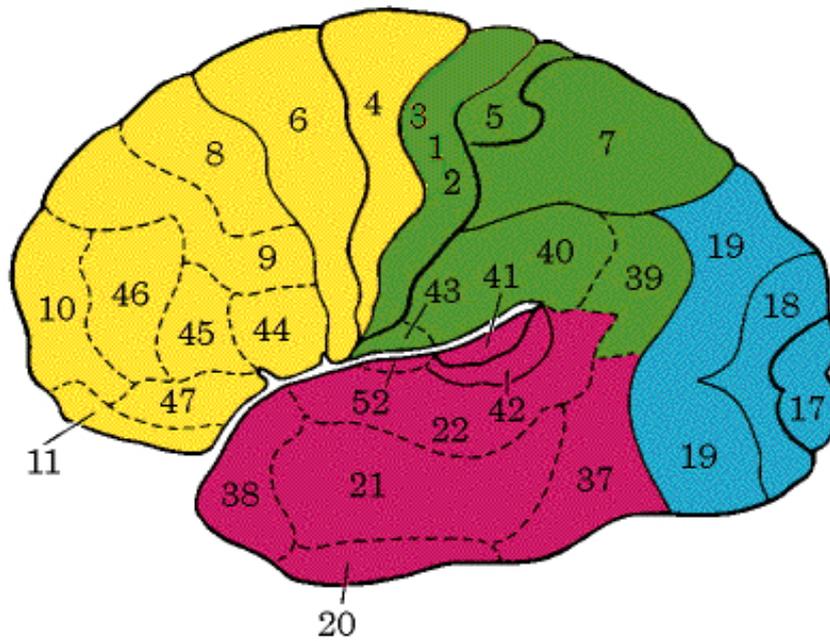
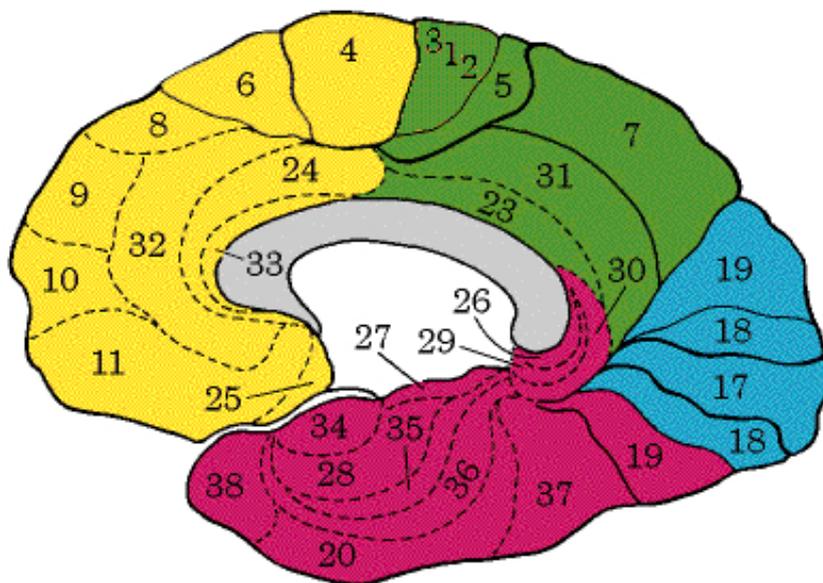


Figure 2.1: Anatomical connectivity of macaque visual areas as shown in Rees et al. (2002) based on the work published by Felleman and Essen (1991). AIT, anterior inferotemporal cortex; BA, Brodmann area; CIT, central inferotemporal cortex; d, dorsal; DP, dorsal prelunate area; ER, entorhinal cortex; FEF, frontal eye fields; FST, floor of superior temporal cortex; HC, hippocampus; LGN, lateral geniculate nucleus; LIP, lateral intraparietal area; M, magnocellular regions; MDP, mediodorsal parietal area; MIP, medial intraparietal area; MSTd, dorsal part of the medial superior parietal area; MSTl, inferior part of the medial superior parietal area; MT, middle temporal cortex (visual area 5); P, parvocellular regions; P-B, parvo-blob; P-I, parvo-interblob; PIP, posterior intraparietal area; PIT, posterior inferotemporal cortex; PO, parieto-occipital area (visual area 6); RGC, retinal ganglion cells; STPa, anterior superior temporal polysensory cortex; STPp, posterior temporal polysensory cortex; TFTH, temporal areas; v, ventral; V1V4t, visual areas; VIP, ventral intraparietal area; VOT, visual occipitotemporal cortex; VP, ventroposterior visual area.



(a) Lateral surface



(b) Medial surface

Figure 2.2: List of Brodmann areas in the brain. Frontal, parietal, occipital and temporal lobes are represented in yellow, green, blue and magenta, respectively. Based on the original image from Brodmann (1909).

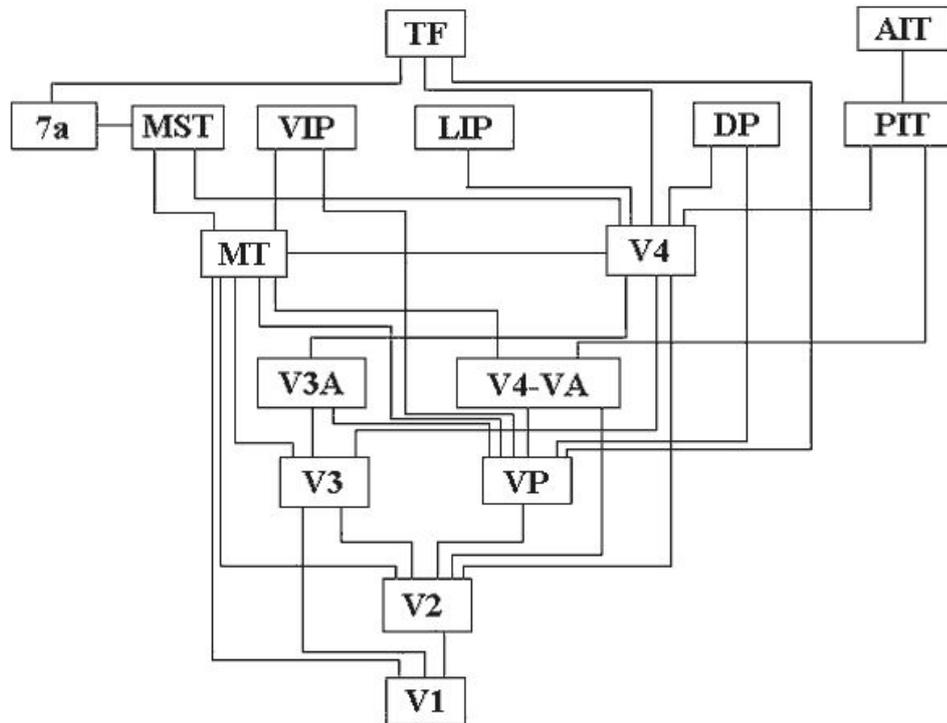


Figure 2.3: Main connectivity of visual areas in primates. The terminology used follows the one presented in Figure 2.1. Adapted from Van Essen and Maunsell (1983)

of actions, see Andersen and Buneo (2002)). The dorsal stream originates in neurons in V1 projecting directly to the V3, located dorsally adjacent to V2 (see below). Neurons in this area are mainly activated by moving stimuli and they project into V5 (also known as middle temporal area, MT). Neurons in MT are sensitive to motion in a particular direction, usually having suppressed activity for movement in the opposite direction.

The Ventral Visual Pathway

The ventral visual pathway, as seen above, originates in the primary visual cortex and performs the identification of shapes, colours and textures, leading to object recognition. It is sometimes referred, as well, as the “what” pathway.

Neurons from V1 project into the neighbouring area of V2 (or second visual area) located in Brodmann area 18 in the occipital lobe. Neurons in V2 are activated by lines and edges, as in V1, although the receptive fields presented by this later stage are larger than in the first one, created by convergent projections of V1 cells into V2 ones (Van Essen and Anderson, 1995). In turn, cells in area V2 project to area V4, containing neurons with larger receptive fields and responses to basic geometric shapes and contours.

At the end of the stream, projections reach the posterior part of the inferotemporal cortex (IT), that, in turn, project to the anterior part of IT or area TE – right hand side of Figure 2.3 – where neurons responding to complex stimuli (e.g. faces) were found, despite small changes in the perspective of the object shown (Tanaka, 1996).

The high selectivity of neurons in the IT cortex, compared to previous stages of object recognition (V2 and V4), varies across its different sub-areas. The specificity and complexity of the stimulus needed for neural activation are greater the more anterior the neurons are located (see Logothetis and Sheinberg (1996) and Tanaka (1996) for reviews). However, the increasing complexity of the objects represented are based on an adequate combination of basic features (Kobatake and Tanaka, 1994; Freiwald et al., 2009). The importance of the neural mechanisms for object recognition in the anterior part of the temporal lobe have also been shown in humans, in a dual way to the results in neurophysiology in monkeys (Tanaka, 1997).

Despite the clear distinction between both visual processing streams, the areas involved are not isolated from each other as can be seen in Figure 2.3. In addition multiple secondary cross projections across different stages exist as can be seen in Figure 2.1 (Felleman and Essen, 1991). These projections are thought to help in the establishment of synchronisation between both functions,

e.g. the recognition of an object and its spatial location (and trajectory). It must be said that the different stages of processing introduced above present a map of feedforward projections that is complemented by a similar structure of feedback connectivity (Van Essen and Maunsell, 1983).

2.2 Anatomy of the Medial Temporal Lobe

In the section above we reviewed the cascade of neural events elicited by the presentation of an object in the visual field until its recognition. The activation of neural aggregates starts in the retina, projects to the back of the brain and follows a ventral pathway until the reach of the anterior part of the temporal lobe. From there, neurons on IT project directly to the input areas of the MTL: the perirhinal and entorhinal cortices (Saleem and Tanaka, 1996; Suzuki, 1996; Lavenex and Amaral, 2000). Similar to the visual system, other high sensory areas (auditory and somatosensory cortices) process information in a hierarchical structure, projecting into the input stages of the MTL (Lavenex and Amaral, 2000).

The MTL is located, as described by its name, in the internal (medial) part of the temporal lobe, located central to the middle of it. The MTL is usually divided in 5 main structures:

- Perirhinal cortex
- Parahippocampal cortex
- Entorhinal cortex
- Hippocampus
- Amygdala

The first three of these structures are, on occasions, grouped together and referred as the *parahippocampal region*, *parahippocampal formation* or *parahippocampal gyrus* (Eichenbaum et al., 2007). These three structures have the layered structure common to most cortical areas, while the amygdala and hippocampus lack this layered organisation. In fact the two later areas are sometimes grouped with other structures in the limbic system, group of areas in the border of the cortical region with multiple connections that are related to the storage and management of emotions (see below).

The **perirhinal cortex** comprises parts of Brodmann areas of the brain 35 and 36 (see Suzuki (1996) for a more detailed description and Figure 2.2 for a diagram of the location of Brodmann areas). Studies in primates have established that the dominant input of the perirhinal cortex originate in adjacent high visual areas, followed by projections from the parahippocampal cortex and minor projections from somatosensory and auditory areas (Suzuki, 1996). In turn, the perirhinal cortex has projections to the entorhinal cortex and amygdala receiving strong feedback connections from these structures. The feedback connections received from these areas are, then, projected back into sensory cortices (Suzuki and Amaral, 1994).

The **parahippocampal cortex** comprises areas TH and TF (Suzuki and Amaral, 1994). As in the case of the perirhinal cortex it receives inputs from different unimodal cortical areas, although on its case the projections are generated from dorsal stream areas (Suzuki and Amaral, 1994; Squire et al., 2004). These connections establish the parahippocampal cortex as the main input of spatial information to the MTL.

From these two structures mentioned above, the information is passed onto the **entorhinal cortex**, located in the Brodmann area 28 (Lavenex and Amaral, 2000). The parahippocampal and perirhinal cortices provide two thirds of the

cortical inputs to entorhinal cortex (see Zola-Morgan and Squire (1993); Suzuki (1996); Lavenex and Amaral (2000) and Squire et al. (2004)). In addition to the inputs from these cortical regions, the entorhinal cortex receives polymodal projections from the orbitofrontal cortex, the superior temporal gyrus, the insular cortex, the cingulate cortex and the retrosplenial cortex (Lavenex and Amaral, 2000). In turn, the entorhinal cortex constitutes the main input to the hippocampus, projecting directly to the *dentate gyrus*, and closing a processing loop receiving feedback projections from the output area of the hippocampus: the *subiculum* (see below). The previously mentioned connections allow the entorhinal cortex to integrate polymodal external stimuli provided by different cortical sensory areas and pass them to the hippocampus. This connectivity map makes the entorhinal cortex the connecting gate of the hippocampus with the rest of cortical areas (through parahippocampal and perirhinal cortices) and a crucial integration point in the MTL complex (Lavenex and Amaral, 2000).

The **hippocampus** is a three layer structure located in the medial border of the neocortex as shown in blue in Figure 2.4. The hippocampus is the main component of the MTL, which is sometimes referred as the hippocampal formation. The hippocampus itself is not a simple structure and it is often subdivided in further substructures, being the most important the dentate gyrus, area CA3, area CA1 and subiculum. The connections between these areas is sequential, with the dentate gyrus receiving all the input projections – mainly from the entorhinal cortex, as mentioned above – and passing the information into CA3, projecting to CA1 which, in turn, projects into the subiculum, which projects back into the entorhinal cortex and, from there through the parahippocampal and perirhinal cortices, to different areas of the neocortex (Squire and Zola-Morgan, 1991).

The **amygdala** is an almond shape structure located anterior to the hip-

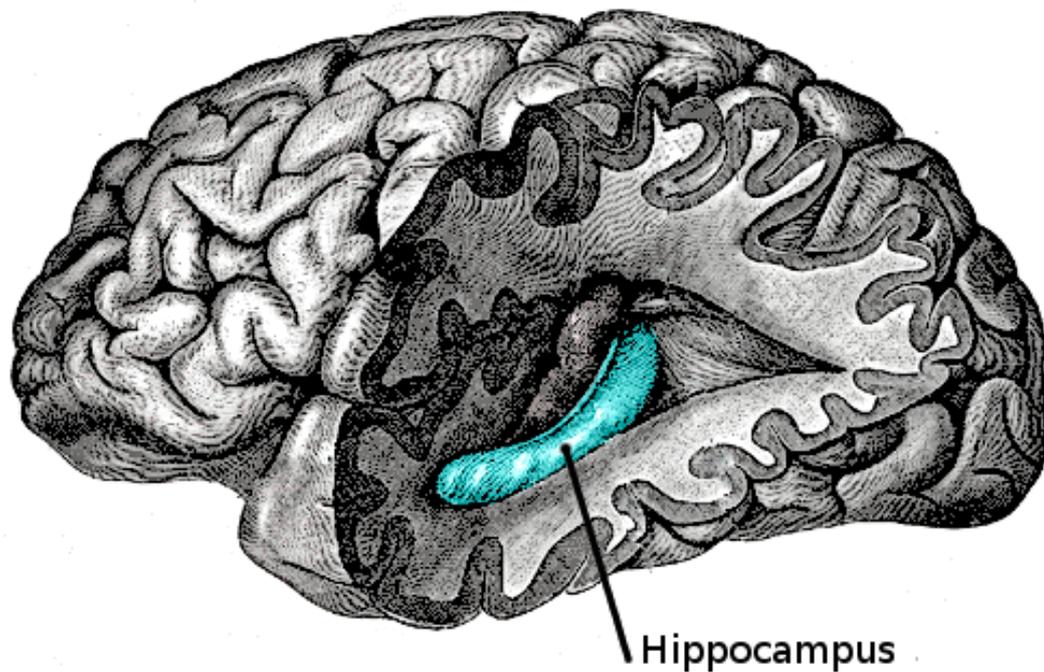


Figure 2.4: View of the left side of the human brain with exposed hippocampus (highlighted in blue). Adapted from Gray (1918).

hippocampus and, as the hippocampus, it forms part of the limbic system: a group of structures forming a border around the brainstem including, apart from the amygdala and the hippocampus, the cingulate gyrus, the hypothalamus and the anterior thalamic nuclei (Gazzaniga et al., 1998; Kandel et al., 2000). The amygdala is subdivided in numerous nuclei, which, in turn, are composed by further subnuclei (McDonald, 1998). Cortical projections targeting the amygdala are generated in all sensory areas: projections from latest stages of auditory and visual cortex, as well as from primary cortex of sensory-motor areas and olfactory bulb (see McDonald (1998) for more details). In addition to sensory inputs, the amygdala receives projections from other polysensory areas: the rest of the MTL and the pre-frontal cortex – related to behaviour and reward

– (Squire, 1986; McDonald, 1998). In turn, the amygdala projects to the hypothalamus, brainstem and striatum (areas related to emotion processing and conditioned behaviour) modulating their activity (McDonald, 1998). It also has feedback projections to the previously mentioned input areas (Squire, 1986; McDonald, 1998). Despite being a structure usually included as part of the MTL, when analysing the connectivity of the MTL it could be noticed that the connections between the amygdala and the other regions described previously, especially hippocampus, are weaker than the main neural circuit conformed by the parahippocampal gyrus, entorhinal cortex and hippocampus. In fact, occasionally, structural descriptions of the MTL do not include the amygdala as part of it (e.g. Eichenbaum et al. (2007)).

2.3 The study of the Medial Temporal Lobe

Despite the relevance of the MTL in neuroscience today, 60 years ago its function and relevance was completely unexplored. However, in an article published in 1957, William Scoville and Brenda Milner reported the case of a patient suffering from profound memory loss after experimental medial temporal lobe surgery (Scoville and Milner, 1957). Since then, the MTL became one of the areas of the brain more studied in neuroscience and the patient reported in the study, H.M., one of the most famous clinical cases in history (Squire, 2009).

2.3.1 The case of patient H.M.

For treating his epilepsy, H.M. underwent a procedure called bilateral temporal lobotomy, consisting of the removal of the hippocampus and part of the adjacent structures of the MTL (Scoville and Milner, 1957). Recent work performing magnetic resonance imaging (MRI) analysis of the affected structures reported

that the surgery resected large areas around the hippocampus (see Figure 2.4), including most of the amygdala, entorhinal cortex and a large portion of the hippocampus (Corkin et al., 1997). On the other hand, the perirhinal cortex and the parahippocampal cortex were spared by the surgery although atrophied after more than four decades since the removal of the rest of the MTL, as did other parts of the brain like the cerebellum and the mammillary nuclei with direct connections to the MTL (Corkin et al., 1997).

After the surgery the patient exhibited impairment in the formation of new memories – known as *anterograde amnesia*. A memory deficit was also shown for remembering episodes of his life closer to the surgery (up to eleven years, see Corkin (1984)), but not the ones from a distant past – referred as *retrograde amnesia* (Milner et al., 1968). Apart from his memory deficit, he did not present any observable intellectual loss, showing an average IQ for a person of his age (Milner et al., 1968; Corkin, 1984). Soon, scientists started to study the extent of the memory loss of H.M.. Despite the impairment presented for the acquisitions of new declarative memories, surprising results arose when scientists completed a series set of tests: H.M. was able to acquire motor skills over large spans of time (Milner, 1962; Corkin, 1968), although he was not able to remember how or when he learnt them and his performance on these tasks was not as good as control subjects' performance. In fact, some tasks proved to be more affected (Corkin, 1965) than others (Corkin, 1968), but H.M. was still showing some degree of improvement on them. Additionally, the condition of H.M. included poor estimation of his own age (he thought he was younger than he was) and not being able to cite the date accurately. He also reported problems recognising himself in pictures taken years after the surgery (Corkin, 1984). When talking about his own life, he said that it was “waking from a dream” with each day being “alone in itself” (Milner et al., 1968).

Although some studies reported that H.M. was able to acquire residual knowledge of his surroundings, this knowledge presented little structure, was incomplete, showed a lack of connectivity to other related events and could not be modified (Milner et al., 1968; Corkin, 2002). For example, H.M. recalled the assassination of John Fitzgerald Kennedy, but he did not know how it occurred. In another example, he was able to give the address and draw a footprint of a house he was living in, even though he moved there after the surgery. However, he reported living in the same house after moving to a caring institution in 1974 (Corkin, 2002). Therefore, if acquired, the new memories were a collection of isolated facts instead of a rich collection of events and experiences (Corkin, 1984, 2002). For the acquisition of this facts, it seemed that H.M. needed continuous exposure to a stimulus, although repeated exposure alone was proven not sufficient for successful learning (Corkin, 1984). One of the latest studies performed on H.M. challenged the commonly accepted view of H.M.'s normal performance for memories occurred long before the surgery (Steinvorth et al., 2005). In this study Steinworth and colleagues revisited the remote memory performance of the patient with a novel interview technique, including a new scoring system, and suggested that H.M.'s recollection of autobiographical memories was impaired, even for the ones originated long before the surgery, although others argued that these deficits could also be caused by other factors such as the absence of rehearsal (Squire, 2009).

In opposition to the under control memory performance of H.M. for facts occurred after the operation, his immediate – or working – memory (see section 2.3.2 below for a list of the different types of memories) matched the one of healthy subjects. His performance matched controls' as far as only elements within his working memory load (about 5 items) were to be managed at the time (Milner et al., 1968; Corkin, 1984; Squire, 2009). This, together with other

series of tests, showed that H.M.'s perception (especially visual perception) was intact (except for odours, see Eichenbaum et al. (1983)), making H.M. aware of the environment to which his attention was fixed upon.

The case of patient H.M. uncovered the vital role of MTL for successful memory formation and the existence of different types of memory. Due to the origin of his lesion (surgical removal of a brain area), the structures affected were clearly identified, allowing a direct correspondence with the collection of processes impaired. The specificity of the deficits observed suggested that particular brain structures must have concrete roles associated to different brain functions (and memory types). H.M. attracted the attention of more than a hundred scientists who studied the MTL and its relationship to memory formation and other brain functions (Corkin, 2002; Squire, 2009). Since then, the history of H.M. – deceased in December 2008 – has been parallel to the understanding of the medial temporal lobe and its role in memory storage and retrieval (Squire, 2009).

