Hans Baldung Three Ages of the Woman and the Death

Age-effects on the functional architecture of the human medial temporal lobe

PhD dissertation
by

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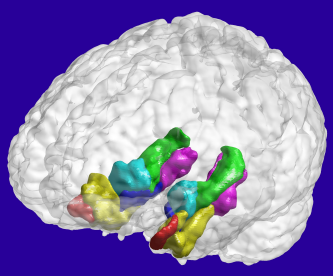

Preface

This thesis is submitted in order to obtain the PhD degree at the Faculty of Health Sciences, University of Copenhagen. The work has been carried out at the Danish Research Centre for Magnetic Resonance (DRCMR) at Copenhagen University Hospital Hvidovre, from September 2004 to December 2007.

Acknowledgement
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Last, but not least, I owe all the gratitude I can give to my family; my wife Majken, and our two boys, Mike and Frederik, for their company, love and support.

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Thomas Z. Ramsøy
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English summary

The aim of the present project has been to study the effects of healthy ageing on the function of the medial temporal lobe (MTL) region. The MTL is today known to be a collection of tightly interconnected yet anatomically diverse regions, and it is thought that these regions play different roles in declarative memory, as well as in different non-memory cognitive functions.

Two articles make up the basis of this thesis: Study I (Regional activation of the human medial temporal lobe during intentional encoding of objects and positions), and Study II (The effects of age on medial temporal lobe activation in intentional encoding of items and positions). In addition, an appendix reviews the methods applied in the present project (The basics of functional Magnetic Resonance Imaging).

The first part of the thesis is a review of the project background. This consists of four smaller sections: the first section reviews age-related changes in brain anatomy and function, the second section treats cognitive changes in healthy ageing, while a third section discusses the relatively new research field the cognitive neuroscience of ageing that combines the efforts from neuroscience and cognitive science. The final section reviews the anatomical and functional aspects of the MTL, and through this argues for the choice of this region as a model for studying age-related changes in brain activation.

Two models attempt to explain age-related changes in brain activation. One of these models claims that changes seen in ageing are suggestive of *compensatory mechanisms*, i.e., that older subjects display altered brain activation patterns that are signs of compensatory mechanisms, and that such mechanisms aim to preserve a normal level of performance. Typically, these changes have been documented in the anterior most parts of the brain (prefrontal cortex), although other studies have demonstrated similar mechanisms in other brain regions. The second model claims that certain age-related changes in brain activation are signs of loss of function, also called *dedifferentiation*. It is claimed that brain regions that are specialized (differentiated) in certain cognitive functions, show loss of specialization with increasing age. Today, there is agreement that both compensation and dedifferentiation can occur, and that they may be descriptive of different mechanisms with different tempi in the ageing brain.

In the present thesis the focus is on the MTL, as this region has shown a high degree of functional specialization (i.e., differentiation), despite its dense interconnectivity. In addition, hemispheric differences have been shown during different cognitive tasks. Furthermore, studies have provided conflicting results concerning the effects of age in the MTL region, and some of this inconsistency may be due to the use of non-optimal approaches for image acquisition and statistical analysis.

The first article in this thesis seeks to establish a combination between a cognitive paradigm and optimal image acquisition and analysis, with the aim to uncover regional differentiation within the MTL in a cognitive process. The second paper sets out from this approach and demonstrates that increasing age is associated with dynamical changes in brain activation during intentional encoding. The results do not show uniform changes with age that correspond to either compensatory mechanisms or dedifferentiation. Taken together, the results suggest that increasing age is related to both loss of function and compensatory mechanisms in the MTL region. In order to uncover the relationship between these findings and changes in other regions of the brain, it is argued that there is a need for studies that combine MTL-optimized methods and whole-brain assessment.

Lægmandsresume (Danish summary)

Formålet med det nærværende projekt har været, at studere effekten af aldring på aktivering og specialisering i den mediale temporallap (MTL). MTL er i dag kendt som et område der består af tæt forbundne men anatomisk forskellige strukturer, og der antages at disse regioner har forskellig betydning for deklarativ hukommelse, såvel som en delagtighed i forskellige kognitive funktioner ud over hukommelse.

Det, der ligger til grund for denne ph.d.-afhandling er to artikler: Studie I (Regional activation of the human medial temporal lobe during intentional encoding of objects and positions) og Studie II (The effects of age on medial temporal lobe activation in intentional encoding of items and positions). Ledsagende til denne afhandling er også en gennemgang af de metoder som er blevet benyttet (The basics of functional Magnetic Resonance Imaging).

Første del af afhandlingen er en gennemgang af baggrunden for projektet. Dette er opdelt i fire mindre sektioner: en første del om de aldersmæssige forandringer man kan observere i hjernen, en anden del om de kognitive forandringer vi kan observere, en tredje del om den relativt nye forskningsretning *cognitive neuroscience of ageing* som netop omhandler kombinationen af viden fra hjerneforskningen og kognitionsforskningen. Sidst gennemgås de anatomiske og funktionelle aspekter af MTL, for herigennem at argumentere for hvorfor netop dette område er valgt som fokus for det forestående projekt.

To modeller beskriver de aldersrelaterede forandringer i hjernens aktivering. En af disse retninger hævder, at hjernens aldringstegn er forenelige med kompensations-mekanismer, dvs., at ældre personer viser ændret hjerneaktivitet for at opretholde det samme performance niveau. Typisk er disse mekanismer dokumenteret i hjernens forreste dele (præfrontale cortex), men der er også studier, der viser lignende mekanismer i andre hjerneområder. Den anden forskningsretning hævder, at visse aldersrelaterede ændringer i hjernens aktivering er tegn på funktionstab, en såkaldt *dedifferentiering*. Her hævdes det, at områder som hos unge er specialiseret til at varetage visse kognitive opgaver, viser et tab af specialiseringsevne i højere alder. Der er i dag enighed om, at både kompensation og dedifferentiering kan forekomme, og at de måske er betegnende for forskellige mekanismer med forskellige tempi i hjernens aldring.

I den forestående afhandling er fokus lagt på MTL, da regionen viser en høj grad af funktionel specialisering (differentiering), trods regionens tætte anatomiske forbindelser, og da der kan forekomme forskelle i hemisferisk aktivering ved forskellige kognitive opgaver. Endvidere har studier af alderseffekter i denne region givet modstridende fund, hvilket kan skyldes ikke-optimale målings- og analysemetoder.

Den første artikel i denne afhandling har søgt at etablere et kognitivt paradigme med optimal billeddannelse og analyse, netop for at afdække relative forskelle i MTL regionens kognitive specialisering. Den anden opgave tager udgangspunkt i denne tilgang, og demonstrerer, at alder er forbundet med dynamiske ændringer i hjernens aktivering, og at alder ikke viser en ensartet forandring med alderen, der enten er forenelig med kompensation eller dedifferentierings-mekanismer. Samlet tyder fundene på, at aldring er forbundet med både funktionstab og kompensationsmekanismer i MTL. For at afdække relationen til aldersbetingede ændringer i ander hjerneområder, er der behov for studier, der benytter både MTL-optimerede og aktiveringsmål der dækker hele hjernen.

1.0 Introduction

In biology, ageing and senescence are terms for the deterioration of organisms with time[2]. Ageing is a manifold of biological processes that, with time, profoundly alter the anatomy, neurochemistry and physiology of all organisms. The impact of ageing affects the whole body, but the effects on the central nervous system are especially dramatic. On mere visual inspection, brains of older people can easily be distinguished from their younger peers, and more specific tests ranging from whole-brain volumetry to studies of neurochemistry and even mitochondria demonstrate substantial age-related changes in the brain.

Until recently, the cognitive and neural mechanisms of age-related changes in cognition were studied independently of each other, i.e. as the *cognitive science of ageing* and the *neuroscience of ageing*, respectively. While the former focuses on age-related changes in behavioural measures of cognition such as attention and memory, the latter studies how age alters the morphology and physiology of the brain. Although it has been reasonable to assume that the observed cognitive changes are consequences of changes in the brain, the direct link between these approaches has only recently been formalized, specifically through the *cognitive neuroscience of ageing*[3]. The advent of modern neuroimaging methods have allowed studies of the effects of healthy ageing on brain morphology and function.