2.3.2 Memory types and brain areas

The identification of different types of memories and the brain structures supporting them, became a hot topic after the first report on H.M.'s condition (Scoville and Milner, 1957), leading to a more comprehensive study of memory. Figure 2.5 shows a diagram with a classification of the different types of memory commonly acknowledged in neuroscience at the present moment. From a classic point of view, there are two main factors differentiating memory types:

1. The time span covered, i.e.: the amount of time a memorised item is available to be used. According to this criterion the memories can be divided in short-term memories – in the range between seconds to minutes

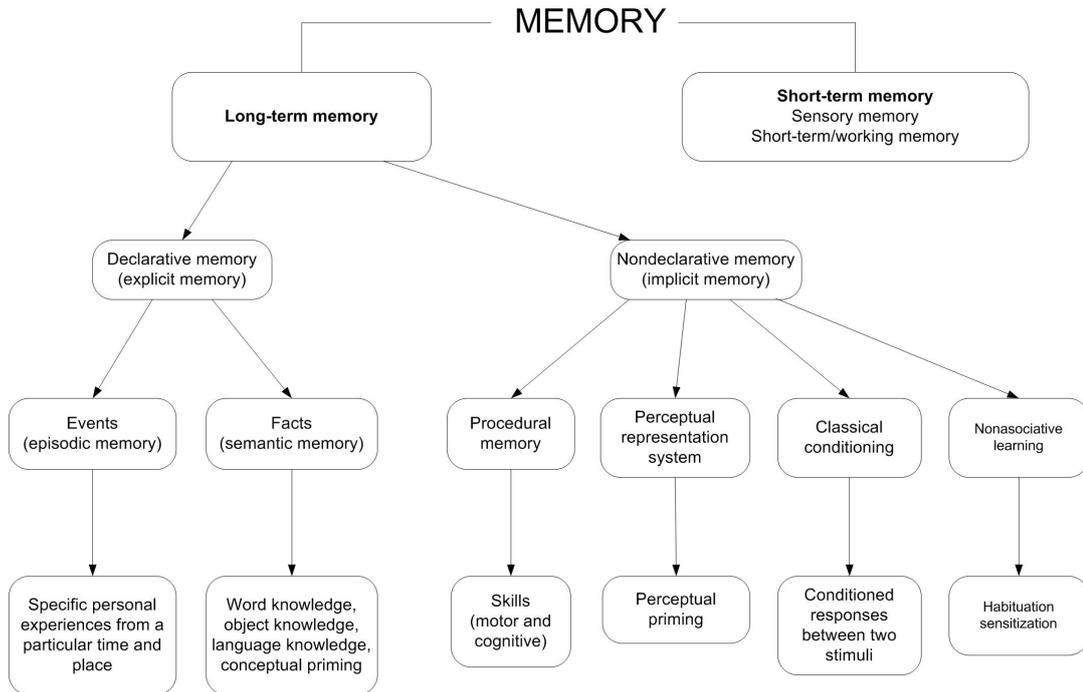


Figure 2.5: Proposed classification of different types of long term memory. Adapted from Gazzaniga et al. (1998).

or even a few hours – and long-term memories – the ones that can be held for hours, days and even years.

2. The consciousness of the acquisition process or how explicit the memory is, i.e.: if memories are consciously acquired experiences / facts or not. When the memory is acquired consciously and it is possible to retrieve it, having a certain knowledge of it, it is defined as a declarative memory (this is the common use for the word memory), while any other type of memory – procedural, sensory, conditioning, etcetera – that is performed but without a conscious use of it is referred as non-declarative.

These two general criteria for dividing different types of memories are agreed among most scientists. However, further division in more specific memory types offers some variation when revising the literature. For example, the

original term of short term memory was defined by George A. Miller in 1956 and established a number of maximum items (from 5 to 9) that could be handled simultaneously by it (Miller, 1956). Since then, the term has been extended into a complete set of mechanisms covering memories with a small span of time and / or elements: sensory memory, phonological loop, working memory and short term memory itself (Squire and Kandel, 1999; Cowan, 2008). Evidence from studies in monkeys suggest that short term memory is related to activity of neurons in the prefrontal cortex (Fuster and Alexandre, 1971; Fujisawa et al., 2008). Other types of short term memories are linked to other brain areas: verbal short term memory (known as the phonological loop) has been related to the perisylvian cortex – the cortical area around the main division between the temporal and the parietal lobes – (Warrington and Shallice, 1969) and sensory memory (lasting a few milliseconds after an stimulus is presented) is related to the “echo” of the neural receptors in the first stages of perception in the brain.

The collection of non-declarative memories presented on Figure 2.5 form an heterogeneous group, depending on different brain complexes. Probably, the most known of them is the classical conditioning, identified by the Russian psychologist Ivan Pavlov at the beginning of the 20th century. The non-associative learning refers to the modification of reflex responses of beings through processes of habituation and sensitisation (processes involving the sobre-exposure to an stimulus changing the innate reaction to it). The procedural memory has been already mentioned when explaining the case of H.M. in the section above: it consists in the learning of a behaviour (expressed as a motor action, like following the contour of a star using a mirror, or in any other manner). Lastly, perceptual priming is the intrinsic learning that the sensory systems made when presenting with a new stimulus, that allows the subject to recognise the given stimulus quicker (more efficiently) the next time it is presented. Priming is

characterised by a reduction of activity of the area involved in the processing of the stimulus between the first time and subsequent presentations (Gazzaniga et al., 1998; Kandel et al., 2000).

Declarative memories refer to the facts and episodes of our life that we are able to recall and use at will in a moment distant from the time moment they were acquired. It is commonly accepted that there are two types of declarative memories: semantic and episodic. Semantic memories are the memories of simple facts like names, words and dates. Basically, it is the system supporting data storage in our brains. Episodic memories refer to those rich, colourful memories, which are full of details including places, people and sequences of events, usually referred to oneself, i.e. autobiographic (Tulving, 1972). This memory system deals not only with memories directly related to oneself, but to any other memory including details of different domains (people, places) inter-related in a particular cross-domain and temporal framework, although they are referred to the personal point of view for their processing and storage. Episodic memory allows past events to be re-experienced, interconnecting different facts of semantic memory into a complete scenario, a conjunction of “what”, “where” and “when” (Tulving, 2002). The case of patient H.M. (presented in section 2.3.1) established a clear dependence of declarative memories and the MTL. In the next section I revise the level of understanding of this relationship between the anatomical structure and its contributions to memory.

2.3.3 The Medial Temporal Lobe and its role in declarative memory

The anterograde amnesia that patient H.M suffered after the surgery that removed his MTL bilaterally was a first indication for scientists that this area was

necessary for the acquisition of new declarative memories. Later cases of patients with MTL damage have presented similar anterograde amnesia to the one presented by H.M., although the exact impairment depended on the anatomical extent of the lesion (Stefanacci et al., 2000; Rosenbaum et al., 2005; Steinvorth et al., 2005).

Through the years a variety of different models have proposed a particular role of the hippocampus, MTL and neocortical areas in the storage and retrieval of memories (Zola-Morgan and Squire, 1993; Nadel and Moscovitch, 1997; Eichenbaum, 2000; Aggleton and Brown, 2006; Bird and Burgess, 2008). General agreements among the different theories could be outlined in two basic points:

- i) the MTL is necessary for memory formation, affecting (to some grade) the acquisition of declarative memories and
- ii) typically, when a lesion is produced, the more MTL areas affected, the more pronounced the impairment in memory results.

The disagreements shown among confronting positions are numerous, leading to interesting and vivid discussions between opposing sides (see Eichenbaum et al. (2008) and Wixted and Squire (2008) for an example). In the view of Squire and colleagues – the **standard model of memory consolidation** – the MTL plays a role as an intermediate buffer for memory consolidation from short term memory into long term memory (Squire, 1986; Squire and Zola-Morgan, 1991; Zola-Morgan and Squire, 1993; Squire et al., 2004). This process could extend for years and the differences in memory deficits depend on the degree of consolidation of memories in the cortex (Squire et al., 2007). In contrast, the **multiple trace theory** proposed by Moscovitch and colleagues (Nadel and Moscovitch, 1997; Moscovitch et al., 2005, 2006) states that not only the ac-

quisition but also the retrieval of memories depends on the MTL. From the moment of perception a unique trace is created for the memory of each episode, coded in a sparse network comprising the different final cortical areas and a link to all of them in the associated aggregate in the MTL. This memory can be retrieved and used by the subject if the complete trace (including all brain areas) remains intact. Factual information is acquired through integration of different traces sharing the same (or similar) concepts (Nadel and Moscovitch, 1997).

Other models of memory included specific mechanisms within the MTL for particular features of memory processing. Aggleton and Brown proposed a functional division between the hippocampus and surrounding cortical areas in the **Dual-process theory** (Brown and Aggleton, 2001; Aggleton and Brown, 2006). On it, they divide the recalling process of declarative memories into recollection and familiarity: the former one would be the complete re-experience of the memory, with full detail and confidence while the later one represents the sensation of “already experienced” lacking the details and confidence of recollection. These two processes would be complementary and supported by two different structures of the medial temporal lobe: i) the hippocampus performs the recollection process and ii) the parahippocampal and perirhinal cortices support processes of familiarity. The view of different systems for familiarity and recollection is also shared by the **relational theory** of Yonelinas, Eichenbaum and colleagues (Yonelinas, 2002; Eichenbaum et al., 2007). In their view, the hippocampus is a major support for relations (Wallenstein et al., 1998). As such, it plays a mayor role in the processing of temporal sequences, cross domain associations and navigation (Eichenbaum, 2000, 2004; Eichenbaum et al., 2007). In general, the theories presented above assume the role of the MTL in its relationship with memory processes, but leave the role of perception to the

specific sensory areas related to each type of stimuli. However, other theories have proposed otherwise, stating that the structures forming the MTL, especially the perirhinal cortex, also play a major role in the perception of complex stimuli, (Buckley and Gaffan, 2006; Murray et al., 2007).

In general, theories on the role of MTL in the formation of declarative memories do not include the amygdala as a major participant. Despite results obtained in the first studies exploring the link between MTL and memory (Mishkin, 1978), it has been later stated that the amygdala plays a different (and complementary) role to the other structures of the MTL: it is a crucial component in the acquisition of emotional memories (Gazzaniga et al., 1998; Zald, 2003; McGaugh, 2004; Phelps and LeDoux, 2005). The amygdala is directly involved in implicit and explicit emotional learning and critical for the recognition of emotional expressions in faces. Although not directly involved in the acquisition of declarative memories, the amygdala is believed to perform a modulation effect over them based on their emotional content (McGaugh, 2004).

2.3.4 The Medial Temporal Lobe and the processing of information

The theories presented above were based on the extensive work performed in the study of the MTL since it was brought into the spotlight of neuroscience in the 1950s. The data collected in humans comes, generally, either from lesions of different nature or from imaging studies. Complementary, in animals, it is possible to do delimited anatomical lesions and different types of invasive recordings. This collection of techniques gives further insight in the study of MTL functions. Invasive recordings are necessary to corroborate the activity of

neurons detected using imaging techniques, which are indirect measures of neural activity (Logothetis et al., 2001; Logothetis, 2008). Combined experimental work explored the role of MTL in the processing of spatial, sensory and time information, offering a more complete picture of the exact contribution of its different structures.

When researching the MTL in animals, recordings in the rat hippocampus by John O'Keefe and John Dostrovsky in 1971 reported a set of neurons called **place cells**: neurons firing to particular locations of an environment, remaining silent for any other location of it (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976). Studies in place cells have led to a better understanding of memory functions, extrapolating the findings in these cells to other cognitive functions (O'Keefe et al., 1998; Eichenbaum et al., 1999; Bird and Burgess, 2008). In fact, recent discoveries have suggested that these neurons could be linked to the experience of the animal, more than to its location, including:

- conditional firing of neurons in their receptive fields depending of the context (Wood et al., 2000);
- sequential firing of place neurons, in addition to while in their receptive fields within a linear maze, before and after going through it (Diba and Buzsáki, 2007);
- activation of a set of neurons, while being on a running wheel, related to a particular part of a maze when planning the next action to take (Pastalkova et al., 2008).

In addition to neurons in hippocampus, only cells in entorhinal cortex have shown spatial-related firing (Hafting et al., 2005). However, the firing presented by entorhinal neurons was in a grid covering the space instead of a

single location. None of the other MTL structures have shown specific firing for locations. These findings agreed with previous works suggesting that the firing of place cells might not be representing the location of the animal, but the experience of the animal there (Eichenbaum et al., 1999). The link between spatial navigation and hippocampus has also been shown in humans by the impairment in navigation skills by an amnesic patient (Maguire et al., 2006) and the activation of individual neurons in epileptic patients (Ekstrom et al., 2003).

The hippocampus has been proved as an important link for processing relationship between different types of information in a collection of studies covering different areas. A recent paper by Olson and colleagues reported that in a study comparing patients suffering from MTL amnesia to controls, the performance reached by the amnesic group was reduced in the case of remembering inter-domain conjunctions (spatial location and object identification) even for very short lags (Olson et al., 2006). In a posterior fMRI study, subjects were showed sets of pictures in different locations. On it, the analysis of the MTL activity stressed the contribution of the hippocampus in the processing of pairs of an old and a new element of the pair object-location (Kumaran and Maguire, 2007). On a similar study, a significant activation of the hippocampus has been also reported for encoding of associations in working memory (Piekema et al., 2009). The learning of the conjunction stimulus-location, has been proved to be also represented at the single cell level in macaque monkeys (Wirth et al., 2003).

Complementary work have uncovered the importance of the hippocampus in the temporal link of individual experiences. A study in three amnesic patients suffering from very early onset of amnesia due to hippocampal damage reported spared ability to acquire factual knowledge at average population level,

but impaired acquisition of episodic memory or a temporal schedule in a daily life basis (Vargha-Khadem et al., 1997). Equally significant is the report of hippocampal activation when recalling a particular sequence of temporal episodes stored in memory – the order of movie sequences – in an fMRI study (Lehn et al., 2009). The role of the hippocampus in memories comprising temporal sequences has been also reported by studies in rats with hippocampal damage performing under control ones in odour sequences tasks (Agster et al., 2002; Fortin et al., 2002).

Single cell recordings in monkey MTL have reported cells responding to visual stimuli on basis of their previous presentation, i.e. their response was based on the recognition (or not) of the visual stimulus (Riches et al., 1991; Xiang and Brown, 1998; Hölscher et al., 2003). These neurons were mainly found in the perirhinal and entorhinal cortices, as well as similar ones outside of MTL as in area TE (Xiang and Brown, 1998). Furthermore, three different populations were found, firing to stimulus according to three criteria: novelty, familiarity and recency (Xiang and Brown, 1998). In total, they represented almost 40% of the activity recorded. Similar responses were found in two studies when analysing the firing of hippocampal neurons, but only in about 5% of the recorded population (Rolls et al., 1993; Xiang and Brown, 1998). This reduced percentage was interpreted in different ways by the two studies. Rolls et al. (1993) suggested that it was a significant sample of the neural population of hippocampus. Meanwhile, Xiang and Brown (1998) considered this percentage to be below the chance level and, therefore, not meaningful in the interpretation of hippocampal activity. This later interpretation would be in line with a recent image study in humans reporting the absence of hippocampal activity in process of familiarity (Eldridge et al., 2000).

A series of works with paired visual stimuli in monkeys showed also that

neurons in the parahippocampal area were related to learning of intra-domain associations. Sakai and Miyashita reported the activation of neurons in the anterior temporal cortex responsive to abstract visual patterns (Sakai and Miyashita, 1991). These neurons were initially responsive to one of the patterns of each pair, but, after learning the association, showed activation for both of the figures of the pair. Additionally, on a posterior study following a similar paradigm, Higuchi and Miyashita showed that the perirhinal and entorhinal cortices were needed for establishing the association of the pairs in infero-temporal cortex – IT – (Higuchi and Miyashita, 1996). Furthermore, these MTL structures were needed for remembering the association of pairs learned before lesions were produced, uncovering the importance of neurons located in the parahippocampal gyrus for both encoding and retrieval. This role was later supported by a posterior work from the same laboratory showing that the fire timing of neurons in areas from IT and perirhinal cortex follow an upward scheme in perception (IT preceding perirhinal firing) while showing the inverse timing scheme for retrieval of memories (Naya et al., 2001).

Studies in recognition and comparison of visual elements on very short time lapses or simultaneous presentation have suggested that the MTL, in particular the perirhinal cortex, could play a role in perception of visual stimuli in addition to the previously mentioned role in memory (see Buckley and Gaffan (2006) and Murray et al. (2007) for reviews).

At the same time studies in both animal and humans deepened the understanding of memory processes and uncovered how perirhinal cortex, parahippocampal cortex, entorhinal cortex and hippocampus interact in the acquisition and retrieval of declarative memories, evidences showed that, as noted in previous sections, the amygdala participated in a different way in the memory processes. Lesion studies soon showed that the role of amygdala was more de-

terminant in fear conditioned responses than in declarative memories (Squire et al., 2004). Since then, responses from amygdala have been extensively studied using imaging techniques (fMRI and PET, for a review see Zald (2003)). The mediation of amygdala in perceptual processes has been documented, although it is believed that it is related to processes of attention and detection of danger (Gazzaniga et al., 1998; Phelps and LeDoux, 2005). One of the most noticeable characteristics of this role of amygdala is the fast habituation of the neural responses, recording a decrease in activation after only a reduce number of exposures (Breiter et al., 1996), although it is a different process than the above-mentioned conscious detection of novelty and familiarity reported for other areas.

2.3.5 Direct recording of medial temporal lobe neurons in humans

The research works presented above provide evidence of the function of MTL either by indirect measures (fMRI, PET or lesion studies) or by animal models of brain functions. The former ones offer valuable information, but only of basic structures and do not report direct activation of neurons (Logothetis et al., 2001) while the later ones offer possible discussion over processes requiring consciousness and biases produced by the training required to achieve the desired performance. Therefore, the exceptional opportunity of recording the activity of single cells in humans developed more than two decades ago (Heit et al., 1988) and used regularly on a more recent set up (Fried et al., 1997) has offered invaluable insight of brain functions for the last years (Engel et al., 2005).

These recordings are carried out on epileptic patients who suffer from severe epilepsy that needs of intracranial monitoring for the correct estimation

of the epileptic focus for the exploration of a possible surgical resection of it (Engel et al., 2005). The type of recording electrode used in this set up is presented in Figure 2.6. A set of microelectrodes of about $40\ \mu\text{m}$ diameter each is added to the tip of the EEG electrode used for clinical purposes.

In addition to the obvious benefits obtained from experiments with human subjects, it is specially interesting to be able to instruct them in the task to be performed instead of long training sessions needed in animal studies. A major advantage of instruction over training on a task to is that the later may change the firing of the neurons or even the brain structures involved on the process (Mishkin et al., 1984).

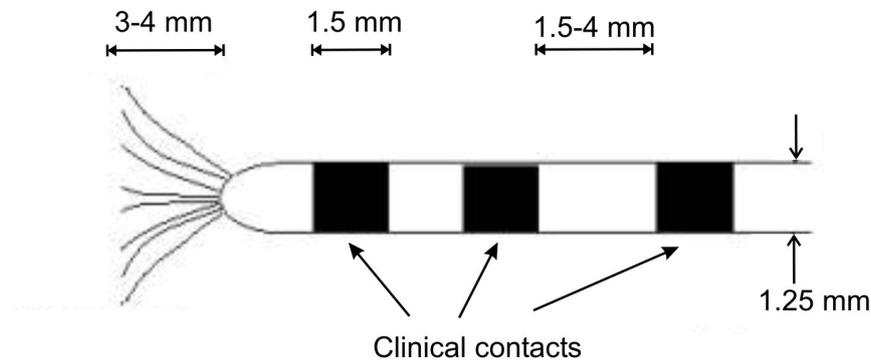


Figure 2.6: Electrode for intracranial recordings. The clinical contacts record the EEG signal for diagnosis purposes while the microelectrodes at the tip register single cell activity (Fried et al., 1997)

As could be expected from the research presented in the previous section, with recordings of single neurons in MTL, neurons with responses elicited by objects and faces in recognition memory tasks were found (Fried et al., 1997).

These responses were selective to either faces or objects (Fried et al., 1997) and, as a later report showed, they could be not only excitatory, but also inhibitory, i.e. the neuron fires significantly below its baseline rate (Fried et al., 2002). In addition, later studies showed that there are also specific populations of neurons contributing to recognition through the identification of novel stimuli: a population with increasing activity for novel items and another one for already presented ones (Rutishauser et al., 2006a; Viskontas et al., 2006).

Further investigation of the neural responses' specificity revealed that they are, in fact, selective to different categories much more narrowly tuned than general faces and objects: animals, food, patterns, spatial locations, cars and famous and emotional faces (Kreiman et al., 2000a). The category selective activity of the neural population seen by Kreiman and colleagues was later found also at the aggregate level by an analysis of the local field potential (LFP) around the tip of the electrodes (Kraskov et al., 2007).

Despite the correlation between responses of single units in the MTL and visual stimuli, further studies revealed a series of characteristics that linked them to higher cognition processes and suggest that they are not involved in perception. In 2002 Kreiman and colleagues showed that in cases of visual rivalry the firing of neurons followed the conscious perception reported by the subject (Kreiman et al., 2002).

A major breakthrough in the analysis of single cell activity in MTL was the report of Quian Quiroga and colleagues of neurons responding to particular concepts when the subjects were presented with a collection of pictures (Quian Quiroga et al., 2005). The most striking characteristic was the *invariant* character of the responses: the recorded activation of a particular unit was linked to the presentation of a concept, rather than an stimulus, i.e. the neurons increased their activity to *any presentation* of a particular idea. The range of

concepts could be from a category (e.g. animals), to a very specific one (e.g. the tower of Pisa, in Italy). These cells represent a subpopulation of neurons within the MTL forming a sparse representation of particular concepts recognised by the patient (Quian Quiroga et al., 2008a). Indeed, the neural population offered a representation of the knowledge space of the subject – in a similar way that place cells represent space information in rat hippocampus –, including the possibility of decoding visual stimulus from the firing of the units recorded (Quian Quiroga et al., 2007).

Further investigation carried in the neurons reported by Quian Quiroga et al. (2005) uncovered a collection of intrinsic characteristics that helped in the understanding of the role played by them. When subjects were tested at very short presentations, the neurons only became active for the trials in which the pictures were recognised, remaining silent in the rest of the occasions (Quian Quiroga et al., 2008b). In an equally revealing study, it was shown that responses in different areas of the MTL presented a different level of invariance for the stimulus presentation of the preferred concept (Quian Quiroga et al., 2009). Responses in hippocampus, entorhinal cortex and amygdala presented invariance also to the mode of presentation (visual or auditory), while responses found in the parahippocampal cortex were responsive only to visual stimuli (Quian Quiroga et al., 2009). These results denoted a hierarchical structure within the MTL, as also suggested by the analysis of the temporal onset of responses for different areas reported by experimenters of the same group, with earlier responses in parahippocampal cortex, followed by the ones in entorhinal cortex with later ones in hippocampus and amygdala (Mormann et al., 2008). These results are in full agreement with the anatomical structure of the MTL (see section 2.2) and previous reports using combined measures from fMRI and EEG (Fernández et al., 1999). This collection of characteristics, together with

the data from MTL presented in previous sections, support the suggested role of these neurons in the link between perception and declarative (probably episodic) memories (Quiñan Quiroga et al., 2008a).

Single cells in the MTL have also been tested on their role in retrieval of memories. Category cells reported by Kreiman et al. (2000a) were also active when the subjects were instructed to imagine the pictures (Kreiman et al., 2000b). In addition, when instructing the subjects to remember word pairs, hippocampal neurons were indicators of the performance in pairs remembering (Cameron et al., 2001). Furthermore, in a paradigm using short videos and instructing the subject in free recall a few minutes later (mimicking the natural functioning of episodic memories) highly selective neurons, similar to the ones reported by Quiñan Quiroga et al. (2005), became active during the presentation of a particular clip (Gelbard-Sagiv et al., 2008). They also increased their activity preceding the subjects' oral report of that particular memory. In a posterior study, the authors analysed the neural activity during repeated visualisation of clips. They reported that neurons recorded in the hippocampus (and not in other areas) presented increased correlation for consecutive time segments within the films (Paz et al., 2010). This correlation, linking successive temporal events, was considered the same that mediates episodic memories. This view can be supported by a recent study that has shown that the responses of MTL neurons are more likely to be elicited by concepts of high personal relevance to the subject (Viskontas et al., 2009), in line with the autobiographical nature episodic events.

2.4 Extracellular recordings in the medial temporal lobe

The data presented above was recorded through electrodes placed within the brain structures of epileptic patients following clinical criteria (Fried et al., 1997). In this section I review the technique utilised and the signal processing methods necessary for the identification of single neuron activity.

Invasive recordings of neural activity can be done at various scales, depending on the concrete target of the study and the availability. An overview of the recording ranges of the different intracranial recording techniques is presented in Table 2.1. **Electrocorticography (ECoG)** and **deep EEG** use similar principles to scalp EEG (non invasive). ECoG consist on a collection of small electrodes that are attached to the brain surface under the scalp. They allow much more precise study of cortical areas than scalp electrodes and they can even be used for tasks requiring high recording precision like brain machine interfaces (Kipke et al., 2008). Deep EEG uses, as well, a similar principle, but it is based on electrodes placed deep in brain areas, needing of a hole to place electrodes similar to the one showed in Figure 2.6 across the brain areas to be recorded. Both of these techniques, as well as the LFP, record the activity of a particular brain area close to the electrode. The activity registered comes from the activity of both inputs and outputs of the neurons in the area. However, although more specific than the scalp recordings, they are not able to provide single unit activity. **LFP recordings** provide, as mentioned, the activity of the population around the tip of an electrode. The recording electrode, however, is the same as the one needed for single unit activity, which is much smaller than the one used in EEG recordings. Thus, individual activity can only be recorded using extracellular or intracellular recordings. While the first one is based on

an electrode placed in the area around neurons, **intracellular recordings** consist in trespassing the cellular membrane and recording from inside a neuron, making sure that the spikes recorded come from a single source.

Recording Technique	Effective recording distance	Recording level
ECoG	\sim cm	cortical column
Deep EEG	\sim cm	brain substructure
LFP	\sim mm	neurons aggregate
extracellular rec.	\sim 100 μ m	several neurons
intracellular rec.	\sim μ m	1 neuron

Table 2.1: Range of the different types of invasive recordings

In the case of **extracellular recordings** a very thin electrode (about 10–50 μ m in diameter) is placed in the brain tissue, in the vicinity of neural bodies. There, it records the electric activity of the surrounding area (see Figure 2.7a and Buzsáki (2004)). Each of the neurons close to the electrode generates a small electric field surrounding its soma when becoming active: a spike (Rieke, 1997; Gazzaniga et al., 1998; Kandel et al., 2000). The spikes generated by different neurons display a characteristic wave shape in the electrical signal recorded, depending, among other factors, on the morphology of the neuron and the relative position between the neuron and the electrode (Rall, 1962; Gold et al., 2006). The contribution of thousands of these neurons generates a complex signal recorded by the electrode (Douglas and Martin, 1991).

The recorded signal contains two types of information: LFP and single and multiunit activity (Douglas and Martin, 1991). From the electrode located in brain tissue (see Figure 2.7a) the signal is amplified and sampled to its digital format for processing and storage. The standard procedure followed from there (as can be seen in Figure 2.7b) is: 1) *high pass filtering* of the signal (200-300 Hz), to work with the band frequency of spikes separating them from the LFP activity; 2) *spike detection*, usually making use of an amplitude

threshold, getting those spikes with higher amplitudes (from neurons closer to the electrode); 3) *feature extraction*, reducing the dimensions of representation of each spike, characterising them by a few parameters that would help in their posterior classification and 4) *clustering* using the features from the previous step and a clustering algorithm to group the spikes detected into individual neuron activity (Lewicki, 1998). The process of spike detection and sorting allows the characterisation of the activity of the basic units of brain functioning (Rieke, 1997).

The units resulting of the sorting process are usually divided in single units and multi-units (Martinez et al., 2009). Single units are those neurons close to the recording electrode – less than $50\ \mu\text{m}$ away from the tip, see Buzsáki (2004) – (neurons red, green and blue in Figure 2.7a). These units are characterised by a high signal-to-noise ratio, which allows clear separation of the neural signals based on the wave shape of the spike. Multi-unit activity is generated by the neurons located slightly further from the electrode, covering up to $140\ \mu\text{m}$ (Buzsáki, 2004), area represented in blue in Figure 2.7a. The spikes generated by the firing of these neurons have a lower amplitude, enough to be detected by the amplitude threshold, but their signal-to-noise ratio does not allow their clustering, therefore they are usually group together.

Enhancements of extracellular recording performance are most important for the neuroscience community. The efforts for improving the recording capabilities are focussed on two directions: 1) the production of optimal electrodes and 2) the improvement of spike sorting algorithms.

The evolution of the recording probes have been noticeable in the last 15 years, providing advanced solutions for the extracellular recording of neurons. The combination of electrodes spatially close – close enough to record from the same sources – has allowed to record the same group of neurons from two

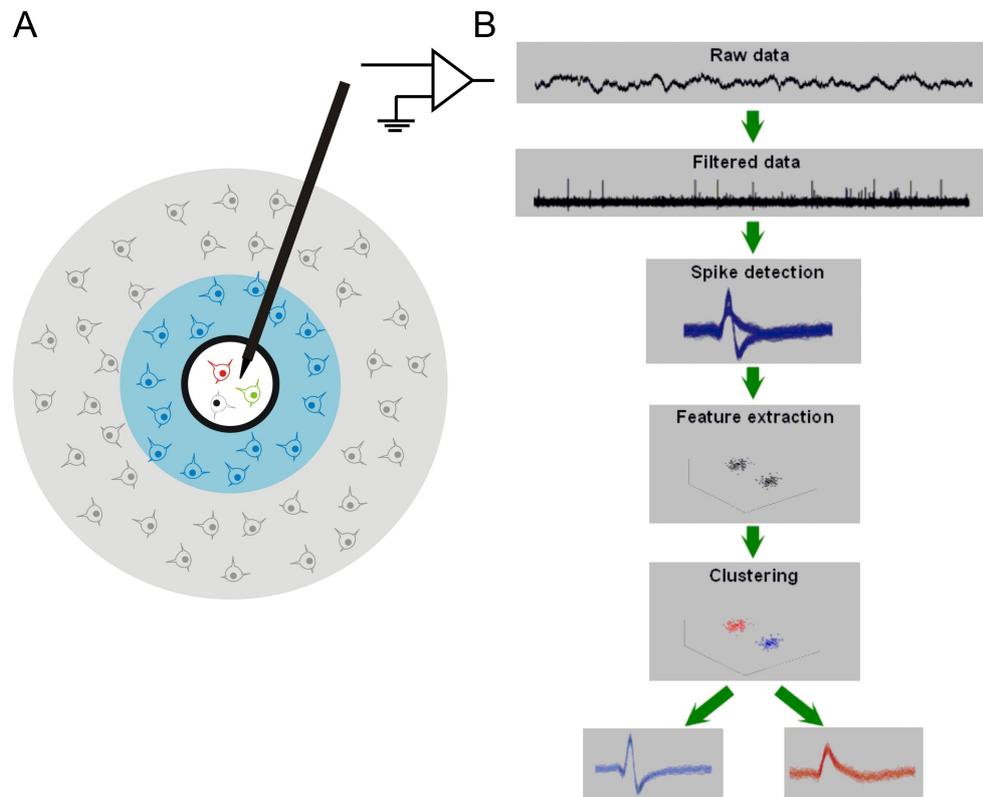


Figure 2.7: Recording electrode and spike sorting steps. A) electrode in neural tissue. B) step by step process of spike sorting.

(McNaughton et al., 1983) and four electrodes (Gray et al., 1995), facilitating the sorting of the neurons (Wehr et al., 1999; Harris et al., 2000; Henze et al., 2000). Furthermore, ambitious designs have included up to 96 electrodes in a single silicon-based probe with interelectrode distances below $100 \mu\text{m}$ allowing recordings of a single neuron from more than four contacts (Csicsvari et al., 2003; Blanche et al., 2005). Other probes have included up to a hundred individual electrodes, for recording of large populations of neurons in cortex (Nordhausen et al., 1996; Maynard et al., 1997; Rousche and Normann, 1998). However, even in these new developed probes, a spike sorting process is necessary for the identification of the activity of individual neurons (Brown et al., 2004; Nicolelis and Lebedev, 2009).

In recent years many solutions have been proposed for the problem of spike sorting (Fee et al., 1996; Chandra and Optican, 1997; Hulata et al., 2000; Letelier and Weber, 2000; Pouzat et al., 2002; Quian Quiroga et al., 2004; Zhang et al., 2004; Rutishauser et al., 2006b; Vargas-Irwin and Donoghue, 2007). All the mentioned algorithms follow the structure presented above (see Figure 2.7b), offering particular solutions on the feature extraction and sorting strategy used. The lack of a common data format and the variety on the testing conditions makes the comparison between algorithms difficult and complex. In general, they are developed in concrete conditions to be used as a tool for a particular experimental set. This, in addition to the training required to perform best of each one of them, makes a real comparison of performances almost impossible. In the following section, I describe the particular algorithm used in the analysis of data recorded from MTL in humans, leading to the results presented in section 2.3.4.

2.4.1 The `wave_clus` algorithm

In a significant part of the studies presented in section 2.3.4 the results obtained were based on the spike sorting performed by the algorithms `wave_clus`, an automatic algorithm developed by Quian Quiroga and colleagues for its use in extracellular recordings (Quian Quiroga et al., 2004). As presented in the scheme of Figure 2.7, the first step consists in high pass filtering of the data. In the case of `wave_clus` this filtering is non-causal to avoid changes in the spike shape induced by changes on phase made by causal ones (Quian Quiroga, 2009).

Next, it performs the spike detection from the resulting signal, by setting

amplitude threshold at:

$$Thr = 5\sigma_n; \sigma_n = median\left(\frac{|x|}{0.6745}\right) \quad (2.1)$$

where x is the bandpass filtered signal and σ_n is an estimation of the standard deviation of the background noise (see Quian Quiroga et al. (2004) for details). Note that the estimation of equation 2.1 is based on taking the median of the signal and it is, therefore, locked to the noise level and is hardly dependent on the firing of large amplitude spikes, as it is when taking the conventional standard deviation of the signal.

After spike detection, features of the spike shapes are extracted by using the wavelet transform, a time-frequency decomposition with optimal resolution in both time and frequency (Mallat, 1989; Quian Quiroga et al., 2004). Each obtained wavelet coefficient corresponds to a feature of the spike shapes at a given frequency and time range. The coefficients that best separate the signal are automatically selected by using a Kolmogorov-Smirnov test (Quian Quiroga et al., 2004), choosing those with the least normal distribution, which likely represent more than one cluster of spike shapes. In the last step the algorithm does an unsupervised classification to group spikes into classes using superparamagnetic clustering, a clustering method from statistical mechanics that is based on nearest-neighbour interactions and does not assume any particular distribution of the data (Blatt et al., 1996; Quian Quiroga et al., 2004).

Figure 2.8 presents an example of spike sorting with `waveclus`. In this particular example the algorithm outcome was four different clusters with a clear separation in the feature space. Figure 2.9 presents another case of spike sorting. In this occasion, the recording contained a high firing neuron (a total of almost 8800 spikes) and a sparse neuron (less than 400). The algorithm had no

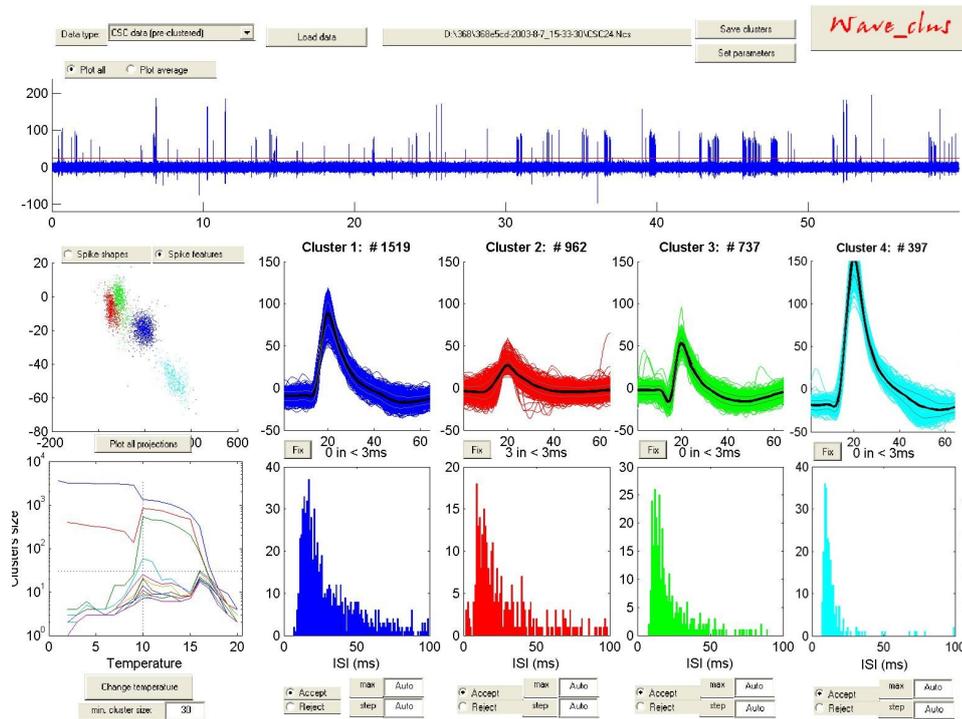


Figure 2.8: Sorting example using wave_clus. On top 60 seconds of the continuous signal. On the left the temperature diagram and a representation of the spikes in the feature space. On the bottom part the four clusters resulting of the clustering process are displayed.

problem separating them in two different clusters. These examples illustrate two of the characteristics of wave_clus: 1) automatic identification of various units and 2) isolation of sparse neurons in presence of other high firing units. Both of these characteristics are of special interest in extracellular recordings of MTL in humans. In these recordings the number of units surrounding the electrodes is completely unknown and it must be determined during the sorting process. The presence of sparse neurons in the MTL, and especially in the hippocampus, has been known for more than 2 decades from the recording of place cells in rat hippocampus (Thompson and Best, 1989). This study reported that only one third of the cells recorded while the animal was anaesthetised were active in a given environment. However, more recent studies have suggested that,

based in physiological data, out of a few hundred neurons around an electrode only four or five, at best, are actually recorded (Buzsáki, 2004; Shoham et al., 2006). This intrinsic pattern on the brain, with a low level of activity for a significant proportion of neurons, presents a great challenge for experimenters and algorithm designers. The recording of the activity of this neurons can be crucial for understanding the extraordinary characteristics of the brain (Shoham et al., 2006), as recordings in the human MTL have started to show (Quian Quiroga et al., 2008a).

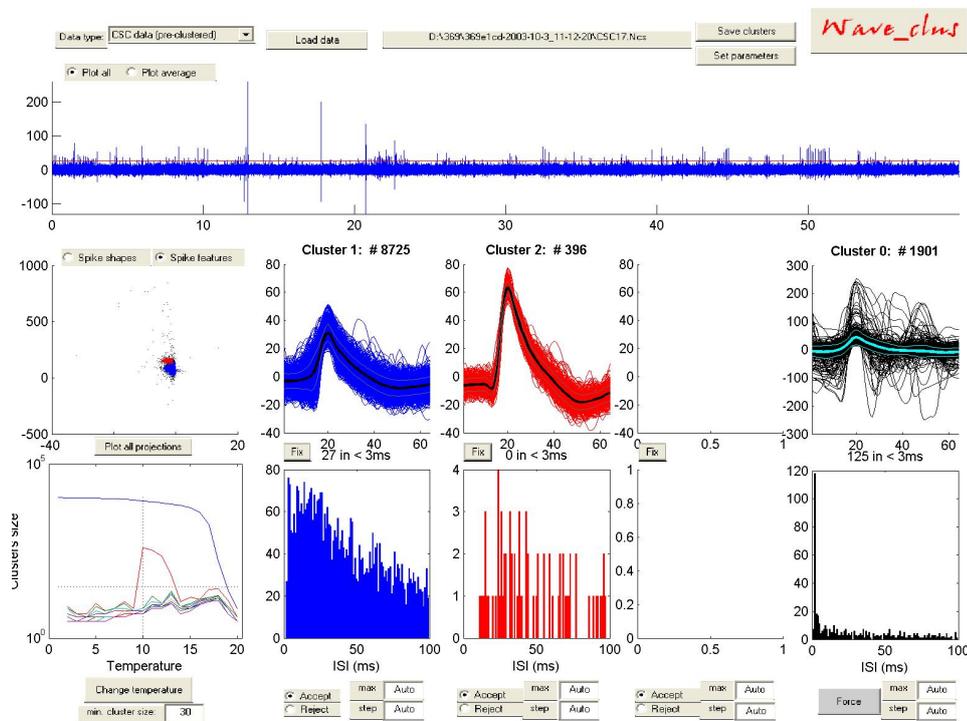


Figure 2.9: Another sorting example using wave_clus. In this case the solution provided contains two clusters: a multiunit and a sparse firing single unit

2.5 Summary of Chapter 2

In this chapter we have introduced the MTL and its relevance for neuroscience. Following the research elicited by what is probably the most famous amnesic

patient in neuroscience – the case of H.M. –, we have reviewed the primary role of the MTL in memory and the discussion established in neuroscience about its role and the ones attributed to the structures conforming it.

We have seen how the experimental work developed in animals have allowed further clarification and understanding of specific functions of the MTL. Next we have seen how it is possible to record the direct activity of neurons in the human MTL. These recordings have shown neurons correlating with conscious perception of concepts, independently of the concrete presentation of it. Further studies have also shown the presence of a population of MTL neurons responding to the relative novelty of the stimulus presented.

To finish, we have reviewed the processes needed for the successful recording of single cell activity from electrodes implanted in the brain. We have also seen the performance and the desirable characteristics to be met by the algorithm.

In the result section I address two main questions related to the themes presented above, related to the neurons recorded in MTL in humans, one in the understanding of the functioning of the abstract neurons in the MTL and one concerning the method used in their recordings:

- How do the very abstract neurons identified by Quiñones Quiroga et al. (2005) vary with the repetition of a certain stimulus? Is there any difference among the neurons of the regions of the MTL when a stimulus is presented repeatedly?
- The spike sorting method introduced performs satisfactory in the recordings reported up to the present point. However, is the spike sorting identifying all the active neurons surrounding the tip of the electrode? Is there a limitation on the performance of the algorithms of spike sorting? Would

we be able to detect more neurons if they were present?

The remainder of this work is dedicated to addressing these questions, developing adequate analysis and computational tools covering the two different areas. The first point is addressed in Chapter 3 where we reproduce the results published in Pedreira et al. (2010). The second one is addressed in Chapter 4 covering the work covered in Pedreira et al. (*in revision*).

Part II

Results

Chapter 3

Analysis of stimulus repetition in MTL neurons firing

3.1 Introduction

In the previous chapter we have seen that the recognition of visual objects is processed along the ventral visual pathway, extending from primary visual areas (V1) to the inferotemporal cortex (IT) (Logothetis and Sheinberg, 1996; Tanaka, 1996). In turn, IT cortex has direct projections to the MTL (Saleem and Tanaka, 1996; Suzuki, 1996; Lavenex and Amaral, 2000), where single cell studies in monkeys reported visual responses, among others, by stimulus-selective neurons which were related to the learning and rehearsal of association between visual cues (Sakai and Miyashita, 1991; Naya et al., 2001; Wirth et al., 2003). As seen in section 2.3.5, it has been shown that, in humans, neurons in the MTL respond strongly to visual inputs (Fried et al., 1997; Kreiman et al., 2000a,b, 2002; Quiñan Quiroga et al., 2005, 2008b, 2009; Gelbard-Sagiv et al., 2008). However, it has been previously stated that the MTL is involved in declarative memory, rather than visual recognition. This raises the question

of why MTL neurons respond strongly to images if this area is not part of the visual perception system. As seen in section 2.3.5 it has been suggested that these neurons link visual perception to memory formation based on i) the well-established findings about the role of the MTL in memory storage, ii) the relatively long latency of MTL responses at ~ 300 ms or longer after stimulus onset (Mormann et al., 2008; Quian Quiroga et al., 2005) compared to ~ 120 ms in monkey IT (Hung et al., 2005) and iii) the fact that MTL neurons encode abstract information and not particular visual details (Quian Quiroga et al., 2005).