Several studies have now documented age-related changes in brain function in a variety of brain regions. One class of these studies has found that increasing age is associated with reduced hemispheric asymmetry in brain activation in a range of brain regions, something that has been suggested to be a sign of *compensatory mechanisms* in healthy ageing. A second class of studies has demonstrated age-related reduction in neural specificity, also called *dedifferentiation*. That is, some regions of the brain seem to loose their modular role in a specific cognitive process. Important to the present work is the suggestion that these two processes may be relevant for different regions of the brain. That is, while hemispheric asymmetry reductions are predominantly found in the prefrontal cortex and parietal lobe (and, to some extent, also in the temporal lobe), the findings of age-related reduction in neural specificity has predominantly been shown in the temporal lobe. This suggests that both asymmetry reduction and dedifferentiation can take place at the same time, albeit in different regions of the brain, and that they may be related processes in healthy ageing.

The present thesis sets out from this diversity of findings. The aim has been to study the effects of age on medial temporal lobe (MTL) function. Recent developments in the study of the functional organization of the MTL region has demonstrated that it consists of a collection of anatomically different yet tightly interconnected regions that may both show both collaboration and specialization in different cognitive operations. This suggests that by employing cognitive paradigms that evoke specific regional activation differences within the MTL, it may be possible to study whether age has an effect on this regional specificity, and determine whether such changes are signs of dedifferentiation or compensation. A brief outline of the aims of this project follows.

2.0 Aims of the project

1. To study the effect of intentional encoding of visual objects or positions on regional activation in the MTL (paper I), and through this establish a test that engages MTL regions differentially
2. To study the effect of ageing on the regional MTL activation during intentional encoding of visual objects and positions (paper II)

3.0 Background

The following chapter is divided into sections that describe the background of this thesis. The first three sections provide a brief overview of the background and some central findings in the cognitive neuroscience of ageing field. This will provide a brief overview of what has been identified as the main problems in the cognitive neuroscience of ageing, and the associated methods that are currently being used. Following this, the fourth section describes the MTL region, its intrinsic anatomy and the debate about its involvement in different cognitive functions. This part also presents the rationale for using the MTL region as a model of ageing. Chapters four and five will review and discuss the two studies that are part of the present thesis, and their relation to central issues in the cognitive neuroscience of ageing. Finally, the appendix describes the methods used in the study of MTL function and age-related changes in this brain region.

3.1 The ageing brain

The dawn of neuroimaging techniques such as Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) and Electroencephalography (EEG) allowed the *in vivo* study of age-related morphological and functional† changes of the brain. These studies have generally demonstrated an age-associated reduction in total brain volume [4]. Both cross-sectional and longitudinal MRI studies have demonstrated a decline in total brain volume in later life [5]. Using a so-called voxel-based morphometry (VBM)‡ approach Grieve et al. [6] showed an average loss of 2.5±5 mL (approximately 0.3%) per year in global brain volume across eight decades. Longitudinal studies have shown that tissue loss is particularly pronounced in healthy individuals over age 60, showing a total brain volume reduction of 5.4±3cm^3 or approximately 1.6% reduction [78]. Thus, there is evidence that the brain decreases in total volume with age.

3.1.1 Morphologial changes

MRI studies of the effects of age on specific brain volumes have shown independent and often non-linear changes with increasing age [9]. In a study of the effect of age on grey and white matter tissues of the cerebrum and cerebellum, Jernigan et al. [10] found that, e.g., the hippocampus showed a significantly accelerated loss relative to grey matter elsewhere in the brain, and that the prefrontal cortex was disproportionally affected by cortical volume loss and increased white matter abnormality. Furthermore, it was found that age-related volumetric changes were better described using non-linear compared to linear analysis. Consequently, some regions showed a 'plateau' phase where age had small effect on regional brain volume, and other age stages where these chages were much more pronounced. The results also showed that the loss of cerebral and cerebellar white matter occurred later than the loss of grey matter, but that ultimately, white matter loss was larger than grey matter loss.

3.1.2 Changes in brain function

In addition to the morphological changes seen in ageing, studies have shown that the neurophysiology of the brain changes with age. In early population studies using SPECT※

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† The term functional neuroimaging here refers to the assessment of brain processes, as opposed to static structural features of the brain. Examples of functional measures include perfusion MRI (such as Arterial Spin Labelling) and BOLD fMRI, both methods being used in this thesis.

‡ VBM is a computational approach to neuroanatomy that measures differences in local concentrations of brain tissue, through a voxel-wise comparison of multiple brain images[1]. The value of VBM is that it allows for comprehensive measurement of differences, not just in specific structures, but throughout the entire brain. The VBM has not been used in the work presented here.

※ SPECT = Single Positron Emission Computed Tomography, which provides a measure of tissue blood flow.

scanning, increasing age was associated with a general reduction in brain perfusion [111213]. Other studies have shown regional preservation of perfusion in ageing [141516]. It was also shown that failure to take age-related morphological changes into account, such as the dilution of ventricals, may lead to false estimates of perfusion [1718].

With the advent of perfusion sequences for MRI, such as Arterial Spin Labelling (ASL), several advances have been made in assessing age-related change in perfusion, or cerebral blood flow (CBF). ASL has a higher resolution than PET and SPECT scanners, and the combination with