Brain imaging studies of stimulus repetition in humans, using both PET and fMRI, showed a decrease in activity for different areas, including the MTL (for reviews see Henson and Rugg (2003); Ranganath and Rainer (2003) and Grill-Spector et al. (2006)). This effect, known as *repetition suppression*, has been associated with the behavioural phenomenon of *priming*, which is generally characterised by the shorter time required to obtain a response of a familiar object compared to a novel one (Schacter and Buckner, 1998; Wiggs and Martin, 1998; Henson and Rugg, 2003). In general, the reduction in neural activity associated to an specific stimulus has been related to processes involving perception, attention and learning (see Grill-Spector et al. (2006) for a review). At the neural level, an analogue effect known as *response suppression* was reported in a series of experimental stimulus repetition paradigms inducing response suppression patterns in monkey IT neurons (Miller et al., 1991; Li et al., 1993). These recordings were collected in awake animals performing a delayed match to sample (DMS) task and the firing of stimulus-selective neurons presented a cumulative decay over trials. In addition, two recent studies have reported similar effects for non-memory tasks (Sawamura et al., 2006; Liu et al., 2009). However, the exact correlation between the effects measured using image tech-

niques and single unit recordings are still to be defined. Given these previous findings, we investigated whether a similar pattern of decreased responses with stimulus repetition was also present in the visual responses in the human MTL. We hypothesise – considering the studies about MTL function reviewed in chapter 2 – that such a finding with neurons in this area may be due to their role in declarative memory.

3.2 Materials and methods

3.2.1 Subjects and recordings

Subjects were 26 patients with pharmacologically intractable epilepsy (15 men; 22 right handed; 17–54 years old). Extensive non-invasive monitoring did not yield concordant findings corresponding to a single resectable epileptogenic focus. Therefore, patients were implanted with chronic depth electrodes for typically 7–10 days to determine the focus of the seizures for possible surgical resection (Fried et al., 1997). All studies conformed to the guidelines of the Medical Institutional Review Board at UCLA. The electrode locations were based exclusively on clinical criteria and were verified by MRI or by computed tomography co-registered to preoperative MRI. Here we report data from sites in the hippocampus, amygdala, entorhinal cortex and parahippocampal cortex. Each electrode probe had a total of nine micro-wires at its end, eight active recordings channels and one reference (Fried et al., 1997). The differential signal from the micro-wires was amplified using a 64 channel Neuralynx system, filtered between 1 and 9,000 Hz and sampled at 28 kHz. Each recording session lasted about 30 minutes.

The data reported here were recorded during 44 experimental sessions.

Subjects lay in bed facing a laptop computer on which pictures of animals, objects, landmark buildings and known and unknown faces were shown. After image offset, subjects had to respond whether or not the picture contained a human face, by pressing the Y and N keys, respectively. This simple task, on which performance was virtually flawless, required them to attend to the pictures (Quiñan Quiroga et al., 2005). Images covered about 1.5 degrees of the visual angle and were presented for 1 second at the center of the screen, 6 times each in pseudo-random order. In a slightly different version of this paradigm, for 13 sessions the presentation time was 500 ms and the key responses were omitted. These sessions were considered together with the 1 s presentation sessions since there were no clear differences in the response patterns. The mean number of images shown to the patients was 114.2 (range 83–192). The number of pictures in each experiment was determined by their presentation time, to reach a total experiment length of approximately 30 minutes, maximum time allowed in a single session with a patient (i.e. the higher number of presentations belonged to the experiments that showed pictures for 500 ms). Of the 44 experimental sessions, 26 corresponded to the first experiment done with each of the 26 patients, so that the first trial for each picture was the first time the patient saw the image at the UCLA ward. The remaining 18 sessions corresponded to second sessions collected from 18 of the 26 patients, carried out on a following day. All the pictures considered from the second sessions were already presented in the first session. Due to the variability of spike shapes, it was in general not possible to follow the activity of single neurons across different experiments.

3.2.2 Data analysis

From the continuously recorded data, spikes were detected and sorted using the ‘Wave_clus’ software package (Quian Quiroga et al., 2004). As in previous studies (Quian Quiroga et al., 2005, 2007), a response was considered significant if it was larger than the mean plus 5 standard deviations (s.d.) of the baseline period (1000 to 300 ms before stimulus onset) for all stimuli, and had at least two spikes in the time interval between 300 and 1000 ms after the stimulus onset. For those pictures eliciting significant responses, we computed the total number of spikes between 300 ms and 2000 ms after stimulus onset for each trial. To account for the fact that different neurons have different firing rates, the responses were normalised by dividing by the maximum number of spikes across trials. For example, if a neuron responded to a particular stimulus 40, 35, 32, 30, 29 and 28 spikes in each one of the trials, we divided all of the trials by the one with a maximum number of spikes (40), obtaining normalised responses of 1, 0.875, 0.8, 0.75, 0.725 and 0.7, respectively. To analyse the change of the response magnitude in each trial, the normalised number of spikes across trials were statistically compared using a one-way analysis of variance (ANOVA, Test 1), where the independent variable was the trial number and the repeated measures were the normalised responses. This provided with a measure of the significance of the differences in the responses obtained for each trial at population level. This analysis was performed for the whole population of responses in the MTL as well as for each of the four subregions separately.

For a further characterization of the response patterns, for each of the significant responses we calculated a linear regression of the number of spikes with trial number within a session. Then, differences in the slope values of these linear fits covering the six trials of an experiment, were compared for the

different MTL areas and between the first and second experimental sessions using a two-way ANOVA (Test 2). The independent variables were MTL area and session number, and the repeated measures were the slopes of the responses (the slope of the linear fit of the six points representing responses for each trial of a session). Post-hoc, we evaluated differences of the slope values to the zero slope response pattern (i.e. a response with the same number of spikes for every stimulus presentation) for each area separately using a paired T-Test (Test 3).

To evaluate the time profile of the responses, the instantaneous firing rate was computed by convolving the normalised spike trains with a Gaussian kernel (sampling period = $0.5ms$, $\sigma = 100ms$). From the average instantaneous firing rate (across all responses) for each trial we defined: i) the peak amplitude; ii) its latency; iii) the onset of the response, as the point where the instantaneous firing rate crossed 4 s.d. above baseline and stayed above for at least 100 ms, and iv) the duration, as the time interval between response onset and offset (Figure 3.1). Offset was defined similarly to onset but crossing the 4 s.d. line downwards and staying below it for at least 100 ms. The effect of stimulus repetition on each of these parameters was assessed using one-way ANOVA with independent variable trial number (Test 4). The repeated measures were the values of the corresponding parameters for each response.

3.3 Results

In 26 first experimental sessions for each patient we recorded from 1210 MTL units (515 single units and 695 multi-units, see Section 2.4), with an average of 46.6 units per session. Of these 1210 units, 262 (22%; 132 single units and 130 multi-units) had a statistically significant response to a total of 725 pictures (an average of 2.77 responses per unit). For the second experimental sessions we

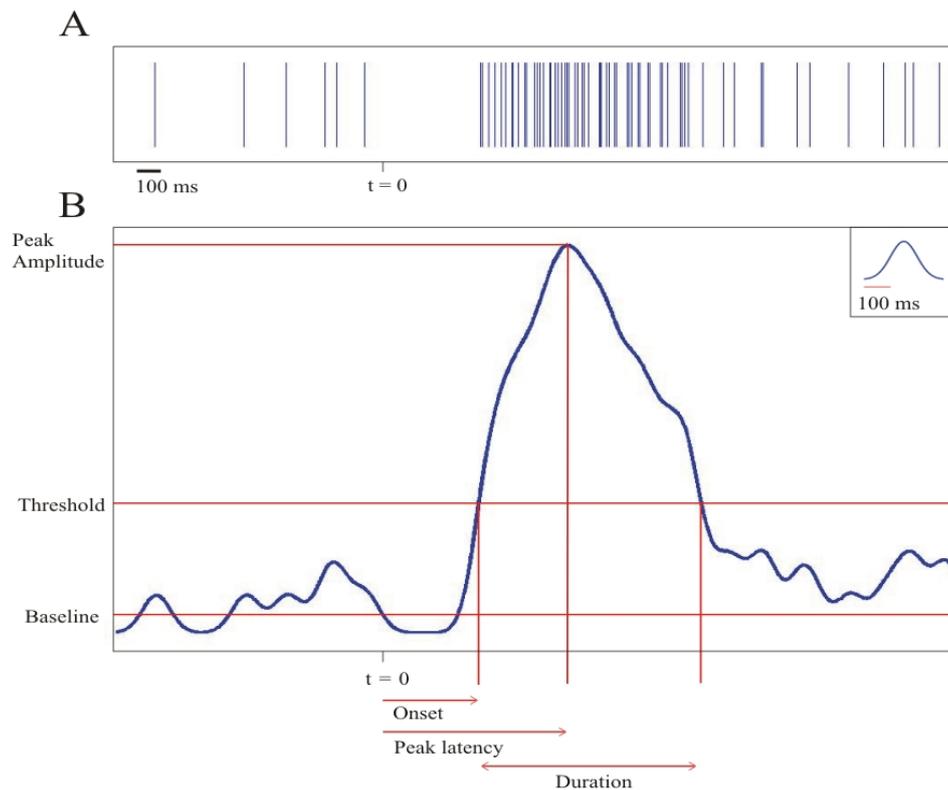


Figure 3.1: Parameters used for timecourse statistical analysis. A: Raster plot of a response from an idealized neuron. B: Instantaneous firing rate corresponding to the raster plot in A obtained by convolving the spike train with a Gaussian kernel with $\sigma = 100$ ms. A threshold was defined as the mean of the baseline plus 4 s.d. The onset of the response was defined as the time when the firing rate curve crossed this threshold and stayed above it for at least 100 ms. The offset was defined similarly, but with the firing rate curve crossing the threshold downward for at least 100 ms. Duration was the difference between the offset and the onset times. Time $t = 0$ ms symbolizes the onset of the picture.

recorded from a total of 745 units (328 single units and 417 multi-units), with an average of 41.3 units per session. Out of these 745 units, 110 (15%; 57 single units and 53 multi-units) had a significant response to a total of 289 pictures (2.63 responses per unit). The decrease in the responsiveness of the recorded units between experimental sessions (22% for the first session against 15% for the second one) was significant (chi-square, $p < 0.001$).

3.3.1 Single cell responses

Figure 3.2 presents four examples of significant responses recorded in four different patients. For each response we display the raster plot (first trial at the top), the number of spikes per trial and the peri-stimulus time histograms. The neuron in panel A was located in the entorhinal cortex. Its average baseline activity was 2 Hz and it fired with up to 20 Hz to the patients own picture. The neuron in panel B was located in the amygdala and from a mean baseline activity of about 7 Hz, it responded with up to 50 Hz to the picture of a monkey. The neuron in panel C was located in the hippocampus and from a baseline of about 3 Hz it responded with 30 Hz to the picture of a squirrel. Finally, the neuron in panel D was in the parahippocampal cortex and it responded to a picture of the World Trade Center with about 45 Hz from a baseline of 10 Hz. All these units increased their firing at least 3 times in response to their preferred pictures. However this change was not equally distributed across the six trials. In fact, in the four examples a clear decay in the number of spikes with trial number can be observed, as shown by the spike counts for each trial.

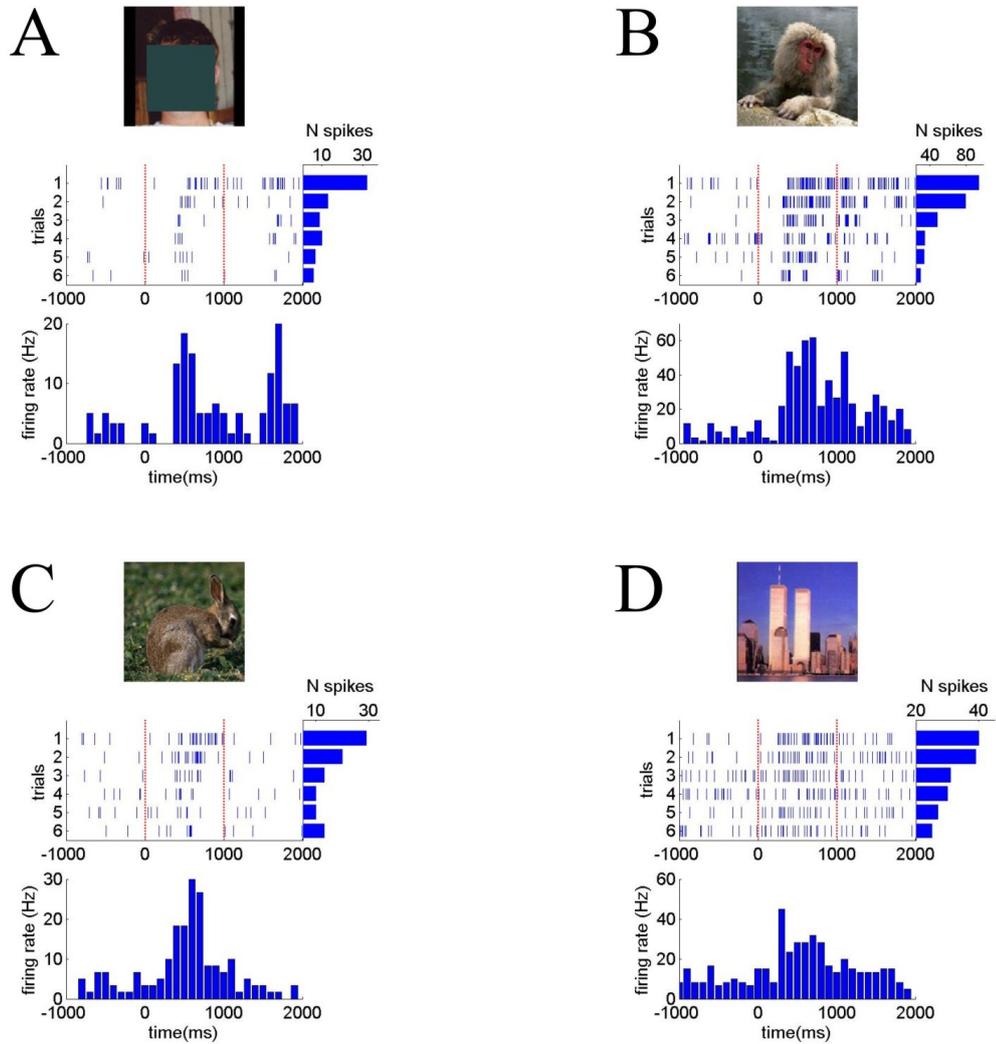


Figure 3.2: Single cell responses. The four panels (A–D) correspond to the responses to the preferred stimulus for 4 different neurons. Responsive neurons were located in entorhinal cortex, amygdala, hippocampus, and parahippocampal cortex, respectively. For each response, we display the raster plot (1st trial at the top), the number of spikes within a 300- to 2,000-ms time window (right) and the peristimulus time histograms (bottom). A decrease in the number of spikes with trial number can be observed for each neuron. The onset ($t = 0$ ms) and offset ($t = 1,000$ ms) of the pictures are marked by red dotted lines.

3.3.2 Population results

For each trial Figure 3.3A shows the mean normalised number of spikes of the 725 responses recorded in the first sessions: 238 from neurons in the amygdala, 311 in the hippocampus, 105 in the entorhinal cortex and 71 in the parahippocampal cortex. As shown in the single cell examples of Figure 3.2, there was a significant decay of the normalised number of spikes with trial number ($F(5, 4084) = 19.34, p < 10^{-15}$, Test 1, see Materials and Methods). Note that the normalised spike number does not reach a value of 1 because the maximum firing rate for different responses was not always at the same trial. Interestingly, this pattern of decay was not the same for all MTL areas, as shown in panels B to E of Figure 3.3. Considering each area separately, this effect was statistically significant for the responses in amygdala ($F(5, 1345) = 16.87, p < 10^{-15}$), hippocampus ($F(5, 1726) = 6.03, p < 10^{-4}$) and entorhinal cortex ($F(5, 588) = 2.52, p < 0.05$), while responses in the parahippocampal cortex ($F(5, 407) = 0.98, p = 0.43$) had no significant dependency on trial number.

To further study differences between the four MTL regions, for each response we computed the slope of the best linear fit and statistically compared the results for different areas (Test 2, see Materials and Methods). As shown in Figure 3.4, there was a significant difference between areas ($F(3, 721) = 7.1, p < 10^{-3}$), which was mainly because of the smaller slope values in the responses from the parahippocampal cortex. In line with this observation, a separate T-test analysis for each MTL location (Test 3) showed that the slope of the responses from entorhinal cortex, hippocampus and amygdala were statistically different from zero ($t = -3.04, d.f. = 104, p < 0.005$; $t = -5.77, d.f. = 310, p < 10^{-7}$; $t = -8.11, d.f. = 237, p < 10^{-13}$; respectively), while the ones

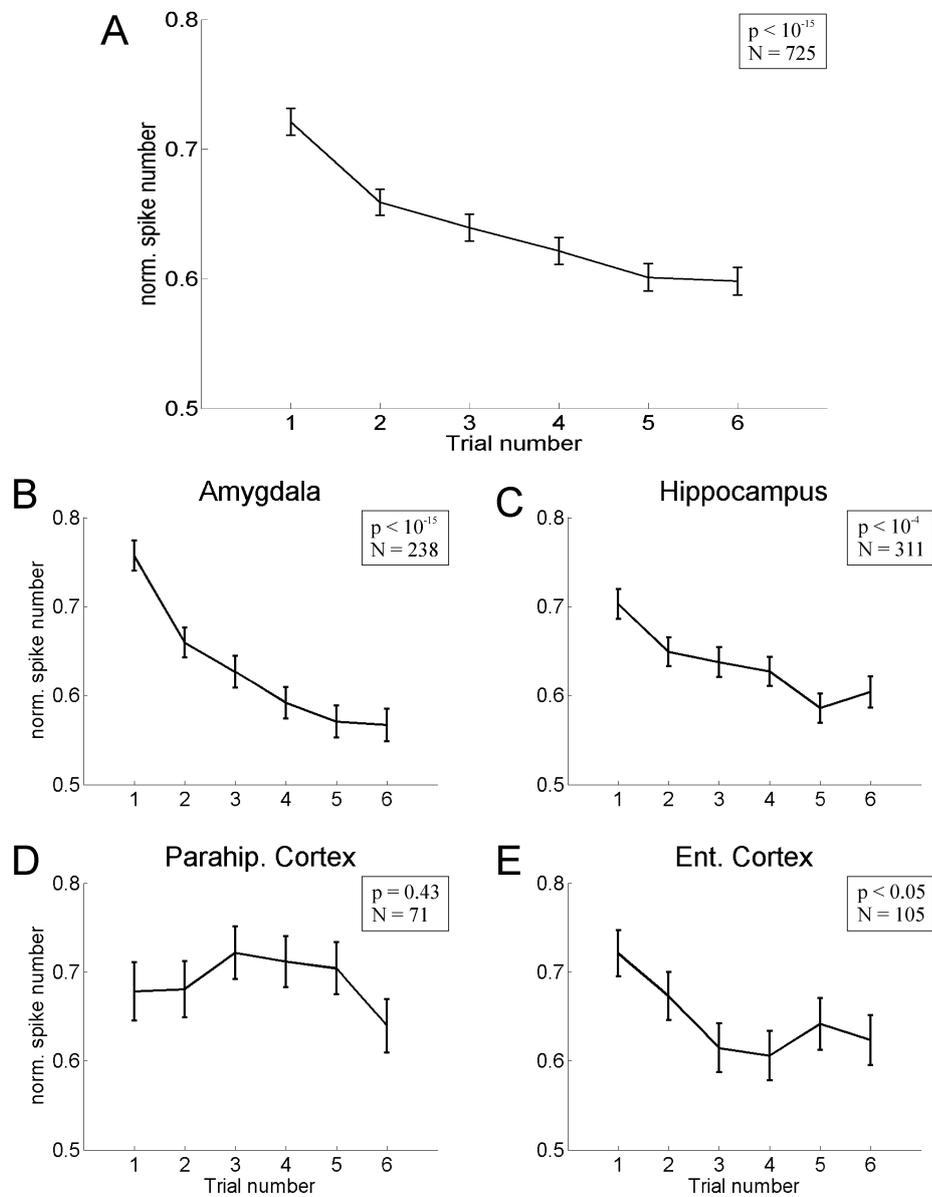


Figure 3.3: Normalised mean number of spikes per trial. A: result for all 725 responses. There was a significant effect of the trial number on the mean firing rate. The mean number of spikes was reduced by 17% from the 1st trial to the last one of the session. B–E: responses divided by area. There was a significant decay with picture repetition for responses in the amygdala, hippocampus, and entorhinal cortex but not for the parahippocampal cortex. N, number of responses. Bars denote standard error of the mean (s.e.m.)