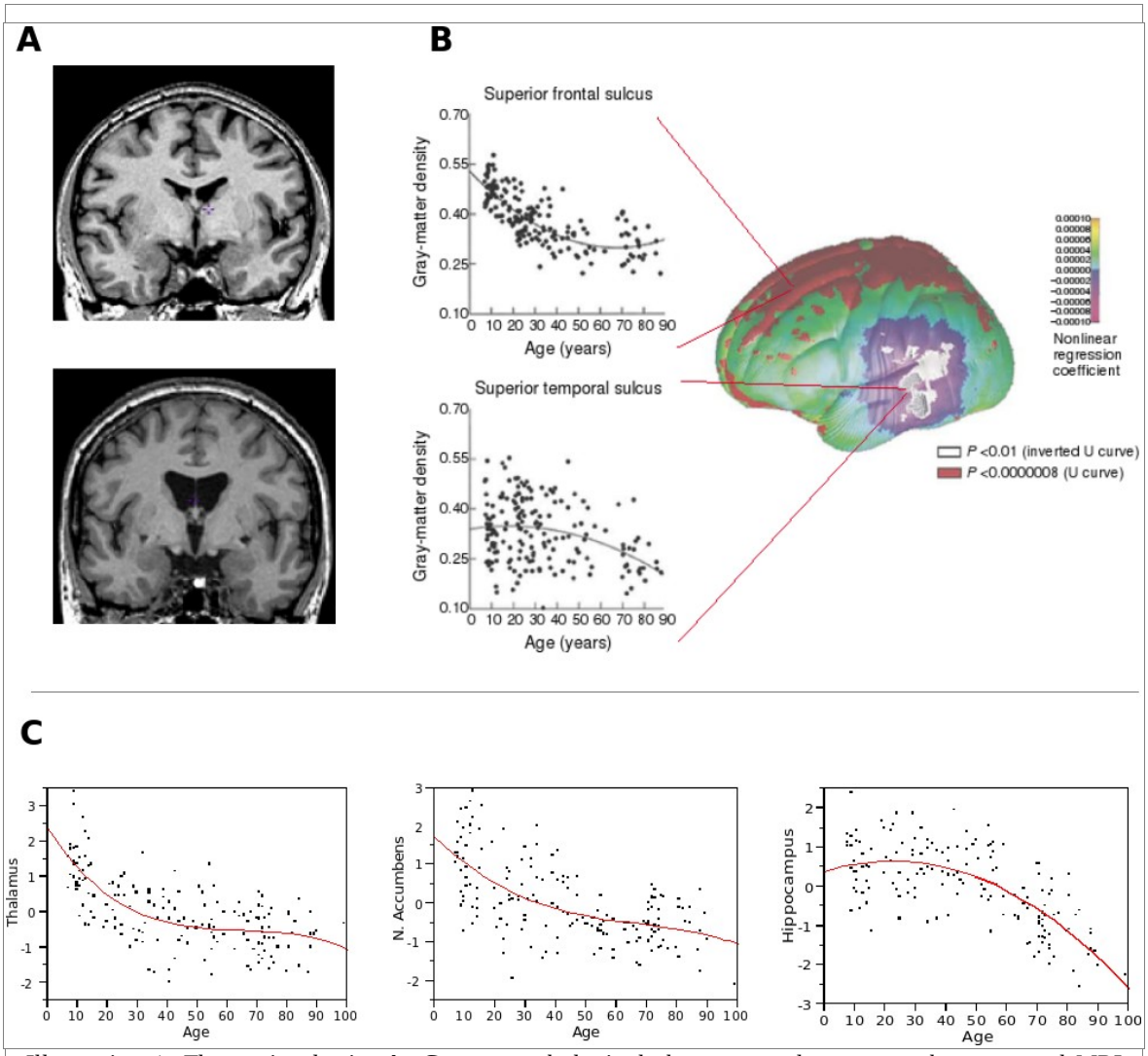

Illustration 1: The ageing brain. ***A:*** *Gross morphological changes can be seen on the structural MRI scan between a young (top) and an old (bottom) subject.* ***B:*** *Brain changes in maturation and ageing can be identified by fitting time-dependent statistical models to data collected from cross-sectional or longitudinal data, and measures such as cortical thickness can be plotted onto cortex using a colour code (from Toga, Thompson & Sowell 2006).* ***C:*** *A more region-specific approach applies the drawing of regions-of-interest, in which anatomically specified regions are outlined and their size relative to total intracranial volume is plotted as a function of age. Plots of different regions (left = thalamus, middle = nucleus accumbens, right = hippocampus) demonstrate regional differences in age-related chages in brain structure. Y axis shows standardized residuals (reproduced with permission from Terry Jernigan).*

structural image acquisition ensures an optimal comparability of structural and perfusion images in coregistration. It is today standard procedure to apply tissue segmented MR images and extract grey and white matter perfusion estimates separately. Combined with the assessment of separate measures for different regions of the brain, a more detailed analysis of age-related changes in

region- and tissue-specific perfusion is now possible. For example, Parkes et al. [19] found an age-related change in grey matter (but not white matter) perfusion at a rate of 0.45% reduction per year. These findings were predominantely found in the frontal and parietal cortex. Furthermore, Biagi et al. [11] recently demonstrated decreases in brain perfusion especially at around age 16, but no age-related changes in brain perfusion during adulthood. However, this study was undersampled in ages above 65 years, thus making interpretation of these results in terms of ageing unwarranted.

In summary, studies of brain perfusion have showed age-related changes in regional brain perfusion across the entire age-span. As measures of brain activation, especially BOLD [†] fMRI, depends upon a combination of physiological factors, including CBF, age-related changes in perfusion are thought to influence the BOLD signal. Consequently, results showing an age-related change in BOLD fMRI can, in part, be explained by alterations in perfusion. Indeed, it has been suggested that studies of age-related changes in BOLD fMRI should include measures of perfusion as covariates. In a recent study, Restom et al. [20] studied the effect of age on both BOLD fMRI and ASL and found an age-related regional reduction in CBF. Furthermore, it was demonstrated that the significantly larger responses in CBF and BOLD were attributable to a lower baseline CBF in older adults. Thus, studies reporting age-related changes in BOLD fMRI may be confounded by a lower baseline perfusion in old relative to younger subjects.

BOLD = Blood Oxygen Level Dependent, i.e., a measure of the relative level og oxygen in the blood. The BOLD fMRI is treated in the appendix on fMRI.

3.2 Cognitive ageing

Apart from the obvious physical changes that accompany increasing age, other well known age-related changes are of a mental and cognitive kind. It has been known that old adults[‡], while still being mentally healthy, become slower and gradually have specific cognitive problems such as reduced performance on different kinds of memory tests. Empirical studies of healthy ageing have long explored these cognitive changes. For example, increasing age has been associated with significant reductions in speed [21], working memory [222324], and long-term memory function and efficiency [252627].

It has been suggested that in older age, it becomes increasingly difficult to ignore irrelevant information or thoughts, and to inhibit dominant responses [28]. This means that old adults may remember items, facts and news relatively well, but may be disproportionally impaired, relative to young adults, in remembering the context in which the information was presented. Consequently, it is possible that age-related decline in working memory performance does not represent a decrease in capacity, but rather a cluttering of irrelevant information due to decreased ability to control working memory contents.

3.2.1 Two views on cognitive ageing

The failure to control the access of irrelevant information to working memory has been suggested to be a mark of loss of functional specialization or *dedifferentiation* that could be a result of reduced neuronal integrity. That is, cognitive functions that are functionally dissociated in young adults are expected to show reduced differentiation in older adults. The dedifferention theory is based on the idea that brain maturation during childhood and adolescence leads to a differentiation both between neural representations and between cognitive functions in general. For example, the processing of different visual stimuli such as faces, houses and letters are thought to become increasingly differentiated during development. Similarly, the representation of this information in working memory is thought to become more differentiated during brain maturation.

At the other end of the age spectrum, it is suggested that the biological changes accompanying increasing age eventally lead to reduced differentiation between perceptual and cognitive functions. Following this idea, one would expect that the functional organization of behavioural and cognitive factors such as speed, working memory, and long-term memory would change as a function of age. That is, age would be expected to lead to a lower performance *and* that the internal organization of this function would change. Contrary to this, Park et al. [29] found that although the general performance on tests of speed, visuospatial and verbal working memory and long-term memory declined as a function of age, the internal organization of each function did not change. The differentiation between verbal and visuospatial working memory showed a high degree of interrelation and shared variance. Indeed, the researchers found that speed and working memory together predicted performance on visuospatial and verbal recall, and that speed was more basic than working memory in this regard. In this respect the cognitive functions do not seem to undergo a dedifferentiation process, but are rather generally affected by speed and WM function, and a relatively spared functional organization.

Despite these findings, one possibility is that the dedifferentiation process is taking place at a 'lower level', i.e., between different information types that are processed in more or less the same way. Thus, instead of afflicting higher cognitive mechanisms such as working memory itself,

! A brief note on age terminology: when discussing age, I will sometimes refer to adults as young (typically 18-35), middle-aged (35-55) or old (>55). These age groups are somewhat arbitrary, but they will serve as references for the discussion. However, through most of this dissertation, age is treated as a continuous factor.

dedifferentiation might be a descriptive of age-related changes in functions such as the processing of houses, faces and letters. In this view, older subjects may show reduced efficiency in differentiating between stimulus types during the same cognitive operation, but with intact internal organization of working memory and other higher cognitive functions. Alternatively, older adults may show both impaired working memory function and a dedifferentiation in other cortical regions, and that these processes should be treated as separate phenomena. Finally, it may be possible that age leads to changes at the neuronal level despite preserved behavioural functions.

Contrary to the dedifferentiation view, it has been suggested that a class of age-related cognitive changes are signs of compensatory mechanisms. According to this *compensation* view, older adults have the ability to counter age-related cognitive deficits when task conditions are supportive of this [128]. Indeed, studies have reported substantial gains from cognitive training in old adults [303132]. For example, in a study by Calero and Navarro [33], 133 elderly subjects were divided into groups and either enrolled into a memory training program or a sham condition, and cognitive performance was measured both immediately after the training and again after 9 months. The researchers found that the training programme significantly improved cognitive performance at both times, supporting the view that compensatory mechanisms can take place in older age in order to produce a relative improvement in performance.