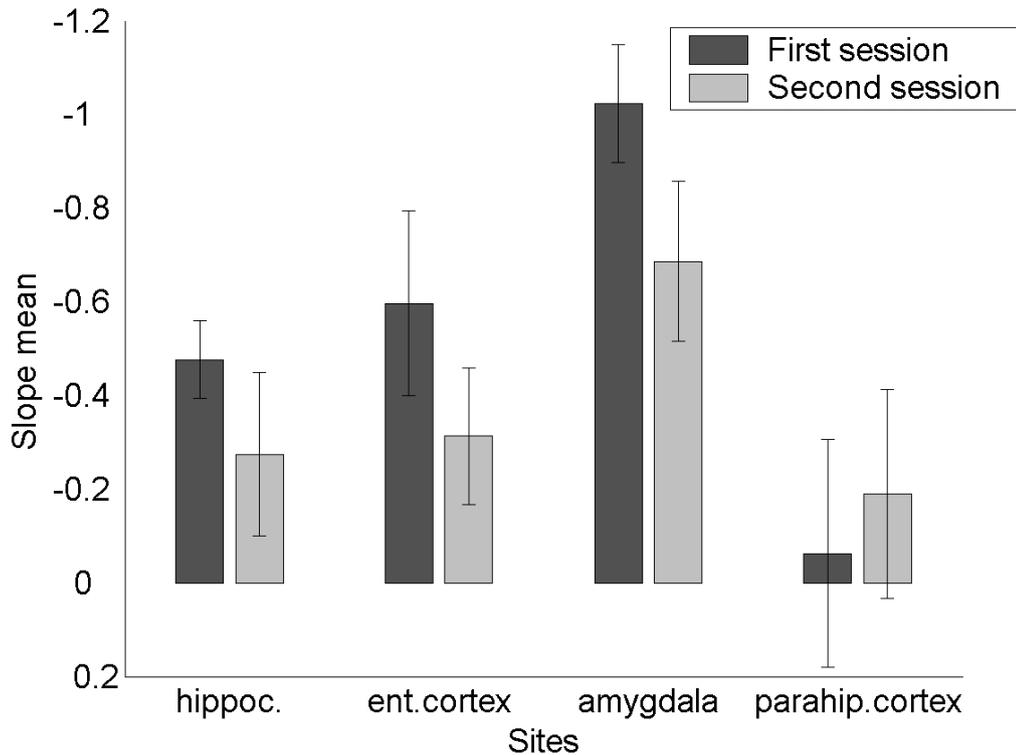


Figure 3.4: Mean slopes of the decay in response magnitude with trial number. Slopes of the responses grouped by location. The 1st and 2nd sessions are represented in dark and clear gray, respectively. The average slope value in session 2 was 50% lower for responses in hippocampus and entorhinal cortex and 30% for the amygdala responses. Parahippocampal responses had flat slopes, reflecting a similar firing for all trials in both sessions. Bars denote s.e.m.

from parahippocampal cortex were not ($t = -0.26, d.f. = 70, p = 0.8$).

3.3.3 Time profile of the responses

Figure 3.5A shows the instantaneous firing rates for each trial (see Materials and Methods) averaged over the 725 responses. Responses for all trials are clearly larger than baseline activity, and the decrease of the number of spikes with trial number shown in Figure 3.3 seems to be caused both by differences in the duration of the responses and differences in the peak amplitudes. Moreover,

it is apparent that for all trials, the onset of the responses does not differ significantly. Figure 3.5, B–E, shows the firing rate of the responses divided by areas. A higher amplitude for the first trial can be seen only in the responses from amygdala. A delayed maximum response in the first trial can be seen in the hippocampus and the entorhinal cortex. To verify these observations, we assessed and statistically compared the onset, duration, peak amplitude and latency of the responses, as defined in Figure 3.1 (Test 4).

Figure 3.6A presents the average peak latency for the entire population of neurons, for each of the six trials. There was a statistically significant decay of the peak latency with trial number ($F(5, 3998) = 6.41, p < 10^{-5}$). The same statistical analysis performed for each area separately, presented in panels B to E, showed significant peak latency shifts with trial number for the responses in amygdala, hippocampus and entorhinal cortex ($F(5, 1422) = 7.82, p < 10^{-6}$; $F(5, 1860) = 7, p < 10^{-5}$ and $F(5, 624) = 3.36, p < 0.01$; respectively, as shown in panels B, C and E). Figure 3.7A shows the analysis of the peak amplitude. As for the peak latency, there were significant differences with trial number ($F(5, 3998) = 2.83, p < 0.05$). However, as can be seen in panels B to E, in this case, a separate analysis for each area showed that this effect was significant only for the responses in the amygdala ($F(5, 1442) = 2.92, p < 0.05$), whereas it was not significant for the responses in hippocampus, entorhinal cortex and parahippocampal cortex ($F(5, 1860) = 0.98, p = 0.43$; $F(5, 624) = 0.91, p = 0.47$ and $F(5, 246) = 0.48, p = 0.79$, respectively). Figures 3.8 and 3.9 show the effect of trial number on the duration and the onset latency of the responses. As observed in Figure 3.5, panel A of Figure 3.8 shows a significant decrease with trial number for the duration of the responses ($F(5, 3531) = 3.09, p < 0.01$), but this effect was only significant for the responses in the amygdala ($F(5, 1205) = 2.43, p < 0.05$, Figure 3.8B). Figure 3.9

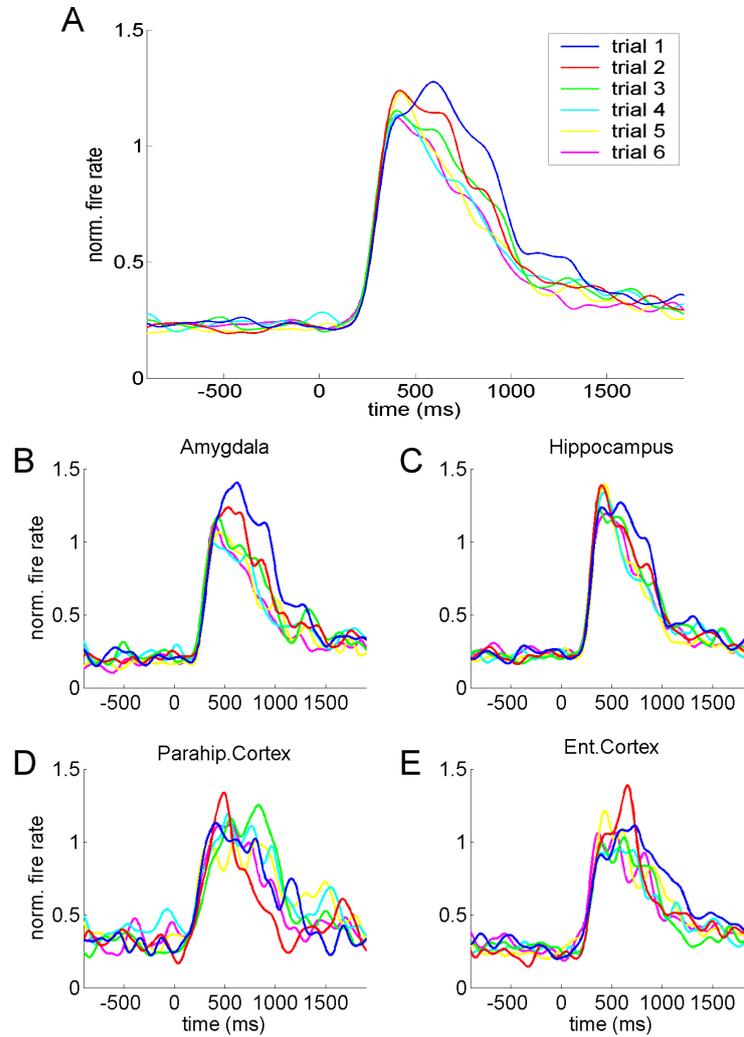


Figure 3.5: Mean instantaneous firing rate for each trial. A: average over the whole set of responses (725). Note that all 6 trials had similar onset latencies. Responses for the 1st trials, especially for trial 1, in dark blue, had a larger duration, as well as a delayed and slightly higher peak value. B-E: data broken down by areas. In B, responses from amygdala (238 responses) showed higher and later peak values for the 1st presentations. In C, responses from hippocampus (311) showed a late peak value for the 1st trial. D: no differences in the responses from parahippocampal cortex (71) were observed for the different trials. E: responses from entorhinal cortex (105) showed a similar delay pattern as those in hippocampus.

shows that there were no significant differences for the response onset latencies both for the entire population of neurons ($F(5, 3531) = 0.26, p = 0.9$, Figure 3.9A) or for data divided by area ($F(5, 1205) = 0.35, p = 0.88$; $F(5, 1505) = 0.66, P = 0.65$; $F(5, 469) = 0.25, P = 0.94$, and $F(5, 334) = 0.13, P = 0.99$; for amygdala, hippocampus, parahippocampal cortex and entorhinal cortex, respectively; panels B to E of Figure 3.9).

3.3.4 Results for the second experimental sessions

Next, we compared the results of the first sessions with those obtained in following experiments, usually performed on a different day. These sessions were available for 18 of the 26 patients. All the pictures considered from these sessions were already presented in the first sessions.

As for the first sessions, we computed the slope of the best linear fit to the number of spikes per trial for each of the 289 responses obtained in the second sessions: from the amygdala (138), hippocampus (68), entorhinal cortex (45) and parahippocampal cortex (38). The slope values for the different locations are presented in Figure 3.4 and the normalised responses analysis divided by area in Figure 3.10. The response pattern was similar to the one obtained for the first sessions, with responses in amygdala having the largest rate of decay with trial number, followed by the responses in entorhinal cortex, hippocampus and finally the ones in the parahippocampal cortex. A T-test analysis of each MTL subregion showed that only responses in amygdala and entorhinal cortex had a slope significantly different from zero ($t = -4.01, d.f. = 137, p < 0.001$ and $t = -2.16, d.f. = 44, p < 0.05$, respectively; Test 3). Note that, in general, the responses in the second sessions had overall lower slope values compared to the ones of the first sessions.

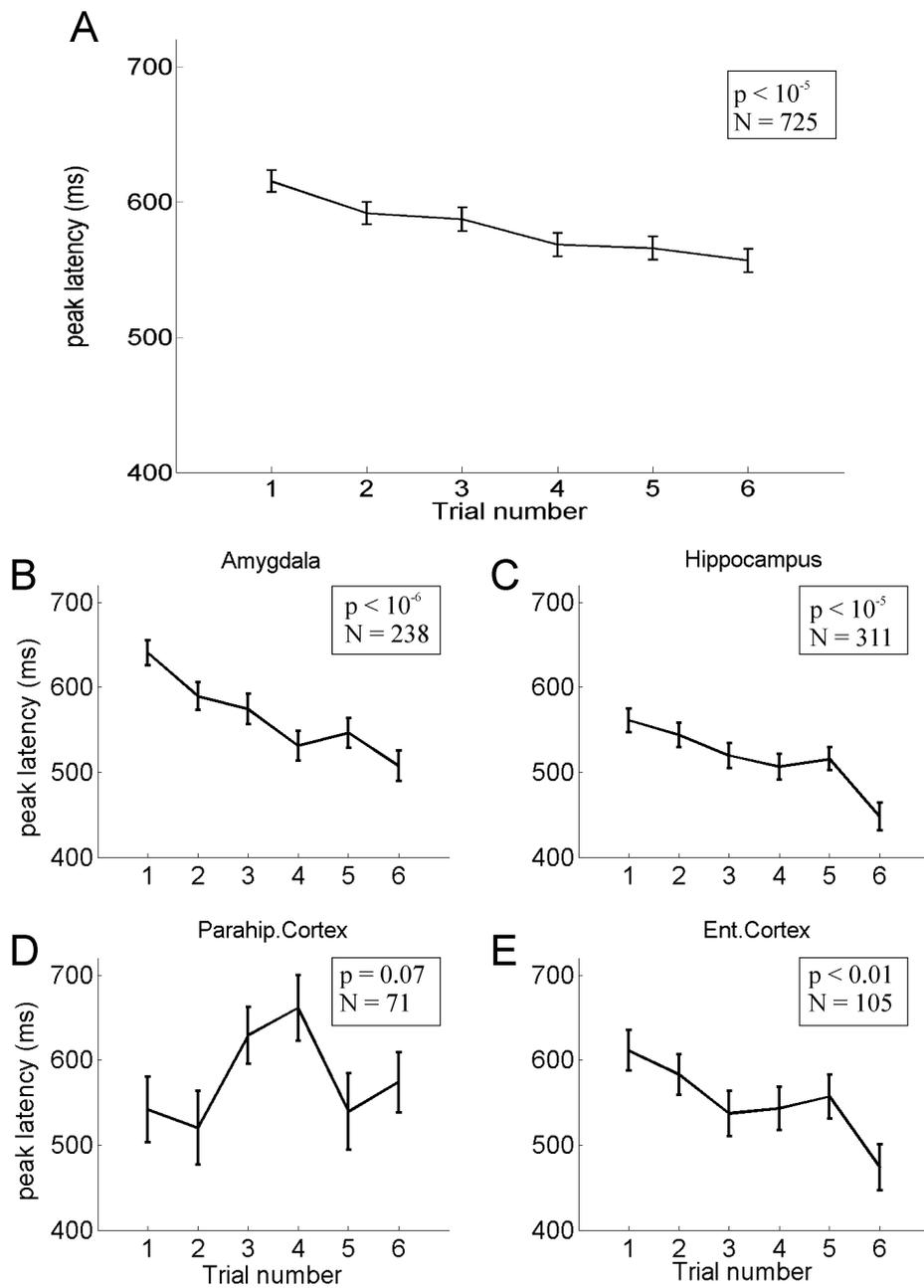


Figure 3.6: Average peak latencies. A: average peak latencies for all the 725 responses. Latencies were significantly earlier for later trials. B–E: the same analysis separated by area. A significant latency decrease with trial number can be seen in the responses from amygdala (B), hippocampus (C), and entorhinal cortex (E) but not in the responses from parahippocampal cortex. N, number of responses. Bars denote s.e.m.

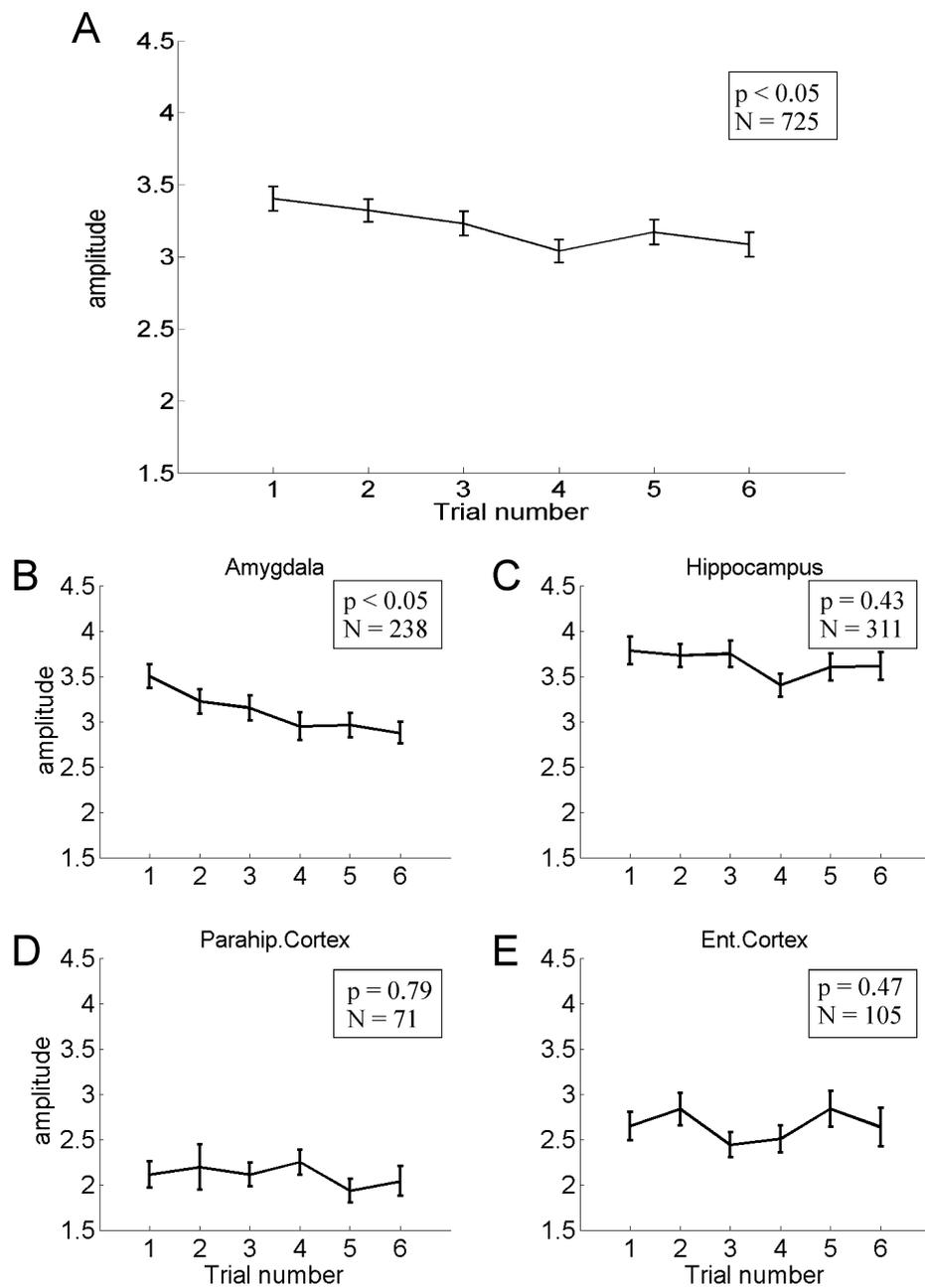


Figure 3.7: Normalised average peak amplitude of the significant responses. A: average peak amplitude for all the 725 responses. The peak amplitude was significantly higher for the 1st trials. B–E: data broken down by areas. Only responses from amygdala showed a significant decrease trial number. N, number of responses. Bars denote s.e.m.

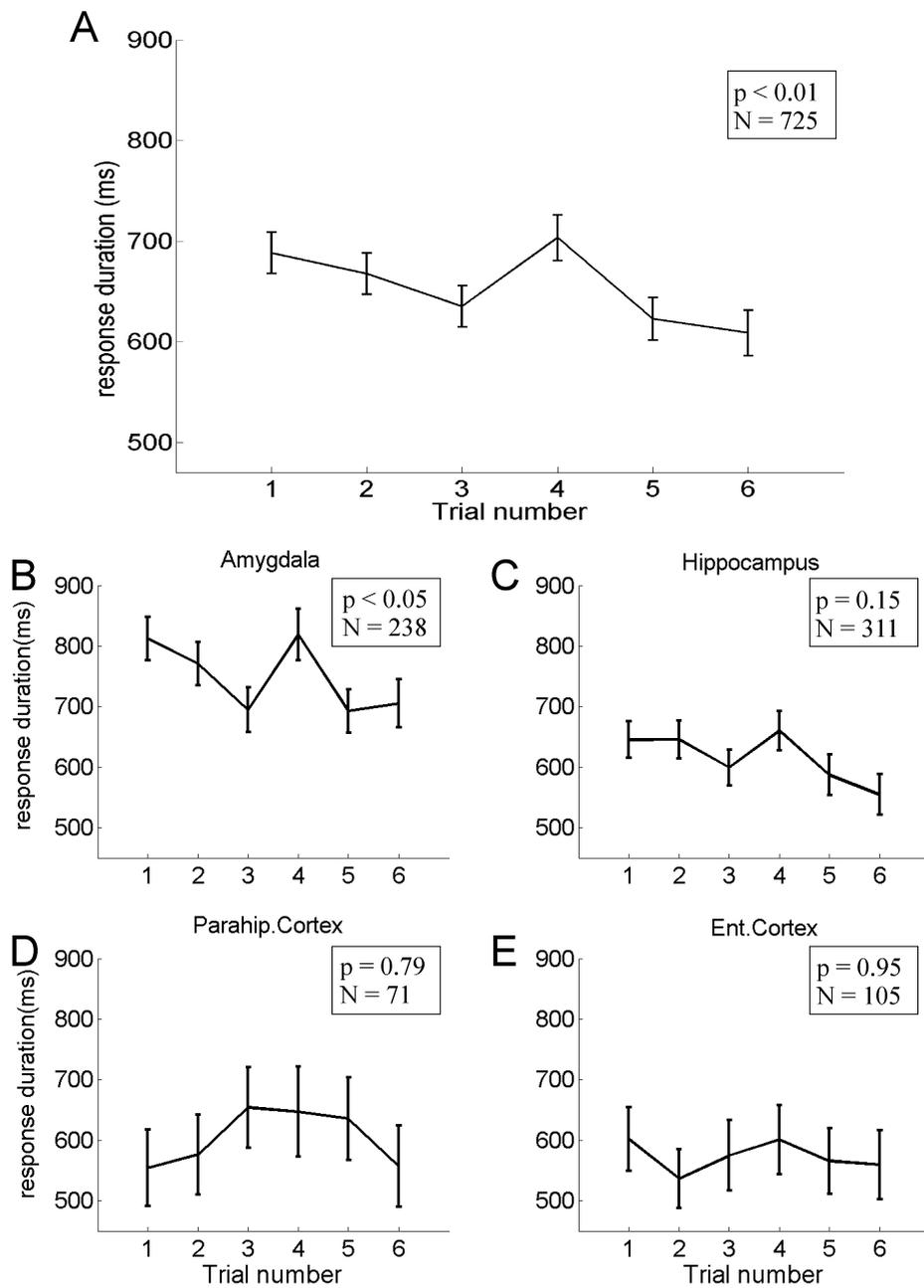


Figure 3.8: Average duration of the significant responses. A: average response duration for all the 725 responses. The duration decreased significantly for later trials. B–E: data broken down by areas. N, number of responses. Bars denote s.e.m.