It should be noted that the effects of such training are larger among subjects that may be considered priveleged in terms of level of education, social activity levels and verbal ability, than those less fortunate [3435]. This has led some researchers to suggest that favourable hereditary and environmental factors increase the *cognitive reserve* which, in turn, may act as a compensation that reduces the effects of ageing [3637].

Compensation can occur in many ways. Typically, the compensatory functions that are suggested in the literature on ageing are taken as an indication of changes in cognitive functions and/or strategies that have positive effects on behaviour. However, it is possible that some compensatory mechanisms have no measurable effect on behavioural outcome, or may even lead to a further reduction in performance. That is, changes may occur at the neuronal level, whilst behaviour remains unaffected.

3.3 Cognitive neuroscience of ageing

As shown, age leads to observable changes in both cognitive functions and within the brain. The obvious task is to relate these two approaches. Despite abundant evidence about cerebral ageing and cognitive ageing, the link between these two domains has been missing. To overcome this, a number of researchers have explicated a new scientific discipline called the *cognitive neuroscience of ageing* [129].

The goal of this cognitive neuroscience of ageing program is to reveal relationships between changes in the brain (as described in section 3.1) and changes in cognition (as described in section 3.2). One can identify three basic methodological approaches to achieve this goal: the neuropsychological, the correlational and the activation imaging approach [128]. The *neuropsychological approach* compares cognitive changes in healthy ageing and in patients with brain damage due to trauma, stroke or degenerative disease. We could also add that the neuropsychological approach includes modelling of age-related changes in cognitive performance, as measured by traditional neuropsychological tests. Second, the *correlational approach* involves associating a neuronal measure, such as structural imaging, to a cognitive measure, e.g., working memory performance. Finally, the *activation imaging approach* measures brain activity in young and old during cognitive performance. It is especially this latter approach that is the focus of the present thesis.

In general, although any change in cognition and behaviour implies a change in the brain, one useful distinction is between two different types of age effects. A *neurogenic age effect* occurs when a change in the brain causes a change in cognition. One example is the relationship found between age-related changes in gray and white matter and decline in working memory performance [3839]. In contrast, a *psychogenic age effect* is the term for age-related changes in cognition that lead to changes in the brain, for example if age-related disuse of cognitive strategies leads to atrophy of certain brain regions. In the present work, our main focus is on neurogenic effects, i.e., we assume that the observed age-related changes in cognitive performance are due to changes in brain morphology. However, it may still be possible that some compensatory mechanisms in older adults may be classified as psychogenetic.

Among the large range of studies that have focused on the effects of age upon functional activation, two types of consistent findings have been made. The first is that older adults tend to show a more bilateral recruitment of the prefontal cortex during demanding task, and that this response is interpreted as a sign of relative preserved or improved performance, compared to old subjects that do not show this pattern. The second class of finding is that age leads to a reduced efficientcy and specificity in functional activation, compared to young adults. Both these classes are reminiscent to the discussion about dedifferentiation and compensation, as discussed in section 3.2.1. Here, the two classes of findings are treated separately, and finally discussed together, in section 3.3.3.

3.3.1 Compensatory mechanisms in the ageing brain

In studies of episodic memory, the memory for personally experienced past [40,41], young healthy individuals display a hemispheric asymmetry for different stages of the mnemonic process. Functional neuroimaging studies have shown that the left PfC is more involved in episodic encoding, whereas the right PfC is more involved in episodic retrieval, a pattern described by the hemispheric encoding/retrieval asymmetry (HERA) model [139]. The role of the left PfC has been attributed to semantic processing [4243], and it is known that semantic processing enhances encoding [4445]. The right PfC has been associated with verification and checking operations [464748]. When left PfC activation has been observed during retrieval, it is often attributed to semantic generation[49].

In studies of ageing, one of the most consistent findings has been an age-related change in asymmetry during episodic memory processing. According to the Hemispheric Asymmetry Reduction in Older Adults (HAROLD) model PfC activity tends to be less lateralized in older than younger adults [50]. The model is now supported by functional neuroimaging, electrophysiological and behavioural evidence in the domains of episodic memory, semantic memory, working memory, perception and inhibitory control. As previously mentioned, in young adults, brain activation during episodic memory retrieval tends to be right lateralized [40]. However, compared to this group, older adults show significant activations in both hemispheres during word pair cued recall [51], working memory [52], and face matching [53].

In general, the bilateral involvement in older adults is interpreted as compensatory and as a sign of preserved cognitive function. That is, to counteract cognitive decline, older adults recruit both hemispheres in a task in which young subjects recruit only one hemisphere. Several studies have demonstrated an association between age-related reduction in hemispheric asymmetry and preserved performance. Here, one can divide between two classes of findings: studies comparing the performance of younger and older adults, and studies in which there is a difference between high and low performing older adults. An example of the former class of findings comes from a study by Mattay et al. [54] who found that even at comparable performance levels during a low load working memory task, older subjects showed a bilateral engagement of the PfC, compared to young subjects. When working memory load increased, older subjects performed worse than young subjects, and showed a reduction in the bilateral recruitment of the PfC. This suggests that, within capacity, compensatory mechanisms such as additional prefrontal cortical activity are called upon to maintain proficiency in task performance. However, as cognitive demand increases older adults are pushed past a threshold beyond which physiological compensation cannot be made and a decline in performance occurs.

The second class of studies on compensatory mechanisms focuses on high- and low-performing older adults. For example, in a comparison of young adults, low-performing old adults and high-performing old adults, it was found that low-performing older adults recruited similar right PFC regions as young adults during memory retrieval, while high-performing older adults engaged PFC regions bilaterally [55]. This suggests that low-performing older adults recruit a network similar to young adults but use it inefficiently, whereas high-performing older adults counteract age-related neural decline through a functional reorganization of neurocognitive networks. Such findings indicate that the older brain can reorganize to better cope with cognitive challenges. Although over-activation may play a compensatory role when cognitive decline is limited, under-activation seems to be the typical pattern when cognitive impairment is in a more progressed state. This pattern of age-related changes suggests that compensation through over-activation is restricted to the early stages of cognitive impairment in ageing.

Although the most reported age-related reductions in hemispheric asymmetry have been found in the PfC, other studies have reported age-related asymmetry reductions in other brain regions, including in the parietal cortex during a test of executive function [56], in parietal and temporal cortices during face memory [5357], and in primary motor cortex during reaction time tasks [5859]. In studies by Maguire and Frith [6061] hemispheric asymmetry reduction in older adults has been found in the hippocampus during an autobiographical retrieval task. Consequently, it has been argued that the evidence of age-related changes in brain activation is generally in favour of the HAROLD model.

Despite this, studies have reported conflicting findings for other cortical and subcortical regions than the PfC. For example, a relationship has been reported between increased bilateral activity in

fusiform gyrus and hippocampus and poorer performance in younger adults [62]. Conversely, in the same analysis, right middle temporal and medial prestriate activation was associated with poorer performance in younger adults and better performance in older adults. These results are difficult to intrepret within the HAROLD model.

As previously mentioned (section 3.2) it has been found that age leads to reduced performance on high-order cognitive functions such as WM and attention despite a preserved internal organization. Compared to the results mentioned previously that show significant alterations in neural activations in both preserved and reduced performance in older adults, the claim for unaffected internal organization is not supported. Instead, several findings now suggest that the neural architecture underlying different cognitive functions is disrupted with increasing age.

3.3.2 Age-related loss of regional specialization in the brain

Contrary to the compensatory model of ageing, others have suggested alternative explanations of the observed age-related changes in brain activation. Rather than seeing an age-related reduced hemispheric asymmetry in the PfC as a sign of compensatory mechanisms, it may signal a failure to keep neural activity confined within a single hemisphere. According to the dedifferentiation model of ageing, old age is associated with a loss of specificity in neural activation. This model follows the idea of a differentiation process during brain maturation in early childhood and adolescence, as originally stated in the early child development literature by Garrett [63]. Here, it was suggested that during maturation, a global cognitive capacity branches into a series of specialized cognitive abilities. More recently, the idea of differentiation during maturation has been supported by functional neuroimaging studies [6465]. The dedifferentiation model, on the other hand, suggests that in old age, neural systems become less functionally differentiated as a consequence of general atrophy and reduced neuronal function. Following this view, the age-related reduction in episodic memory and other cognitive functions may be caused by dedifferentiation in related structures.