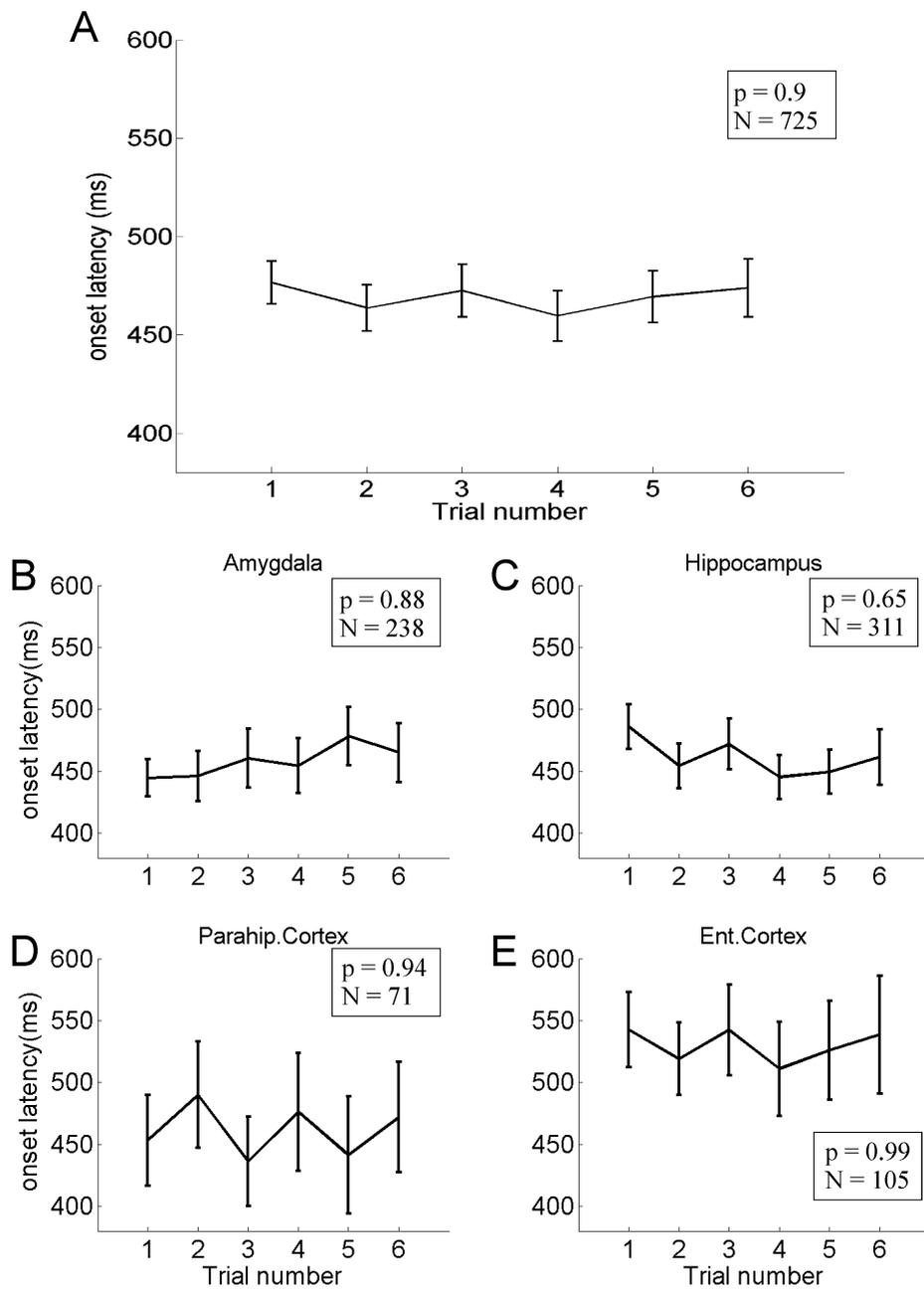


Figure 3.9: Average onset latencies of the significant responses. A: averaged onset latencies for the 725 responses. B–E: data broken down by areas. There were no significant differences with trial number. N, number of responses. Bars denote s.e.m.

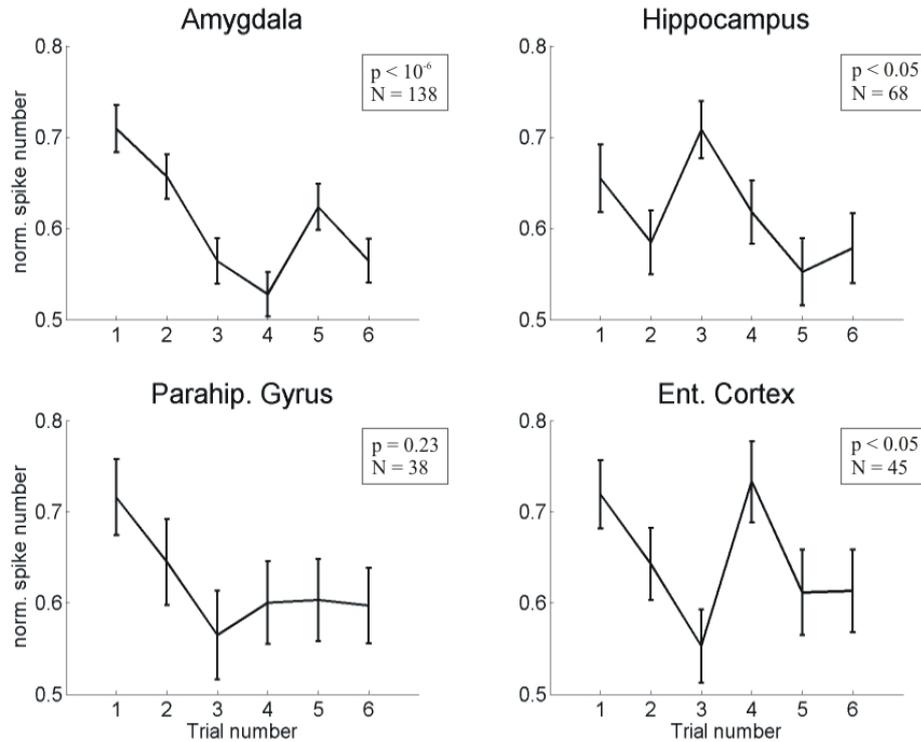


Figure 3.10: Mean number of spikes per trial for different MTL regions in the second session. Responses had a significant decay with picture repetition for amygdala, hippocampus and entorhinal cortex ($F(5, 754) = 7.55, p < 10^{-6}$; $F(5, 373) = 2.5, p < 0.05$; $F(5, 249) = 2.68, p < 0.05$, respectively) but not for parahippocampal gyrus ($F(5, 219) = 1.38, p = 0.23$). N refers to the number of responses for each particular region. Note that the patterns for the second session were not as clear as the ones found in the first experimental session, in agreement with data shown in Figure 3.3. Bars denote s.e.m.

Statistical differences in the slope values between the different brain areas and between the first and the second sessions were evaluated with a two-way ANOVA (Test 2). There were significant differences across the different MTL regions ($F(3, 1006) = 8.28, p < 10^{-4}$). The comparison between the first and the second sessions showed a general trend nearly reaching significance with smaller slope values for the second sessions ($F(1, 1006) = 3.55, p = 0.06$). Because responses in the parahippocampal cortex did not show an effect with stimulus

repetition, we repeated the analysis excluding these responses and found that the difference of the slopes between the first and second sessions was statistically significant ($F(1, 899) = 4.68, p < 0.05$).

3.4 Discussion

In this study, we showed a decrease of the number of spikes fired by neurons in the human MTL in response to repeated picture presentations. This effect was not homogeneous across the different MTL areas. In particular, a decrease in the response peak amplitude with trial number was significant only for the amygdala responses. Moreover, there were significant decreases of the response peak latencies for the responses in the amygdala, hippocampus and entorhinal cortex (but not for parahippocampal cortex). Given that the onset of responses was not different for the different trials (see Figure 3.9), the decrease in peak latency can be attributed to a time-sharpening of the responses – i.e., responses were more localized in time for the later trials – in agreement with the pattern observed in the instantaneous firing rate curves shown in Figure 3.5. The fact that in our study the time-sharpening of the responses was accompanied by a decrease in duration only for the amygdala neurons can be attributed to the less accurate estimation of the response durations, which accumulate inaccuracies in estimating both the onset and offset of the responses. In agreement with the previous observations, there was a decrease of the total number of spikes elicited in response to the stimulus for responses in the amygdala, hippocampus and entorhinal cortex.

Long-term response suppression effects have been reported by recent studies in monkey IT cortex during visual fixation and stimulus classification tasks (Freedman et al., 2006; Anderson et al., 2008). Interestingly, as seen in section

2.2, IT cortex has large projections to the MTL areas we recorded from. More related to our findings are reports of short-term response suppression in mere visual fixation tasks (Sawamura et al., 2006; Liu et al., 2009).

3.4.1 Novelty and familiarity in single cells in the human medial temporal lobe

There has been extensive research on response suppression in humans using non-invasive techniques such as EEG and functional MRI (fMRI) (e.g. Breiter et al. (1996); for reviews see Grill-Spector et al. (2006) and Ranganath and Rainer (2003)). However, it has to be noted that these studies can only give indirect evidence about the activity of single neurons, since they only measure the activity of large populations (Logothetis et al., 2001; Logothetis, 2008). Closer to our findings, using single cell recordings in patients implanted with intracranial electrodes for clinical reasons, two recent studies have reported novelty and familiarity effects in human MTL neurons (Rutishauser et al., 2006a; Viskontas et al., 2006). In these studies, previously unseen pictures of unknown places and faces were shown in a two-session protocol. In the first session, a set of these pictures was presented and subjects were instructed to remember them. In the second session, the previously presented pictures were mixed with some novel ones and subjects were asked to remember whether the picture had been shown previously or not. In the study of Viskontas et al., neurons from the hippocampal and parahippocampal regions showed, in general, a higher firing rate for novel pictures. Moreover, neurons from the hippocampus presented a decrease of their firing below the baseline activity for subsequent presentations. Rutishauser et al. described two subsets of cells in hippocampus and amygdala: one group of cells that increased their firing when the stimulus presented

was new and another one that increased their firing when it was shown few moments before (Rutishauser et al., 2006a). Although related, there were two main differences between these two studies and the one presented here. First, the neurons described by Rutishauser et al. (2006a) and by Viskontas et al. (2006) were significantly less selective than the ones presented here. In the study by Rutishauser et al. (2006a) the firing of the neurons presented was independent of the stimulus presented (see Figure 2 of the paper). In the study by Viskontas et al. (2006) the neurons presented either not selectivity at all or selectivity to faces or places – representing a broad category selection, covering about 50% of the stimuli presented – contrasting to the specific responses of the neurons presented here – the selectivity of our units is 2.77 responses out of more than 100 presented in average, including numerous pictures of faces. This reduced selectivity compared to our study can be attributed to the fact that: i) we used familiar stimuli, which are more likely to elicit responses (Viskontas et al., 2009); ii) we used an optimal spike sorting algorithm that is particularly suited to detect sparsely firing neurons, which typically have very low baseline firing rates (see e.g. Quiñero et al. (2008b, 2009)) and iii) we recorded continuous data and used optimal offline analysis. Note that to avoid large data volumes many acquisition systems detect spikes online based on amplitude thresholds set by hand. These thresholds may be set to non-optimal values, especially if the experimenter is dealing with a relatively large number of channels. In particular, this approach may miss or non-optimally detect very selective neurons because these may be silent when the thresholds are set. A similar “dark matter” problem arises when using movable electrodes because silent neurons may not be identified as the electrode passes by, unless the right stimulus is shown (Olshausen and Field, 2004; Shoham et al., 2006; Quiñero et al., 2008b). Although we currently have no direct evidence to assess

the contributions of each of these factors for comparing our responses with those described in Rutishauser et al. and Viskontas et al., it is likely that due to these differences our study describes a different set of cells with much higher selectivity. Following the terminology adopted by Bogacz et al. (2001), it could be said that we described the behaviour of representation neurons, while previous studies would be describing *familiarity detection neurons* (FDN). Understanding the role of these different types of neural responses with stimulus repetition effects is a subject for further investigation. The second main difference with the works from Viskotas and colleagues and Rutishauser and colleagues is that in their case the neuronal responses were elicited by an active memory task, whereas in our case the responses occurred in a nearly-passive viewing task. However, the fact that we did not have an explicit memory task does not rule out a memory effect because subjects can still remember seeing a particular picture at the UCLA ward even if not explicitly asked to do so. Interestingly, it has been recently shown that these percepts can trigger strong responses when later recalled (Gelbard-Sagiv et al., 2008).

3.4.2 Novelty and familiarity in the monkey medial temporal lobe

With the very few notable exceptions mentioned above, direct studies of the response patterns of single neurons with stimulus repetition have only been done in animals (see Ranganath and Rainer (2003) for a review). In a series of studies using delayed matched-to-sample (DMS) and recognition memory tasks, Brown and colleagues reported neurons in the infero-temporal (IT) cortex and the MTL – more specifically the perirhinal and entorhinal cortices and the hippocampus – that responded differentially based on the familiarity, recency

and novelty of the stimulus (Riches et al., 1991; Fahy et al., 1993; Xiang and Brown, 1998). Rolls et al. (1982) described neurons in the anterior border of the macaque thalamus that responded only to familiar stimuli in a recognition task, and in another study with a similar task, they found that about 2% of the recorded neurons in the hippocampus responded differently to novel and familiar stimuli (Rolls et al., 1993). The finding of neurons with these response patterns has been interpreted – both by Brown and colleagues and by Rolls and colleagues – as related to recognition memory processes. It has also been proposed that this effect leads to a tuning of the neural population towards a sparse representation of the stimuli (Rainer and Miller, 2000).

3.4.3 Repetition suppression in monkey infero-temporal cortex

It has to be mentioned that the neurons described in all these studies were probably, as in the cases of Rutishauser et al. (2006a) and Viskontas et al. (2006) mentioned in section 3.4.1, FDN and, as stated by Xiang and Brown (1998), despite some stimulus selectivity, the reported results were independent of the particular responsiveness of the neurons to different stimuli on the set. On the contrary, the responses of the MTL neurons we record from are representation neurons (Quiñan Quiroga et al., 2007), with highly selective responses (only responses with firing, at least, five times bigger than the baseline level are considered) and they do not simply reflect the familiarity or novelty of the pictures, as it was the case for the previous two single cell studies in humans and the abovementioned studies in monkeys. Interestingly, the nonselective novelty-dependent neurons reported in the MTL (both monkeys and humans) were obtained during explicit memory tasks. Our results are more reminiscent

of studies in IT cortex in monkeys, which showed a decreased firing of single neurons when a stimulus was shown repeatedly while performing a DMS task (Miller et al., 1991; Li et al., 1993; Desimone, 1996). In the later case, since these responses were selective to specific novel stimuli, it has been claimed that they are not just novelty detectors – i.e. they do not respond to any novel stimuli – and instead, they act as adaptive mnemonic filters providing a signal of a novel stimulus deserving attention. In addition to this, recent single cell recordings in monkey IT while performing repeated visual fixation and stimulus classification tasks (Anderson et al. (2008) and Freedman et al. (2006), respectively) have shown a long-term decrease of neural firing. Furthermore, two other studies in monkey IT during a visual fixation task reported neurons showing a time-localized response suppression without a change in response onset, as in our case (Sawamura et al., 2006; Liu et al., 2009).

3.4.4 Selective medial temporal neurons and the formation of memories

Even though response patterns in these studies are similar to the ones reported here, the main difference is that the neurons reported here were recorded in the human MTL. Given the role of MTL neurons seen in chapter 2, it is plausible to infer that the novelty effect reported here is correlated to memory formation processes, in agreement with previous claims from Quian Quiroga and colleagues suggesting that these neurons are making the link between perception and memory (Quian Quiroga et al., 2005, 2008a). In particular, decreased firing with stimulus repetition may reflect the decrease of relevant information to be stored into memory after each presentation since the amount of information is larger the first time the picture is seen than after several presentations (Bogacz

et al., 2001). In other words, subjects may remember seeing a particular picture during the experiments but after several repetitions not much relevant information that could be stored in memory is added by any further presentation of the same image. A mechanistic explanation of how these neurons know what the relevant information is goes beyond what can be inferred with current data. However, it is in principle possible that relevant information is selected by attention mechanisms in upstream areas or that the MTL neurons described here interact with the less selective MTL neurons described by Rutishauser et al. (2006a) and Viskontas et al. (2006) to assess stimulus novelty or familiarity – which would behave similarly to the perirhinal cortex familiarity detection neurons proposed in the computational model of Bogacz et al. (2001). Our experimental design did not include control conditions – like showing some of the pictures for the first time half way through the experiment – to rule out arousal effects. It is therefore possible that the actual arousal state of the patients may have contributed to the repetition effects described in our study. However, it seems not likely that such pattern of responses can be attributed to an overall effect of tiredness within a recording session because: i) we found stronger repetition effects for the first sessions compared with the ones observed in sessions performed on following days and ii) decreases in firing were not uniform for the different MTL areas. In particular, there was no stimulus repetition effect for the neurons in the parahippocampal cortex, thus rendering a general “lack of arousal” explanation less plausible. In line with these results showing different responses patterns for different MTL areas, a recent study reported a lower selectivity (i.e. neurons fired to more images) and earlier responses in parahippocampal cortex compared with the other MTL areas (Mormann et al., 2008).

3.5 Summary of Chapter 3

In chapter 2 we have seen that the role of the different areas of the MTL is still under discussion. Adding to the evidence of functional specialization within the MTL, our results show a dissociation in the response pattern of neurons in the parahippocampal cortex compared to the other MTL areas. In particular, parahippocampal neurons were the only ones that did not show a decrease in firing with stimulus repetition. Considering the abovementioned explanation that MTL neurons may be providing the link between perception and long term memory formation, the lack of a stimulus repetition effect in parahippocampal neurons may be showing that this area is not involved in such a process.

Chapter 4

Limitations of spike sorting algorithms and sparse neurons

4.1 Introduction

We have seen in chapter 2 how complex brain processes are encoded by the activity of neural networks that can be recorded using extracellular recordings, as in the case of chapter 3. The study of these processes can largely benefit from the simultaneous recording and analysis of the firing patterns from wide populations of neurons (Douglas and Martin, 1991; Harris et al., 2003; Brown et al., 2004; Buzsáki, 2004; Quian Quiroga and Panzeri, 2009). Applications making use of Brain Machine Interfaces and neural prostheses could also significantly benefit from advances in registering a wide collection of neural activity, thus allowing more complex and precise movements based on the activity of larger number of identified cells (Donoghue, 2002; Carmena et al., 2003; Chapin, 2004; Musallam et al., 2004; Velliste et al., 2008; Nicolelis and Lebedev, 2009). The development of multiple electrode recording probes (Maynard et al., 1997; Blanche et al., 2005; Keefer et al., 2008) and spike sorting algorithms (Letelier

and Weber, 2000; Pouzat et al., 2002; Quian Quiroga et al., 2004; Zhang et al., 2004; Rutishauser et al., 2006b; Vargas-Irwin and Donoghue, 2007), have provided an increasing number of identified neurons recorded simultaneously from extracellular recordings. Recent developments in this area allowed to go from detecting 2 to 3 neurons per channel (e.g. Fee et al. (1996)), to the identification of large number of neurons over long periods of time (Carmena et al., 2003; Buzsáki, 2004; Blanche et al., 2005; Jackson and Fetz, 2007; Tolias et al., 2007). In recording from epileptic patients, such technical improvements have allowed the recording of up to 6 neurons from a single electrode (Quian Quiroga et al., 2005, 2007). However, theoretical calculations based on the neural density in brain tissues, spike amplitudes relative to noise and signal attenuation, suggest that it should in principle be possible to record the activity of few hundred individual neurons from a single electrode (Henze et al., 2000; Buzsáki, 2004; Shoham et al., 2006). Different factors have been proposed to account for the relatively low number of recorded neurons. In particular, it was suggested that this could be due to tissue damage caused by the insertion of the electrodes in the recording area (Claverol-Tinture and Nadasdy, 2004), or the electrical insulation caused by the substrate of the probe (Moffitt and McIntyre, 2005). Nonetheless, even the combination of these effects cannot fill the gap between the units predicted from physiology and the recording results. Another possible reason for this mismatch could be due to the presence of sparse neurons, which are rarely detected because they are silent most of the time (Buzsáki, 2004; Shoham et al., 2006). However, it is also possible that the relatively low number of detected neurons could be due to limitations in the spike sorting algorithms.

As we saw in section 2.4 the quantification of spike sorting performance requires the use of simulations. Simulated data also offer the possibility of

controlling parameters such as the signal to noise ratio, the firing rate of the units and the characteristics of the different spike shapes, thus allowing a reliable measure of the algorithm response under different recording conditions. However, spike sorting performance has usually been tested with a few simulated neurons. To our knowledge, there have been no studies exploring how many cells the algorithms mentioned above could detect. Therefore it is still unknown if spike sorting algorithms would cope with a higher fraction of the cells expected from the physiological arguments detailed above. In this study we have systematically evaluated the performance and limitations of spike sorting for different number of neurons present in extracellular recordings using simulated data, with special attention to the detection of sparse neurons.

4.2 Materials and Methods

A simplified scheme of the recording scenario of an electrode implanted in neural tissue can be seen in Figure 4.1. In the area surrounding the tip of the electrode (region A, with white background) the magnitude of the neural signals is considerably higher than the background noise and it is therefore possible to detect and identify their activity with high accuracy. The neurons in red, green and cyan represent active neurons in this area – up to 50 μm from the electrode tip (Buzsáki, 2004) –, recorded by the electrode and sorted by the algorithm. The remaining neurons in this area (in grey) represent the neurons whose activity is not detected, probably, because the sparse nature of their activity (Shoham et al., 2006). The area in light grey (region B) contains neurons close enough to the tip of the electrode to produce spikes larger than the overall noise level, but too small to be individually sorted. The spikes fired by these neurons are usually grouped together as a multiunit cluster. Neurons further away from the

electrode tip (outside area B, i.e. more than $140 \mu\text{m}$ (Buzsáki, 2004)) produce spikes too small to be detected and they only contribute to the background noise of the recorded signal.

To evaluate the spike sorting performance with different numbers of neurons, we created a total of 95 simulations of 10 minutes of extracellular recordings. Each simulation contained background noise, multiunit activity and between 2 to 20 neurons (5 simulations for each case). Following previous studies from our group (Quian Quiroga et al., 2004; Martinez et al., 2009), the noise, multiunit and single unit activities were generated using a database of 594 different average spikes compiled from recordings of monkey basal ganglia and neocortex, as detailed below. In the simulated data, spike labels and times for the multiunit and the single units were stored for subsequent evaluation.