One problem with this interpretation is its difficulty in explaining that asymmetry reductions are associated with *higher* performance in older adults. The dedifferentiation view would predict that reduced asymmetry is a sign of *reduced* neural (and behavioural) performance. Other studies, however, suggest that dedifferentiation may occur at a more basic level. For example, functional neuroimaging studies have demonstrated an age-related loss of regional specialization in visual perception. Park et al. [29] studied the regional activation during perception of visual stimuli such as houses, faces, chairs, and pseudowords. In young adults these regions are known to produce specific and dissociable responses in occipito-temporal regions [65]. By comparing functional activations in young and old subjects, it was found that older adults displayed significantly less neural specialization for each stimulus category in ventral visual cortex compared to young adults.

Taken together, although the dedifferentiation model falls short of an interpretation of age-related reduced hemispheric asymmetry, it may explain the reduced neural specificity observed in other regions of the brain. It is possible that dedifferentiation is a sign of age-related changes that are due to stochastic processes, and that this induces 'noise' in the neuroarchitecture of cognitive functions.

3.3.3 Compensation or dedifferentiation?

As mentioned previously, it is possible that compensation and dedifferentiation is taking place simultaneously in the ageing brain, and that the two intrepretations are not mutually exclusive. Consequently, it has been suggested that PfC hemispheric asymmetry reduction is a compensatory response to an age-related reduced function and dedifferentiation in other cortical regions.

For example, in a study of the effects of age on brain activation during the intentional encoding of

scenes, older adults showed less activation than young adults in the left and right parahippocampus and young adults showed more activation in the middle frontal cortex [66]. Interestingly, it was found that older adults who showed the least engagement of the parahippocampus activated inferior frontal areas the most, thus suggesting that prefrontal regions could serve a compensatory role for declines in medial-temporal activations with age. Similarly, in an fMRI study, Payer et al. [67]showed that increasing age was associated with reduced regional specialization for visually presented houses and faces in the ventral visual cortex. At the same time, the middle and inferior frontal cortex displayed increased activation in older adults compared to the young. Taken together, studies suggest that increasing age leads to changes in brain activation that signal both dedifferentiation and compensation.

In the study of age-related changes in brain activation, most studies have been focusing on the prefrontal cortex, while fewer studies have been specifically aimed at other regions, such as the parietal and temporal lobes. However, in recent years, studies of the MTL, have shown that this region is highly specialized in different cognitive functions. This region-specific specialization is found both between structures within the same hemisphere, as well as between hemispheres. As such, it is possible that this region may serve as a good model for testing the effect of age on regional activation. In the next section, we focus on the medial temporal lobe, its anatomical constituents and ideas about functional specialization.

3.4 The medial temporal lobe

The MTL region is a densely packed and interconnected region on the inside (medial) part of each temporal lobe. The study of this region has been, and still is, under intense scrutiny, both in terms of our understanding of anatomical regions, their internal connections and especially their role in cognitive functions. The following section is a brief review of the anatomy of the MTL region, followed by a review of the debate on what cognitive operations this region is thought to support.

3.4.1 Anatomy of the MTL region

The MTL concept is an example of neurojargon that has traditionally been rich in clinical and behavioural meaning, but sparse in neuroanatomical basis except for topography [68]. Traditionally, at least three anatomical entities qualify as components of the MTL. These include the hippocampal formation, the parahippocampal cortices, and the amygdaloid complex, although this is still considered somewhat controversial [136123]. These structures can be subdivided into more specific regions. Although there is a lack of consensus relating to terms describing the hippocampus and the adjacent cerebral cortex, the term hippocampal formation generally applies to the dentate gyrus, the Cornu Ammonis fields CA1-CA3 (and CA4, frequently called the hilus and considered part of the dentate gyrus), and the subiculum. The CA1, CA2 and CA3 fields make up the hippocampus proper. The hippocampus is also often subdivided in to three regions; head (anterior end), body, and tail (posterior end). This also holds for the amygdala, which should not be thought of as one structure at all, but rather as a composite of parts of the cortex, striatum, and claustrum [122]. Indeed, the amygdaloid complex is composed of more than ten nuclei with different cytoarchitectonic, chemoarchitectonic, and connectional characteristics. The cortex of the parahippocampal gyrus includes the subiculum, perirhinal and entorhinal cortex, all of which are directly and indirectly connected to the hippocampus. In the anterior end of the parahippocampal gyrus we find the

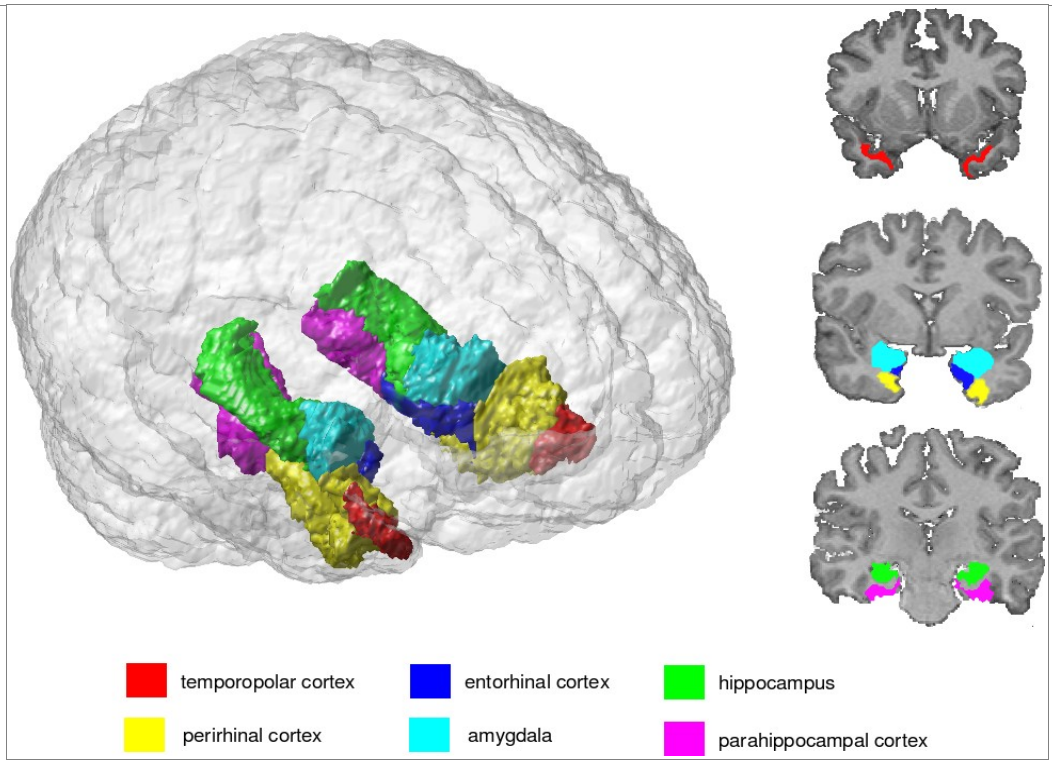

Illustration 2: The extended MTL model used in the present project. Left: 3D view of regions shown within a glass brain model. Right: coronal sections showing the placment of through the anterior (top) to posterior (bottom) axis. Legend shows colours used for each structure.

entorhinal and perirhinal cortex, while the posterior most portion is named (posterior) parahippocampal cortex [69].

In addition to these regions, the temporopolar cortex has been suggested as a part of the primate perirhinal cortex [71] However, this region has received less attention both in anatomical and functional studies compared to the parahippocampal region and its connections to the hippocampus. Recent developments in anatomical delineation of the temporopolar cortex in MRI research have allowed the assessment of the function of this structure. Such studies now suggest a role in novelty processing [101], memory [72] and semantic processing [100]. An illustration of the extended MTL model used in this project is shown in Illustration 2.

The major source of cortical inputs to the hippocampal circuit is the entorhinal cortex. Two main input routes to the hippocampus have been found, both of which arrive via entorhinal projections: (1) a ventral route via the occipito-temporal cortex, including the fusiform gyrus and perirhinal cortex, projects to the (anterior) hippocampus through the lateral entorhinal cortex; and (2) a dorsal processing route through the occipito-parietal cortex and posterior MTL regions such as the parahippocampal cortex, which projects to the (posterior) hippocampus through the medial entorhinal cortex. It should be mentioned that the projections are not merely relayed through the entorhinal cortex, but are rather undergoing further processing in this structure (see next section for a brief review of MTL functions). Anatomical studies of the MTL region thus suggest that visual processing is divided into at least two processing streams, as shown in Illustration 3. However, as this figure shows, there is also a proportion of overlap between the dorsal and ventral streams. The functional consequences of this remains to be resolved.

3.4.2 Functions of the MTL region

A traditional and widely held belief has been that the MTL is solely dedicated to the formation of episodic memories. It has been thought that the hippocampus is the most critical component of the MTL memory system, although to some researchers, lesions to any part of the MTL have been hypothesized to produce identical deficits [13174]. The MTL is typically thought of as needed at the time of learning, in order to establish functional connections with widespread areas of the neocortex. The strong version of this view assumes a high degree of functional homogeneity and serial organization within the MTL, such that double dissociations between individual structures should not be possible. In this sense, lesions to the perirhinal, entorhinal or parahippocampal cortices have been hypothesised to produce the same memory deficits. This view was supported by several early experiments, where lesions to non-hippocampal MTL structures produced deficits comparable to hippocampal damage. Indeed, a decade ago, Squire wrote: (&) the idea that the medial temporal lobe lesions selectively impair memory now rests securely on quantitative neuropsychological data (133, p. 187).

3.4.2a Perirhinal cortex

The past decade has seen a substantial criticism of the uniform view of MTL function. Here, the perirhinal cortex has been the most intensely studied structure in the debate about the functions of the MTL region. Consequently, the review of studies of the perirhinal cortex will be treated first and most comprehensively. From one line of research, studies have suggested that the structures within the MTL play separate roles in memory. Animal lesion studies have shown that lesions to the perirhinal cortex lead to impairments that are separable from those to the hippocampus. For example, aspiration ablation of the perirhinal cortex alone, or together with the entorhinal cortex, cause dramatic deficits in the ability of rats and monkeys to perform a delayed-matching (or non matching-) to sample task (DNMS) [757677].

Based on studies demonstrating deficits in perceptual judgements following damage to the PC, it is claimed that the perirhinal cortex plays a role in object perception (in addition to memory). For example, in a meta-analysis of the effect of selective hippocampal lesions on visual recognition in monkeys, Baxter and Murray [83] demonstrated that, although selective hippocampal damage does yield a net mild impairment in recognition memory (a DNMS task), there is a significant inverse correlation between the extent of hippocampal damage and the magnitude of the impairment in recognition memory. Paradoxically, large lesions of the hippocampus are associated with little or no recognition impairment. Conversely, greater damage to the rhinal cortex (i.e., the perirhinal and entorhinal cortex) is associated with a greater magnitude of recognition impairment. These results suggest that the rhinal cortex and hippocampus make different contributions to recognition memory. In a third approach, the perirhinal cortex is hypothesised to play a significant role in object-level discrimination, a task that follows the processing of visual input through the ventral stream in occipito-temporal regions. Studies demonstrating a role for the perirhinal cortex in encoding are today seen as rather uncontroversial, but recent studies of perirhinal ablation in monkeys have additionally demonstrated selective deficits in object perception and discrimination [78,79]. Following this, the perirhinal cortex is thought to be involved in both episodic memory and visual perception. In their model, Murray and colleagues have proposed a perceptual-mnemonic/feature conjunction model of perirhinal cortex function [80]. In their line of studies Murray and her colleagues have documented that monkeys lesioned bilaterally in the perirhinal cortex are impaired at tasks involving feature ambiguity [81], delayed non matching-to-sample task [82] and single-pair discrimination learning and recognition memory [83,84]. These and other results have been confirmed by other researchers, and extended to involve visual object identification [85] as well as configural learning and paired-associate learning [86].

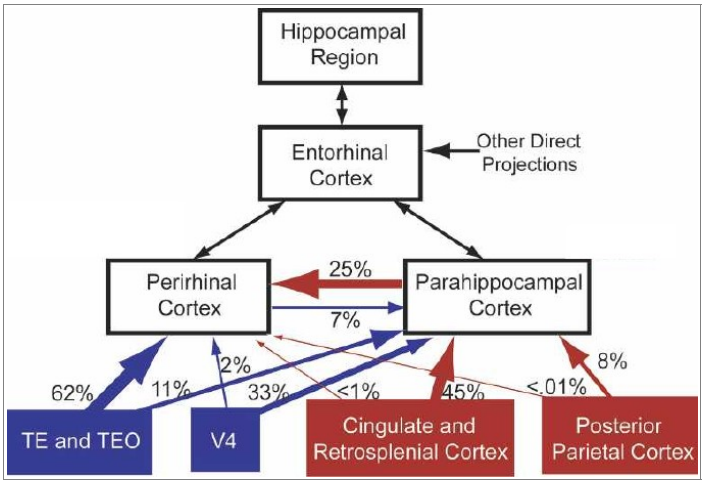

Illustration 3: A schematic diagram of unimodal and polymodal cortical input to the MTL region. Percentages of cortical input are from Suzuki and Amaral (1994b). Line thickness represents approximate percentages of cortical input. This overview of anatomical connectivity suggests the existence of two processing routes, where the perirhinal cortex may be more involved in object processing, while the parahippocampal cortex is more involved in spatial processing. However, the figure also suggests that there is an overlap in this functional connectivity, i.e., the parahippocampal cortex receives some projections from areas TE, TEO and V4; and perirhinal cortex receives input from areas that predominantly project to the parahippocampal cortex. From Buffalo, Bellgowan and Martin (2007). It is worth noting that this figure does not distinguish between entorhinal regions (medial and lateral) nor the projections from these regions to hippocampus.

From this view, the perirhinal cortex plays a specific role in encoding and memory, and even more interesting: it is thought to play a role in non-mnemonic processes such as object perception at the object level . The term object level here refers to processing that occurs above the single feature level. The perception of a face is a good example of this; while the face consists of several features such as eyes, mouth and nose, and one can discriminate between these features, a face is also thought to be processed at a higher-order level, where it is perceived as a single object (just like an eye, at the lower level, consists of several sub-features, yet is perceived as a single object). Thus, when the perirhinal cortex is thought to be involved in object-level perception and memorizing, this refers to the ability to discriminate and perceive at this higher-order level, or at even more complex ones.

Contrary to the findings by Murray and Gaffan, Buffalo et al. [87] observed good performance on tests of object recognition memory at short delays in monkeys with perirhinal cortex removal and in humans with damage to the MTL including the PC. Deficits emerged only when delays between initial and subsequent (test) exposures to objects were increased. The researchers concluded that the perirhinal cortex was not important for object perception, but functioned only in object memory. However, as Murray and Richmond have responded [88], intact performance on object discrimination or object matching tasks with short delays does not necessarily indicate intact perception of all kinds of objects under all circumstances, but only for the objects presented. Thus, the results of Buffalo et al. may not generalise to tests in which the objects are designed to tax visual perception.

3.4.2b Parahippocampal cortex

According to its connectivity, the posterior part of the parahippocampal gyrus labelled the parahippocampal cortex is thought to be involved in the processing of spatial information. For example, in an fMRI study of spatial processing and retrieval, Ekstrom and Bookheimer [89] recently found that retrieval of spatial information led to greater activation in the parahippocampal cortex compared to the hippocampus (and other MTL regions). Similarly, Rosenbaum et al. [90] found increased activity in the parahippocampal cortex, but not the hippocampus, during a spatial navigation task. Indeed, a number of studies have suggested the existence of a 'parahippocampal place area' [919293], non-specifically located within the posterior parahippocampal region that responds more strongly to place stimuli than to other kinds of stimuli. For example, Epstein et al. [94] found that PPA activity was affected by scene novelty, but did not change as a function of perceived motion.