To replicate the fact that in real recording conditions spikes occur at continuous time points – introducing misalignments and making spike sorting more challenging –, the data was first generated with a sample rate of 96000 Hz and it was then decimated by a factor of 4, thus giving a sampling rate of 24000 Hz.

4.2.1 Simulation of background noise

The first step for each simulation was to generate background noise by modelling the overall contribution of distant neurons (neurons at a distance larger than $140 \mu\text{m}$ from the electrode tip; outside the circular area represented in Figure 4.1). For this, we superimposed at random times a large number of spikes from randomly selected neurons from the database. As in previous works (Quian Quiroga et al., 2004; Martinez et al., 2009) the total number of superimposed spikes was half the number of samples during the generation of the signal, prior

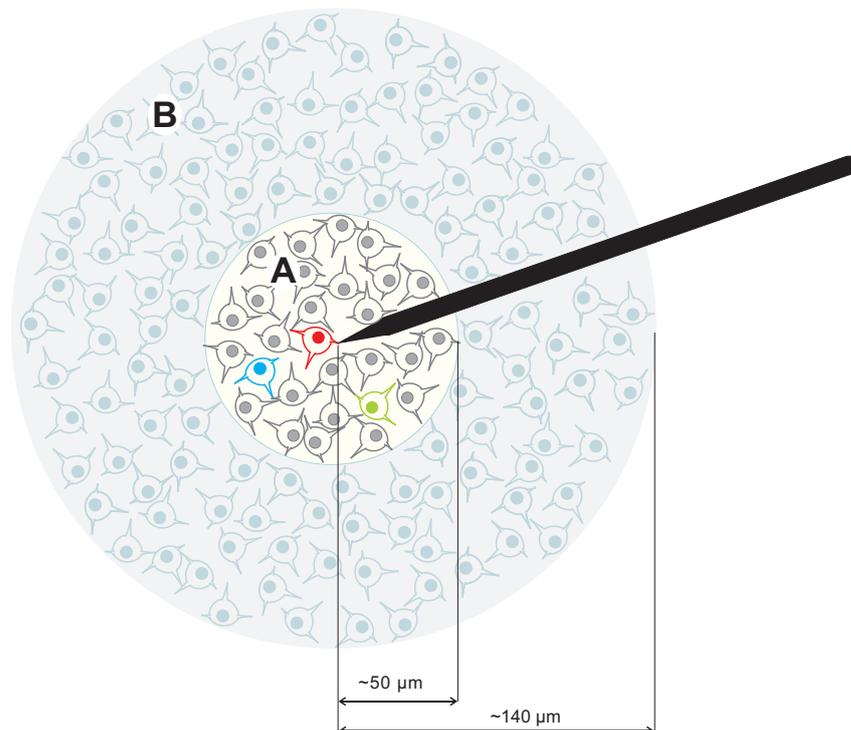


Figure 4.1: Scheme of an extracellular recording with an electrode in the neural tissue. In the area close to the tip of the electrode (region A) the magnitude of the neural signals is considerably higher than the background noise, and it is therefore possible to detect and identify activity of the single neurons in this area with high accuracy. The neurons in red, green and cyan represent active neurons recorded by the electrode and sorted by the algorithm, and the ones in grey represent those that fire less and are not detected. The neurons in the light grey area (B) produce spikes that are larger than the background activity, but not large enough to be individually sorted. The spikes fired by these neurons are usually grouped together as a multiunit cluster.

to the downsampling process, i.e. each second of simulation was generated by the superimposition of 48000 spikes. The amplitude of each of these spikes was scaled by a value randomly selected from a normal distribution ($\mu = 1, \sigma = 0.2$). After superposition of all the spikes, the mean of the resulting signal was then subtracted and scaled to a standard deviation of 0.1.

4.2.2 Simulation of multiunit activity

The simulated signals also included multiunit activity, generated by the spikes from neurons located in region B of Figure 4.1, which can be detected but not sorted. Although the aim of our study was the analysis of clustering performance for single units, the multiunit activity was generated to create a more realistic scenario where the presence of large multiunit clusters increased the complexity of the sorting process.

To replicate the contribution of these neurons in the simulations, on top of the background noise, we added the contribution of 20 different spike shapes randomly selected from the database. The amplitude of the spikes generating the multiunit was fixed to 0.5, a value close to the detection threshold (see below). As typically found in the experimental data, the total firing rate of the multiunit was set to 5 Hz, i.e., each of the 20 neurons generating the multiunit followed an independent Poisson distribution with a mean firing rate of 0.25 Hz.

4.2.3 Simulation of single unit activity

Single unit activity is given by the firing of neurons close to the tip of the recording electrode in extracellular recordings (region A of Figure 4.1). The firing of single units was modelled following a Poisson distribution with a mean firing rate randomly selected between 0.1 and 2 Hz (with uniform probability).

The amplitude of each unit was determined independently and was randomly selected from a normal distribution ($\mu = 1.1$, $\sigma = 0.5$), capped to values within the range 0.9 – 2 to resemble the amplitude distribution (related to the noise level) of single units found in real recordings (Quiari Quiroga et al., 2007).

4.2.4 Performance evaluation

Three expert operators performed an independent (and blind) spike sorting of the 95 simulations – each with 2 to 20 neurons – using ‘Wave_clus’ (Quiari Quiroga et al., 2004). This software offers a combination of properties specially suited for our study: i) the detection threshold is set proportionally to the median of the signal, offering a robust estimation of the background noise and reducing the dependence of the amount (and amplitude) of spikes present on the signal; ii) the number of clusters obtained is only dependent on the spikes distribution and determined automatically by the algorithm; iii) it does not make suppositions *a priori* about the shape of the clusters; and iv) it is able to identify clusters of very different sizes, which is well-suited for the study of sparse neurons.

For each spike sorting outcome, we quantified the number of correctly and incorrectly identified neurons using the following criteria. Hits referred to correctly identified clusters, which matched two conditions: 1) at least 50% of the spikes from an identified cluster corresponded to the same neuron; and 2) the number of spikes detected in 1) were, at least, 50% of the number of generated spikes for this particular neuron. Misses were the total of generated neurons minus the number of hits. False positives were clusters (incorrectly) identified by the expert, which did not fulfil conditions 1) and 2).

Given the well known problems in clustering groups with relatively large

size differences (Ott et al., 2005), we also divided our units into sparse and non-sparse, according to their firing rate, and performed a separate analysis. We defined sparse neurons as those with a firing rate below 0.5 Hz (less than 300 spikes in the 10 minutes of simulated data) and non-sparse neurons as the rest, and evaluated the performance for both populations separately. Due to the random mean firing rate assigned to each unit, for the simulations with a certain number of units we obtained different number of sparse and non-sparse neurons. Therefore, for the comparative analysis of both populations we defined ratios relative to the amount of units present within each group: performance ratio was defined as the number of hits in a simulation corresponding to neurons of a particular group (sparse or non-sparse), divided by the total number of them present in that simulation. Ratios for misses and false positives were defined in a similar way.

4.2.5 Statistical analysis

The spike sorting performance for the sparse and non-sparse neurons was compared using a two-way ANOVA (Test 1). The two independent variables were the number of neurons in each simulation (from 2 to 20) and the neuron type (i.e. sparse or non-sparse). The repeated measures were the corresponding performance ratios (hits, misses or false positives).

4.3 Results

4.3.1 Single case examples

An example of simulated data and spike sorting performed by one of the operators is shown in Figure 4.2. At the top, 60 seconds of raw signal are shown and

at the bottom the (correctly) sorted units: 3 single units (red, green and cyan) and 1 multiunit (blue). The simulation contained the background noise, multiunit activity composed by a total of 2916 spikes, and 3 single units – classes 1, 2 and 3 – with amplitudes 1.38, 1.69 and 1.22 and firing rates 1.91, 0.52 and 0.35 Hz producing a total number of 1063, 317 and 194 detected spikes, respectively. For the multiunit, only 1338 out of 2916 spikes were correctly identified. The multiunit cluster contained 50 false detections and the remaining 1522 spikes did not cross the detection threshold.

The detection and sorting performance of single units was, as expected, much more accurate than the one of the multiunit. For cluster 2 (red) the spike sorting expert detected 1062 of 1063 generated spikes from a single unit plus a single spike generated as part of other unit. For cluster 3 (green) the algorithm detected 316 of 317 generated spikes. For the last cluster (4) the expert detected 194 of 194 generated spikes of a single unit, plus 3 extra spikes generated from other units of the simulation.

Figure 4.3 shows a more complex simulation with 1 multiunit and 6 single units. In this case, the simulation was originally generated with a multiunit containing a total of 2870 spikes and the single units (classes 1 to 6) had amplitudes 1.95, 1.35, 1.19, 1.08, 1.57 and 1.3 and firing rates of 0.24, 0.85, 1.54, 1.02, 0.43 and 1.19 Hz, respectively. The operator clustered a multiunit (red cluster) and 4 single units (clusters blue, green, cyan and magenta). For clusters 3 and 4 (green and cyan, respectively) the operator perfectly identified 676 and 508 spikes, respectively. For cluster 5 (magenta) the operator detected 134 of 134 spikes generated by a single neuron and 2 spikes from another neuron. In contrast, cluster 1 (blue) was incorrectly identified as a single unit. In fact, this cluster contained the activity of 3 different neurons with quite overlapping shapes that appeared as a single cluster to the operator. This mistake was exac-

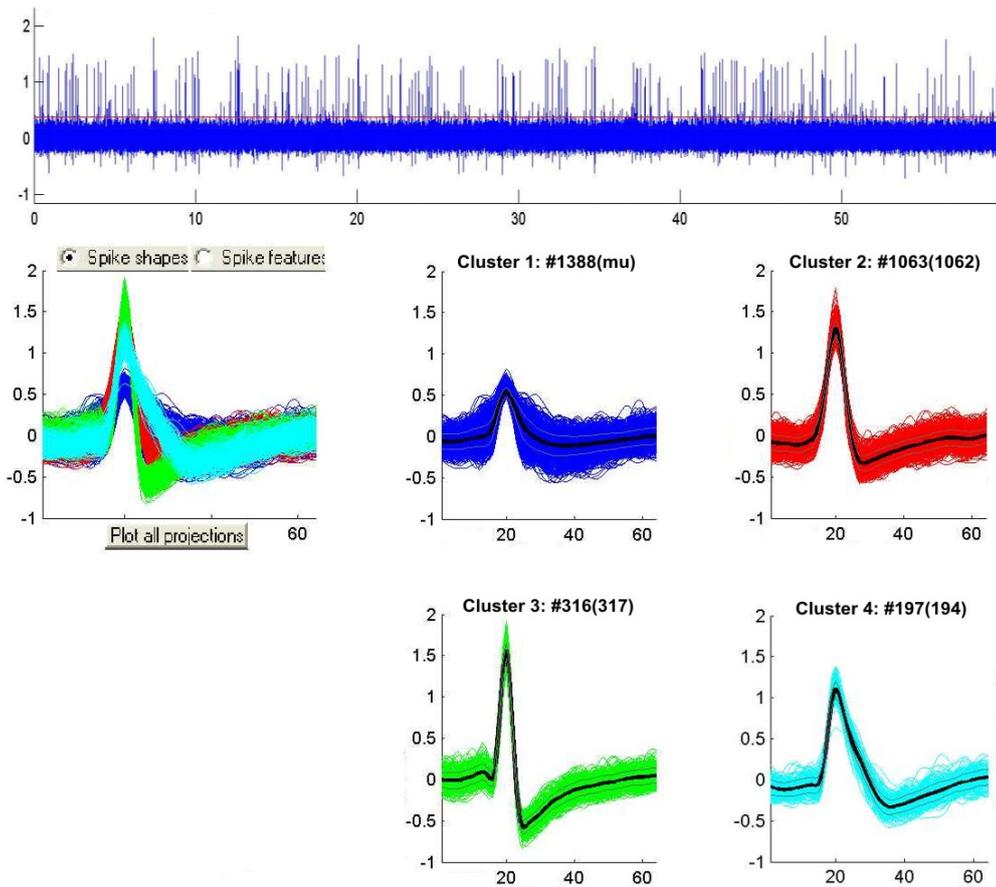


Figure 4.2: Example of spike sorting of simulated data. At the top, 60 seconds of continuous data. The bottom part shows the superposition of all the spike clusters (left) and the sorted clusters in different colours: a multiunit (blue) and 3 single units (red, green and cyan respectively). The number on top of each plot indicates the number of spikes detected and generated (in brackets).

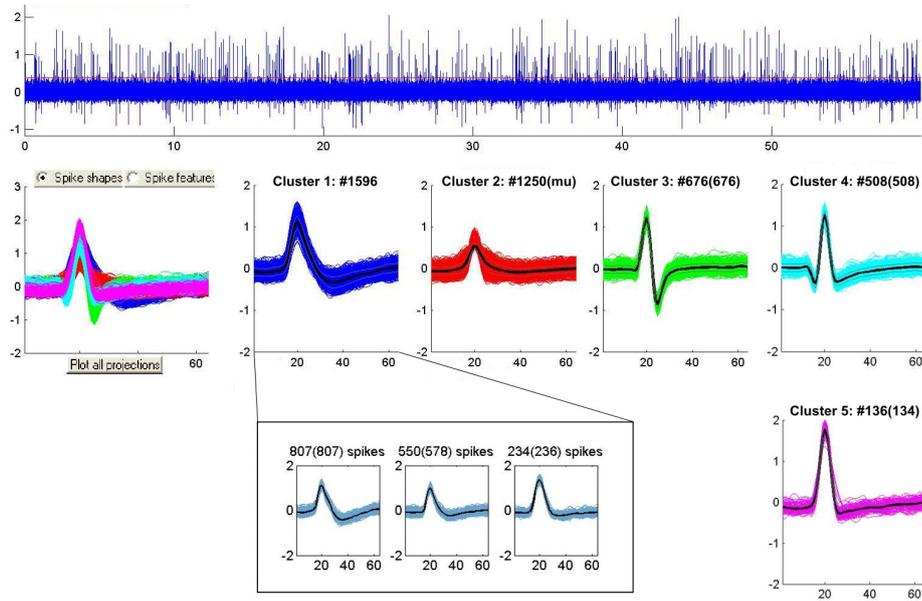


Figure 4.3: Example of a simulation with a large number of units. The operator identified a multiunit (in red), and 4 single units (blue, green, cyan and magenta). However, the blue cluster was formed by 3 different neurons (inset).

erated by the presence of the rest of neurons on the simulation, as a clustering of only these 3 spike shapes alone gave a correct result (see Figure 4.4).

4.3.2 Users performance

Three different operators blindly sorted the 95 simulations generated for this study (see section 4.2). Table 4.1 shows the number of hits for all simulations, and each operator separately. Despite some individual differences all experts reached a similar asymptotic average number of hits in their sorting results. This suggested that the results obtained for the different number of neurons were related to the inherent characteristics of spike sorting process and were not significantly influenced by the subjectivity and potential biases of each operator. Table 4.2 presents the number of false positives and it can be seen that this overall number was low, thus indicating that overclustering (i.e.: splitting

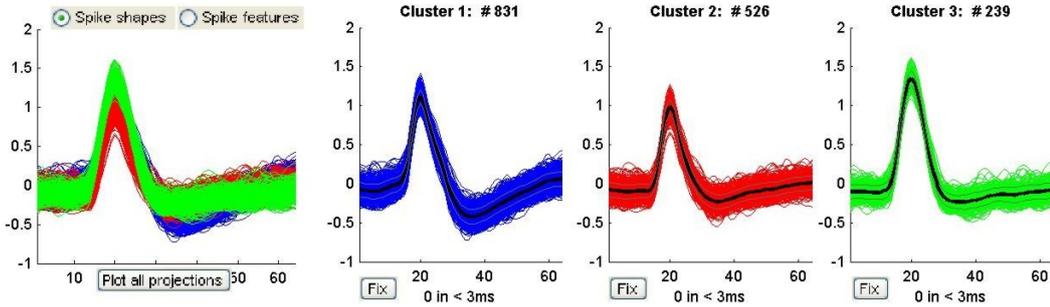


Figure 4.4: Sorting of the spikes identified as cluster 1 in Figure 4.3. The left of the figure shows all the spikes superimposed. When sorting the spikes on their own (no multiunit or other single units included in the spikes to be sorted) the algorithm reached the optimal solution, extracting the three units present in the cluster with great accuracy (clusters blue, red and green).

a single cluster into 2 or more) was relatively rare.

4.3.3 Number of neurons identified

Figure 4.5a shows the average number of hits as a function of the number of generated neurons. For simulations with a low number of neurons the number of detected clusters was nearly perfect (the ideal performance marked by the dashed line). For simulations with large number of neurons the performance decayed reaching an asymptotic value between 8 and 10 correctly identified neurons. Figure 4.5b displays the number of misses and false positives for the different number of generated neurons. The misses followed a complementary behaviour to the number of hits, being negligible for low number of units and rising notably as the number of neurons increased. In fact, the dotted line in Figure 4.5b ($y = x - 8$) fits closely the number of misses in agreement with the asymptotic behaviour showed with the hits. Meanwhile, the number of false positives also increased with the number of units present in the simulation, but it remained below 3 cases per simulation. These two results indicate that the

Neu.	Operator 1			Operator 2			Operator 3			Total		
	Av.	Min	Max	Av.	Min	Max	Av.	Min	Max	Av.	Min	Max
2	1.8	1	2	1.8	1	2	1.8	1	2	1.8	1	2
3	2.8	2	3	2.8	2	3	2.8	2	3	2.8	2	3
4	3.8	3	4	3.6	3	4	3.6	3	4	3.67	3	4
5	4.2	3	5	3.8	2	5	4.4	3	4	4.13	2	5
6	5.2	4	6	5.2	5	6	5.2	5	6	5.2	4	6
7	5	3	7	4.6	2	6	4.8	2	7	4.8	2	7
8	6.8	6	7	6.6	6	7	6.2	6	7	6.53	6	7
9	7.2	5	8	6.8	5	8	7	4	9	7	4	9
10	6.4	3	9	6.2	4	9	6.6	5	8	6.4	3	9
11	8	6	9	7.8	6	10	7.6	6	9	7.8	6	10
12	8.4	7	11	7.2	6	9	7.2	6	9	7.6	6	11
13	8.6	5	11	8.2	3	11	7	3	9	7.93	3	11
14	7	5	11	7.2	4	11	6.6	4	11	6.93	4	11
15	8.2	6	11	7.8	5	10	7.8	5	10	7.93	5	11
16	10.6	10	11	9.6	8	11	10.8	9	12	10.33	8	12
17	7.4	5	9	5.8	5	7	6.4	4	8	6.53	4	9
18	9.2	6	12	9.2	6	13	7.4	4	12	8.6	4	13
19	8.4	6	11	8.2	5	11	6.4	5	8	7.66	5	11
20	9.4	7	12	7.8	5	11	7.8	5	11	8.33	5	12

Table 4.1: Correct Detections (Hits). Minimum, maximum and average of correct detection (hit) performance for the 3 experts. The left column indexes the results by the number of units present in the simulation. Performances for different experts were similar, showing consistency of the results obtained. The last three columns display global statistics, averaging over the three partial results.

decrease in detection performance was due to grouping two or more neurons into single clusters, as exemplified in Figure 4.3.

4.3.4 Sparse neurons

For a better characterization of the spike sorting performance for different types of neurons, we divided our data into sparse and non-sparse neurons (with a firing rate lower / higher than 0.5 Hz, respectively) and compared the results for both groups. Figure 4.6a shows the hit ratio for both types of neurons (sparse in dark

Neu.	Operator 1			Operator 2			Operator 3			Total		
	Av.	Min	Max	Av.	Min	Max	Av.	Min	Max	Av.	Min	Max
2	0.2	0	1	0	0	0	0.2	0	1	0.13	0	1
3	0	0	0	0	0	0	0.2	0	1	0.07	0	1
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0.4	0	1	0.4	0	1	0.2	0	1	0.33	0	1
6	0	0	0	0	0	0	0.2	0	1	0.07	0	1
7	0.6	0	2	0.6	0	2	0.6	0	2	0.6	0	2
8	0.4	0	1	0.2	0	1	0.6	0	1	0.4	0	1
9	0.4	0	1	0.6	0	1	0.8	0	1	0.6	0	1
10	0.6	0	1	0.4	0	1	0.4	0	1	0.47	0	1
11	0.4	0	2	0.2	0	1	0.2	0	1	0.27	0	2
12	0.8	0	1	1.2	1	2	1.4	1	2	1.13	0	2
13	0.8	0	2	1	0	3	1.2	0	3	1	0	3
14	1.8	1	3	1.8	0	3	2.4	0	4	2	0	4
15	1.6	0	3	1.4	0	2	1.2	0	2	1.4	0	3
16	0.8	0	1	1.2	1	2	0.4	0	1	0.8	0	2
17	2.6	2	4	3.4	2	4	2.8	2	4	2.93	2	4
18	2.4	2	3	1.6	0	2	2.2	2	3	2.07	0	3
19	2.4	1	3	2	1	4	2.8	2	4	2.4	1	4
20	2.2	1	3	2.6	1	4	2.8	2	4	2.53	1	4

Table 4.2: False Positives. Minimum, maximum and average of false positive detection performance for the 3 experts. The left column indexes the results by the number of units present in the simulation. Results for different experts were similar, showing consistency of the values obtained. The last three columns display global statistics, averaging over the three partial results.

grey and non-sparse in dashed light grey) and Figures 4.6b and 4.6c the number of misses and false positives. It can be seen that the performance for non-sparse neurons was better than for the sparse ones. For the cases with a large number of neurons, the ratio of misses for the sparse neurons was around 80%, while for the non-sparse neurons it reached values around 50%. These differences were statistically significant ($F(1, 511) = 104.08, p < 10^{-15}$, $F(1, 511) = 129.76, p < 10^{-15}$, Test 1, for hits, or misses, and false positives, respectively). These results uncover the tendency of sparse neurons to be masked by multiunit activity or high-firing rate cells.