3.4.2c Entorhinal cortex

Studies of the entorhinal cortex suggests that it is involved in a range of different processes, including odour perception [9596], visual perception and memory [9798], and multi-modal integration [99]. As previously mentioned, the entorhinal cortex is today subdivided into two regions a medial region receiving input from the dorsal visual stream , and a lateral region receiving signals from the ventral visual stream . Thus, the entorhinal cortex is thought to be involved in the complex integration of visual information about both complex visual objects and visuospatial information, including scenes and the relation between objects.

3.4.2d Temporopolar cortex

The temporopolar cortex has so far received far less attention than other MTL regions. This may have several causes, such as the fact that it only recently has been suggested to be part of the MTL (through its connections to the perirhinal cortex). Second, it is only recently that a firm development of the anatomical understanding and accompanying criteria have been made, thus making it available to high-resolution in vivo studies. Finally, the temporopolar cortex is among the regions most affected by susceptibility artefacts in fMRI scanning, something that only recent developments have sought to solve. Nevertheless, studies from neuropathology have suggested a role for the temporopolar cortex in semantic processing [100], as part of a face-object recognition network [101], and in recognition awareness, especially for old (consolidated) memories of objects [102].

3.4.2e Hippocampus and amygdala

These studies of the perirhinal, entorhinal, parahippocampal and temporopolar cortices are relatively new in the study of the MTL region, especially compared to the preceding years with studies of the hippocampus and amygdala. Both these structures have well-known and well defined functions. The hippocampus is best known for its role in declarative memory, including episodic [103] and semantic memory [104105]. Other studies have found that the human hippocampus is involved in

spatial navigation [106], which has long been known from the animal literature [107108].

The amygdala is typically known as a MTL structure that primarily involved in emotional processing [109110]. Fear responses, stress and anxiety are known to be associated with amygdala activation, as measured by in vivo functional neuroimaging tools such as fMRI [111112], as well as studies of patients with lesions to the amygdala [113114]. However, studies have recently shown that the amygdala is also (just as) involved in positive affect [115116]. In addition to the role in emotional responses, the amygdala has also been implicated in memory, in particular with a modulatory role on memory formation and retrieval [117118119], which is thought to be supported by the close anatomical bonds between the amygdala and hippocampus.

Recent explorations into subregions within both the hippocampus and amygdala have provided new insights and a more complex understanding of their roles. First, it has been suggested that the hippocampus should be divided into three regions head (anterior end), body (middle), and tail (posterior end) [120]. Specifically, the anterior end is thought to be a part of the ventral visual stream , receiving input from the perirhinal cortex through the lateral entorhinal cortex. In contrast, the hippocampal tail is suggested to be involved in spatial processing, as it receives input from the parahippocampal cortex via the medial entorhinal cortex. Recently, Lee, Scahill and Graham [121] demonstrated increased activations in the anterior hippocampus, along with the perirhinal cortex, during unfamiliar trial-unique face oddity judgements, and that novel visual scenes were associated with increased activation in the posterior hippocampus and parahippocampal cortex. Furthermore, it was found that repeated presentations of these stimuli (faces or scenes) were associated with a decrease in activations in those regions responding to when the face or scene stimuli, respectively, had been unfamiliar.

Similarly, the amygdala is known to be a collection of more than ten different neural structures, which makes some researchers claim that the amygdala should not be considered as a single structure at all [122123]. Indeed, based on clinical data [124125] and neuroimaging studies [126], it has been suggested that the amygdala should be subdivided into regions especially for the processing of visual and auditory input, although this is still debated.

Taken together the MTL region consists of several anatomically different yet highly interconnected regions that both collaborate in specific cognitive operations, as well as play separable roles in different cognitive functions. Table 1 briefly summarizes some of the suggested roles of MTL regions.

The brief overview gives way to some suggestions. For example, it seems that all regions are implied in visual memory. However, as regions are quite distinct in their involvement in other regions, it is likely that the same structures make different contributions to visual memory. Also, as the results presented here will show, visual memory should be divided according to both memory processing stage and content.

3.4.3 fMRI studies of MTL function

Several studies have focused on the functions of the MTL region, and results have been varying and sometimes conflicting. As these studies also vary with respect to the methods being applied including paradigm design, image acquisition, preprocessing and postprocessing stages conflicting results may be attributable to different use of methodology. As the present project is using one of many possible methods for assessing MTL activation and cognitive function, a brief review of other studies may illustrate how the methods and results in the present project should be understood. First, we briefly review and compare studies of MTL function. Second, these studies

are compared in terms of their methodological approaches i.e., for scanner parameters and data processing choices.

There is a large range of possible paradigms for testing MTL function, and for the present purposes, we choose to focus on studies of intentional encoding. Here we include nine studies, as shown in Table 2. As can be seen from this brief and limited overview there are diverging findings concerning, for example the involvement of the perirhinal cortex during visual object encoding. This may well be due to the use of different cognitive paradigms and number of subjects. In addition, some studies, like Strange et al. [127] distinguish between anterior and posterior sub-regions of the hippocampus

Function / Structure	visual item perception	visuospatial perception	odour perception	multimodal	visual memory	novelty	emotion	semantic
temporopolar cortex	•				•			•
entorhinal cortex	•	•	•	•	•			
perirhinal cortex	•	•			•	•		•
parahippocampal cortex		•			•	•		
hippocampus	•	•		•	•			
amygdala					•	•	•	

Table 1 Brief overview of suggested roles of distinct MTL regions.

Study	Subjects	Task	TC	EC	PC	PHC	HP	AM
de Zubicaray et al. 2001	10	DNMS encoding	-	-	-	-	-	-
		DNMS retention	-	-	-	-	-	-
		DNMS retrieval	-	-	•	-	-	-
Strange et al. 2002	14	Successful semantic encoding	-	-	•	-	•	-
Reber et al. 2002	10	Picture encoding	-	•	•	••	••	-
		Word encoding	-	-	-	-	•	-
Pihlajamäki et al. 2003	12	Encoding of novel object pairs (vs. old object pairs)	-	-	•(•)	•(•)	-	-
Kirwan & Stark 2004	13	Successful face-name pair encoding	-	-	-	•	•	•
		Successful retrieval	-	•	•	•	•	-
Strange et al. 2005	12	Encoding novel associations	-	-	•	-	• (a)	-
		Encoding familiar associations	-	-	-	-	• (p)	-
Chua et al. 2007	18	Face-name associative encoding (remembered > forgotten)	-	-	•	•	•	-
Buffalo et al. 2007	11	Object encoding	-	-	•(•)	•	-	-
		Position encoding	-	-	•(•)	•(•)	-	-
Ramsøy et al. (submitted)	27	Object encoding	••	••	•	-	•	•
		Position encoding	-	-	-	-	-	-

Table 2 Mini-review of fMRI studies of object encoding. Single dots mean unilateral findings, double dots bilateral findings. Parenthesis signifies trends in data that did not meet statistical threshold. (a) = anterior, (p) = posterior.

However, given the differences in image acquisition, preprocessing steps, and analysis methods, differing results may well be explained by such differences in technical parameters. Indeed, as shown in Appendix I, image acquisition, preprocessing and analysis in BOLD fMRI have been shown to induce significant changes in results. Consequently, a brief overview of the technical specifications in the studies just reviewed may demonstrate, albeit indirectly, how differing results may be caused by such factors.