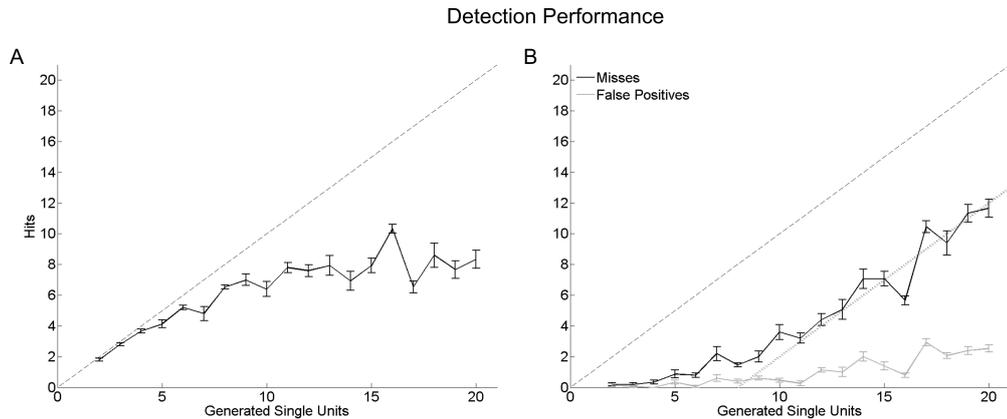


Figure 4.5: Mean detection performance as a function of the number of single units generated in the simulations. A) average number of hits. B) Number of misses (black) and false positives (dashed grey). For the misses, the dotted grey line in B shows the linear function $y = x - 8$; i.e. a line parallel to the diagonal but displaced 8, the maximum number of neurons detected. Bars denote s.e.m.

4.4 Discussion

Studies in recent years have drawn attention to the apparent discrepancy between the number of neurons one expects to see following anatomical and physiological considerations – in the order of few hundred – and the number of neurons typically detected in experimental conditions – up to 6 – (Buzsáki, 2004; Shoham et al., 2006). This difference has been commonly attributed to the sparseness of neural firing (Henze et al., 2000; Olshausen and Field, 2004; Shoham et al., 2006; Fujisawa et al., 2008), although other explanations such as damage of the neurological tissue around the electrode have been proposed (Claverol-Tinture and Nadasdy, 2004). In addition to the issue of silent neurons and the possible effect of the damage to the neural tissue, here we showed that the relative low number of detected neurons can be also attributed to limitations in spike sorting algorithms.

In this study we have shown that for few simulated neurons the number of correctly classified units remained close to the number of neurons present in the

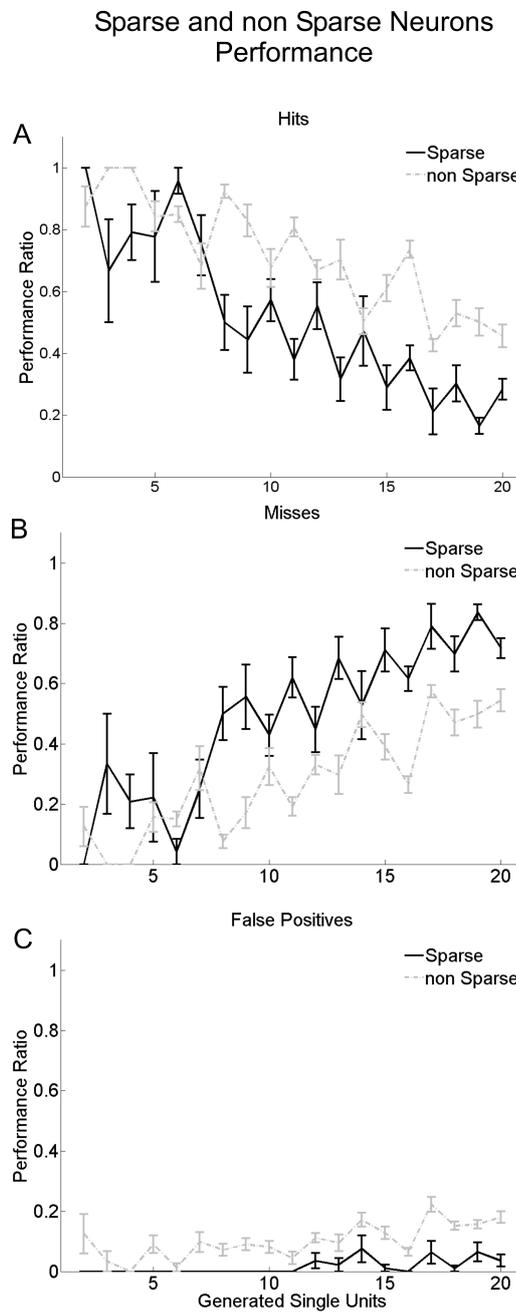


Figure 4.6: Hits and false positive ratios for the sparse and non-sparse neurons. A) Number of hits for sparse (dark grey) and non sparse neurons (light grey), B) Number of misses for the same groups and C) False positives. Bars denote s.e.m.

simulation, as in previous works quantifying the performance of spike sorting algorithms (Letelier and Weber, 2000; Quian Quiroga et al., 2004; Zhang et al., 2004; Rutishauser et al., 2006b; Vargas-Irwin and Donoghue, 2007). These results agree with the maximum number of neurons reported for single channels in experimental conditions (Fee et al., 1996; Nordhausen et al., 1996; Maynard et al., 1997; Quian Quiroga et al., 2007). However, the spike sorting performance reached an asymptotic value between 8 and 10 neurons for larger number of simulated units. Interestingly, there were relatively few false positives. Hence, the sorting mistakes were mainly produced by grouping 2 or more neurons into a single cluster. This process affected more noticeably the sparse firing neurons than the ones with higher firing (see Figure 4.6). Altogether, these results show the need of further improvements of the spike sorting methods presently available, paying special attention to the performance in presence of sparse neurons. In addition to this, no characterization exists of spike sorting performance in the particular scenario where the units to be detected have significant differences in their firing rate. Hence, spike sorting algorithms have not yet been systematically studied and optimized to address the identification of sparse neurons. These neurons usually remain in a resting state with a baseline firing almost negligible, increasing significantly their firing rate when they become active under the presence of an adequate stimulus (Shoham et al., 2006; Quian Quiroga et al., 2008a).

4.4.1 Simulations with large number of neurons

The use of simulations has become a necessary checkpoint for the development of spike sorting algorithms (Letelier and Weber, 2000; Quian Quiroga et al., 2004; Zhang et al., 2004; Rutishauser et al., 2006b; Vargas-Irwin and Donoghue,

2007). However, studies based on simulated data so far have used a relatively low number of neurons (between 3 and 5 neurons per simulation). Hence, the question of how many neurons could be identified by spike sorting algorithms in a situation where many of them became active along a recording remained unsolved.

Here we implemented a set of simulations covering a larger range of single units in order to study how spike sorting algorithms perform in a more challenging scenario not yet explored. We created simulations of extracellular recordings containing noise, multiunit activity and a range of single units, following a similar scheme to a recent study (Martinez et al., 2009) and providing a more challenging scenario than the simulations in the abovementioned studies, mimicking the working conditions of spike sorting algorithms in real recordings.

4.4.2 Sparse Neurons

Our simulations included single units covering a complete range of firing rates similar to the ones found in real recordings (Musallam et al., 2004; Quian Quiroga et al., 2005; Fujisawa et al., 2008). Then we divided them in two populations: sparse and non-sparse neurons, according to their firing rate. The sorting results for both sets of neurons were significantly different, showing a better performance for high firing neurons than for sparse neurons when the number of neurons was increased, despite the reported ability of the algorithm to find these kind of neurons in real recordings (Quian Quiroga et al., 2005, 2007, 2008a). These results agree with the reported problems for detecting sparse neurons in real recordings (Buzsáki, 2004; Quian Quiroga et al., 2008a), although it is usually argued that the missing of these neurons is due to the almost inexistent firing in resting state.

Our results highlight the difficulty of sparse neuron detection, in line with claims that this type of silent neurons could account for mismatch between the number of recorded and the number of theoretically expected neurons (Henze et al., 2000; Buzsáki, 2004; Shoham et al., 2006; Fujisawa et al., 2008). Sparse neurons are not only present in responses related to high level cognitive processing (Quian Quiroga et al., 2008a), but they can also be found in sensory inputs (Olshausen and Field, 2004) and, in general, all over the neocortex (Kerr et al., 2005; Shoham et al., 2006). The activity of sparse neurons in the brain usually carries high amount of information per spike (Quian Quiroga et al., 2007; Quian Quiroga and Panzeri, 2009) and their responses can present striking characteristics such as invariance (Quian Quiroga et al., 2005) or multimodality (Quian Quiroga et al., 2009). Furthermore, the bursting (a high frequency train of spikes in a short period of time) of a single neuron can modify the global brain state (Li et al., 2009) or even the behaviour of an entire being (Brecht et al., 2004; Houweling and Brecht, 2008). In fact, in the case of the studies carried by Brecht and colleagues, the high significance of single neuron firing has been related to a sparse representation for somatosensory and motor activity. Hence, although the multiunit activity recorded by a single electrode contains a great amount of information and it is a valid input for certain applications (Chapin, 2004; Stark and Abeles, 2007), for a complete understanding of the principles of neural coding, the identification of sparse neural activity is crucial.

4.4.3 Limitations in Real Recordings

With a few notable exceptions, for example with simultaneous intra- and extra-cellular recordings were taken (Wehr et al., 1999; Henze et al., 2000), the information about which spike corresponds to which neuron is not available in

in vivo recordings. The utilization of multielectrode probes provides additional information to the extracellular recordings being possible to obtain a more accurate sorting than the one offered by a single electrode. This type of recordings showed that spike sorting methods for single channel recordings identify fewer neurons than using the signal provided by the tetrode, even when the amplitude of the spikes is in the range of single unit activity of the single electrode (Gray et al., 1995; Harris et al., 2000). Interestingly, in a recording of cortical neurons in vivo using a 54 contact probe (Blanche et al., 2005), a comparison between sorting performances for a virtual tetrode and a larger number of contacts was performed. In the case of the tetrode, the study reported a correct sorting of 7 single units, plus a cluster containing spikes from 3 different units – identified in the sorting performed with higher number of active contacts. This recording result – merging different single unit activity into a same cluster – resembled the one found in our study when increasing the number of units in the signal, expressing an intrinsic behaviour of spike sorting algorithms.

4.5 Summary of Chapter 4

In summary, the results presented here uncover the necessity of further improvement in spike sorting algorithms in order to provide experimentalists with adequate tools for recording large ensembles of neurons. The technological development of new recording probes including, for example, the improvement of the quality of the signal transference from the tissue to the electrode and the reduction of the noise present in the signal (Keefer et al., 2008), can increase the number of identified neurons from extracellular recordings. However, it is not only the number of units to be identified, but particularly, the performance for sparse neuron detection that should be improved in the spike sorting algorithms.

Hence, efforts should be made to address robust detection of sparse neurons, as they are easily masked by high firing neurons, which might be encoding some of the most striking characteristics of brain functions.

Part III

Conclusions

Chapter 5

Summary and discussion

The main objective of this thesis was to further the comprehension of the role of MTL neurons in humans during a natural processing of information. Additionally we wanted to investigate the methods used in their recording and the possible influences of limitations in the analysis of neural data.

The main contributions of the present work cover, consequently, combined aspects of data analysis and recording methods. The main contribution to both areas were: 1) we reported new characteristics of neurons representing abstract concepts in the MTL and 2) we quantified intrinsic limitations in spike sorting algorithms affecting the number of neurons that is possible to identify using a single electrode. Here we review these two achievements and discuss their relevance to the neuroscience community.

5.1 Neural firing in the medial temporal lobe

In chapter 3 we presented results obtained from recordings of single units in the human MTL. The recordings were performed in epileptic patients with clinical electrodes implanted prior to a surgical treatment to locate the focus of the

epileptic seizures. This is a rare opportunity and the data obtained is extremely valuable as revealed by a noticeable number of publications reporting exciting findings about the very nature of memory and consciousness in recent years (see Quiñan Quiroga et al. (2005) and Gelbard-Sagiv et al. (2008) for examples and section 2.3.5 for details).

In particular, we studied the effects that the repetition of stimuli have in MTL neurons firing to particular concepts. We found that the firing of neurons to their preferred stimuli was modulated by the novelty of presentation of the stimulus. This modulation presented a series of properties of special interest:

1. The responses decreased their magnitude and duration significantly for repeated presentations, but they were still well above the baseline activity of the neurons, being specific to a particular stimulus.
2. The changes induced by repeated presentations were different on a subsequent experiment, suggesting an underlying cumulative process through sessions. The variation across different experimental sessions also suggests that this process was different from awareness or patient tiredness, as the latter ones should be equal for both experimental sessions.
3. Different MTL structures manifested different changes (or no change at all) in the firing of the neurons recorded under the same conditions, supporting views of different roles for particular areas of the MTL.

Changes in neural activity as a consequence of repetition of particular stimuli have been largely documented in different areas of the brain (see Grill-Spector et al. (2006) for a review). For example, single neurons responding to particular visual stimulus in monkey IT presented a similar pattern to the one showed here, interpreted as a ‘saliency mode’ for detecting new elements

in the visual field (Li et al., 1993). However, the suggested role of the neurons considered in our study as a link between conscious perception and memory imply a complete different role in our case.

We hypothesised that the results presented in chapter 3 could be representing the processes of memory consolidation, linking the memory of the picture presentation of a known and significant concept to the experience of staying in the hospital ward (Viskontas et al., 2009). Following this hypothesis, the repetition suppression seen in neurons of hippocampus, entorhinal cortex and amygdala would be an effect of the decrease of information to be added to the memory trace of the event of the presenting of the picture under those conditions, i.e. while in the first one or two presentations a complete new episode must be coded into memory (presenting a stimulus in the hospital), in the subsequent presentations no “extra” information is added to the episode. This effect contrasts with the formation of a new temporal episode of combined stimuli, in which the neurons participating on it change their firing, increasing their temporal correlation (Paz et al., 2010).

The differences noted between different areas of the MTL, in particular the absence of repetition suppression in responses from parahippocampal cortex, suggested a functional differentiation across MTL structures. Interestingly, the parahippocampal cortex has been identified as part of a system supporting stimulus familiarity by the dual-process theory (Aggleton and Brown, 2006) or the relational theory (Eichenbaum et al., 2007) viewed in section 2.3.3. These statements are not in disagreement with our results, because we used familiar pictures – i.e. the persons or objects used were well known to the patients before the experiment. Following this view, while the firing of parahippocampal neurons would be related to a process of recognition of the presented stimulus, the complex formed by the entorhinal cortex and the hippocampus would

be involved in coding the necessary elements for its posterior recollection, as suggested by Eichenbaum et al. (2007). Another view would be considering an approach closer to the view from Buckley and Gaffan (2006). In this one while the hippocampus, entorhinal cortex and amygdala would be contributing to the formation of the episodic memories, neurons in the parahippocampal cortex would be participating on a different process, probably more linked to the perception of stimuli.

The previous report of cells performing firing related to the familiarity of the stimulus presented (Rutishauser et al., 2006a; Viskontas et al., 2006), but not selective to particular stimuli, contrasts with the firing of highly stimulus specific neurons studied in chapter 3 in the very same areas. In fact, the firing of neurons responsive to familiarity in most areas of the MTL, including hippocampus, has been reported previously in monkeys (Rolls et al., 1993; Xiang and Brown, 1998). Although it could be claimed that the different results were obtained due to a different experimental paradigm, we consider that the collection of neurons reported in those two studies were a different population from the ones presented here, supporting particular cognitive processes although possible interactions between the two populations should not be discarded. However, any of the claims above would need further validation with future experiments analyzing the firing of this population of selective neurons with memory related tasks.

5.2 Limitations in spike sorting algorithms

In chapter 4 we have presented the limitation of present algorithms of spike sorting when utilising them to sort higher number of neurons than the ones used normally for performance tests. The results uncovered the need of further

improvement on the field of spike sorting. The correct characterisation of neural responses generated by single cells is of crucial importance for more advanced studies of neural activity, which suppose perfect sorting on their formulations (Brown et al., 2004).

For our study, we developed and used a complete set of simulations that allowed us to explore algorithms' properties in a realistic environment while keeping complete track of the activity present on the signal. Using a wide range of parameters in the generation of simulated data allowed us to test the spike sorting performance for different population of neurons. The results obtained showed that the limitation found in the sorting process affected especially the population of sparse neurons. Concerns about the detection of sparse neural activity have been recently raised (Buzsáki, 2004; Shoham et al., 2006). Our results showed that, in addition to their tendency to remain silent most of the time, sparse clusters tend to be merged with neurons recorded from the same electrode and with a much higher firing rate.

The reported numbers of neural activity in different areas of the brain have shown that the amount of silent neurons could be ten times the number of units recorded – see Table 1 in (Shoham et al., 2006). Furthermore, the silent neurons population seems to be more significant in the case of pyramidal or principal neurons (Fujisawa et al., 2008). In addition to the amount of these neurons, it should be considered that their firing probably provides more information for researches (and probably within the brain) than high firing ones (Quian Quiroga and Panzeri, 2009). Therefore, improving the algorithms to record this very sparse activity could introduce a large step forward in the understanding of brain functioning. In fact the abstract neurons in MTL are indeed sparse neurons themselves, being activated only by a handful set of stimuli and remaining silent during baseline (Quian Quiroga et al., 2008a).

Furthermore, the improvement of spike sorting algorithms should not only satisfy the needs of scientists unravelling the secrets of our brain, but also to more practical approaches of brain recording. As shown by recent studies with monkeys, the movement of artificial limbs from direct recording of single units is achievable – see (Velliste et al., 2008) for an example –, but it is still far from its final stages of development (Nicolelis and Lebedev, 2009). The future of the field of brain machine interfaces relies on the maximisation of the number of units recorded per electrode, providing stable long lasting recordings, necessary to reach a minimum operational level of precision in the operations performed (Becedas and Quian Quiroga, 2010).

Chapter 6

Suggestions for future work

The work presented in this thesis has covered the objectives and questions presented in the first part of the document. However, as in most occasions in science, the advances presented here have opened new questions and horizons to explore. Here we outline opportunities for further work in the fields covered, taking the results of this work as an starting point.

6.1 Recordings on the medial temporal lobe

6.1.1 Subnetworks interactions

We have denoted the presence of, at least, two populations of neurons in the human MTL. One represents abstract concepts in sparse networks. The other one (described by Rutishauser et al. (2006a) and Viskontas et al. (2006)) forms a population of markers for the detection of familiar and novel stimuli, or familiarity detection neurons (Bogacz et al., 2001). One of the questions that arose in the study of stimulus repetition was if the two sets of neurons interact in some way. Do the familiarity neurons modify the firing of the abstract neurons? Do

the familiarity neurons also modify their firing after multiple presentations of an already familiar stimuli? Does the activation of the abstract neurons associated to known concepts activate the firing of familiarity detection circuits? The study of these questions will require the development of new paradigms involving new stimulus and already known concepts. A memory-based task could be used for exploring the firing of the recorded neurons.

The study should include two different criteria:

1. stimulus selectivity, with a similar criteria to the one presented in this study. This should include the presence of several trials for each particular stimulus, to ensure the statistical significance of the results.
2. novelty detection, analysing if any of the neurons recorded (and not only the selective ones) presents differential firing to novel and familiar stimuli. This analysis would provide a population of novelty and familiarity detectors. For this analysis the presence of completely new pictures during the experiments will be necessary to ensure the firing of novelty detectors; with concepts not familiar to the patient in contrast with the highly significant ones eliciting selective response (Viskontas et al., 2009).

These two separate analysis will provide two sets of populations. The following analysis should include the comparison of this two populations, in order to clarify if they are separate ones or there is an intersection group for both populations. The complete analysis would provide fractions of stimulus selective, novelty detectors and familiarity detectors and their respective joint groups. Then a complete firing correlation of these neurons should be computed, in order to establish the corresponding relationships between different groups and possible hierarchical organisation.

6.1.2 Parameters affecting firing of abstract neurons

We have reported that the repetition of a static stimulus decreases the firing of the neurons responding to abstract concepts, while a parallel study showed that if the concepts are displayed on a movie the firing is altered creating new neural firing correlations (Paz et al., 2010). We hypothesised that the decrease in firing was related to the lack of new information to be added to the memory trace. It would be of interest to explore what other parameters could influence the firing of these neurons. One of the possibilities would be to increase the information related to the picture, expecting that it would increase this firing. It would be also of interest to explore if changes in the background of the picture would also affect their firing. Another factor to consider is if an active memory task would modify the neural firing, on basis of the conscious character of declarative memories and the change induced by attention modulation. All these effects would need to be quantified as the variations the obtained response. Mutual correlations and combined effects will need to be measured to identify if the final modulation is an addition of the individual effects or if there is some hierarchy of processes.

6.1.3 Development of a neural tracking system

One of the problems encountered when studying the effect of time and exposure to stimuli in the MTL neurons recorded in humans is that it is not possible to record neural activity over long periods of time. Thus, it is necessary the development of a robust method that would allow to track neurons between sessions, in order to perform studies of memory further than a half hour recording. It is important that the method would not rely in the responses associated to the neurons, as it would be necessary that the same neurons are recorded in

order to study second order effects such as firing modulation, which depends intrinsically in the firing properties of a particular neuron.

6.2 Improvements in sorting algorithms

6.2.1 Iterative sorting of data

In chapter 4 we have seen that the detection of single units in a recording depends on the total number of them that are present in the recording. A possible strategy to deal with a growing number of units would be to iterate over the sorting algorithm with each of the clusters resulting from the first sorting. It would be similar to having a scenario with a smaller number of neurons. The idea of sequential clustering has been suggested recently for clustering process in general, but has not been applied to spike sorting yet (Ott et al., 2005). Figure 4.4 showed that, in fact, this process could be applied to the sorting of neural spikes with successful results. However, some points must be taken in consideration: 1) the number of iterations to be done has to be limited 2) the existence of a risk of overclustering 3) the amount of computational effort invested by each of the iterations, considering a possible compromise between number of iterations and effort in each of them. In addition, the increase of time consumption by each iteration of the algorithm could penalise its use for some applications in which time is critical, in special for online ones, such as neural prosthetics.

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