Study	Design	Slices	In-plane resolution	Normalization	Smoothing	p cutoff	Other
de Zubicaray 2001	Event-related	5 mm	3.44 x 3.44	yes	6 mm	< .05 uncorrected	
Strange 2002	Blocked	2 mm	2.5 x 2.5	yes	6 mm	<.005 uncorrected	Narrow FOV
Reber et al. 2002	Blocked	6 mm	3.75x3.75	yes	no	<.005 uncorrected	ROI analysis
Pihlajamäki 2003	Blocked	5mm + 1mm gap	4 x 4	yes	8 mm	< 0.05 uncorrected	Data also analyzed in native space
Kirwan & Stark 2004	Blocked	3 mm + 1 mm gap	3 x 3	yes	4 mm	<.03 uncorrected	Normalization = predefined anatomical regions
Strange 2005	Event-related	3.3 mm	3 x 3	yes	6 mm	<.0001 uncorrected	
Chua 2007	Blocked	5mm + 1mm gap	3.75x3.75	yes	8 mm	<.005 uncorrected	
Buffalo 2007	Randomized blocks	4 mm	3.75 x 3.75	no	no	<.05 uncorrected	Analysis in individual space
Ramsøy (submitted)	Randomized blocks	2 mm	3 x 3	no	no	<.05 corrected, trends reported	ROI analysis in native space

Table 3 – Technical specifications in the 9 studies showing different results on MTL activations in visual object encoding paradigms. While the majority of studies are similar with respect to some parameters, e.g. the use of spatial normalization, other factors, such as statistical cut-off and spatial smoothing show large differences.

Taken together, this brief overview demonstrates that the study of MTL function in visual memory formation is subject to large differences in all aspects. Since several studies show diverging results for the role of the MTL in visual memory, it is suggested here that such differences may, at least partially, be explained by the large variance in methodology, and that these variables should be factored into any review of this literature. The approach developed and applied in the present project (see the appendix on fMRI) is suggested so as to avoid many of the technical problems associated with spatial normalization and smoothing, and thus produce a more valid estimate of MTL function.

3.5 Rationale for studying the MTL region and ageing

The cognitive neuroscience of ageing is focusing on a large number of diverse topics, including changes in working memory, perception, speed, language and long-term memory [128129130]. To this end, such models of cognitive and neural changes allow the testing of specific hypotheses of the effects of age on the brain. In the present thesis, the chosen model is the MTL region and its region-specific involvement in cognitive processes. From the previous discussion, the following factors make the MTL region an interesting model to the study of age-related changes in brain function.

First, the study of MTL anatomy in humans and non-human primates, as well as other mammals, has only recently provided sufficiently detailed and consistent definitions of MTL anatomy. Combined with the translation of this knowledge into operationalized protocols for the drawing of regions of interest in MRI studies, it is now possible to perform a detailed analysis of volumetric and functional changes in the MTL regions in different settings, including ageing and degenerative disease.

Second, advances in the assessment of ventromedial regions of the brain using high-field fMRI has allowed more comprehensive analysis of changes in MTL regions. At high-field strengths at and above 3 Tesla, fMRI studies of these regions are troubled by severe susceptibility and motion artefacts due to local field inhomogeneity. With the use of recent developments in image sequence optimization and the application of ROI based analysis (see the appendix on fMRI), more reliable and region-specific assessment of local changes in brain activation are possible.

Finally, recent developments in cognitive neuroscience have moved the view of MTL regions from being a homogenous, functional unit, to a heterogenous view encompassing functions such as high-order visual perception, novelty and multi-modal integration. This development, still to be considered an ongoing debate, has provided new tools and topics to study the effects of ageing on regional activation in the MTL.

Taken together, the use of the MTL region as a model to study the effects of age is interesting on several grounds. The current project has thus applied a combination of the most recent anatomical guidelines, an optimized acquisition approach of MTL activation, and a cognitive approach thought to evoke region-specific activation within the MTL. The appendix on fMRI describes the background and specific considerations and work in this project in the choice of scanning parameters, image processing and statistical analysis. Next, we will review and discuss the two studies conducted as part of this thesis.

3.6 On method

In this project, there has been a need to develop and combine several approaches of image acquisition and processing, identification of specific MTL regions, and statistical analysis. In appendix 3these considerations and developments are described in more detail.

4.0 PAPER 1 – Regional activation of the human medial temporal lobe during intentional encoding of objects and positions

The MTL region is composed of tightly interconnected regions that have long been thought to serve the same cognitive function declarative memory. Previous theories have stressed a unimodal function of the MTL in this role [131132], and have suggested that a lesion to any part of this system will lead to the same dysfunctions, and that lesion extent, rather than location, determines the severity of the dysfunction. More recent studies in animal lesion models [88] and neuroimaging in healthy subjects [133134] suggest that the MTL region is more heterogeneous than previously thought, both in terms of regional contributions to declarative memory and in other perceptual and cognitive functions.

4.1 Motivation for Paper 1

Although several studies have found that MTL regions are different in their contributions to declarative memory, such as episodic memory, less is known about the specific role of each structure in processing of different types of context. It has been suggested that anterior regions such as the perirhinal cortex is more involved in object encoding, and that posterior regions such as the parahippocampal cortex is more involved in spatial encoding. Studies exploring this have generally succeeded in documenting these effects[135], although there are conflicting findings [136]

It is possible that the conflicting findings in the literature may be caused by substantial differences in methodological issues, including use of cognitive task, image acquisition, preprocessing steps and the setting of statistical cut-off. Taken together, the neuroimaging literature is still too diverse and has yet to apply optimal approaches in MTL assessment, to allow any firm conclusions to be drawn. Specifically, although previous studies have suggested that the perirhinal cortex is more involved in object processing, and that the opposite is true for the parahippocampal cortex, direct comparisons between regions have not yet been performed.

In this study, we sought to develop a cognitive paradigm thought to engage separate regions of the MTL, especially to test the relationship between perirhinal and parahippocampal cortex in intentional encoding of objects and positions. In addition, we developed a pipeline consisting of optimization in image acquisition, data preprocessing and post-processing analysis approaches that together are thought to provide a better estimate of MTL function.

Specifically, our hypothesis was that the left perirhinal cortex would be more involved in object encoding than position encoding, and that the opposite relationship was expected for the left parahippocampal cortex. This further led to the of a double dissociation between the perirhinal and parahippocampal cortex. Our reason for focusing on the left hemisphere only attributed to studies showing a hemispheric asymmetry between encoding and retrieval, in that encoding is thought to engage left hemisphere regions more, whilst retrieval is thought to predominantly involve right hemisphere regions [137-139].

4.2 Methods & results

We used fMRI and a region of interest (ROI) approach to study the effects of content (object and position) on activation within the MTL region. Information about scanning parameters for structural and functional images, as well as on paradigm design can be found in Paper 1. Put simply, our

functional scans were optimized to the MTL region using a 3x3x2 voxel dimension, where the 2 mm slice thickness (with no interslice gap) was used to maximize the signal to noise ratio (SNR). This meant that the scans had a smaller field of view than the whole brain. By experimenting with slice orientation of the method suggested by Deichmann et al. [140] we found that a slice orientation 20° oblique to the transverse plane, making the slices roughly parallel to the long axis of the temporal lobe, gave the optimal SNR for the MTL region. Vital to the present analysis is the fact that the effects of content were performed without differences in the stimuli. That is, based on the stimuli alone subjects would not be able to distinguish between the two tasks, and only the preceding instruction would provide information about the task at hand. This means that the analysis of the encoding stage (and the preparation and rehearsal stages) is also a study of mind-setting.

The behavioural results suggested that the object and position tasks did not differ with respect to difficulty. Performance on both tasks was comparable, although the finding of briefer reaction time during the object task could be indicative of slightly lower task demands during recognition.

Our analysis was focused upon the encoding stage (the effect of content during preparation and rehearsal for the young group is reported in Paper II), with a particular focus on the left perirhinal cortex. Here, we found that, as expected, the left perirhinal cortex was significantly more involved in object encoding than position encoding. Contrary to our predictions, the left parahippocampal cortex did not significantly differentiate between object and position stimuli.

A vital analysis was the direct comparison between left perirhinal and left parahippocampal cortex, which showed that the left perirhinal cortex was significantly more involved in object perception than the left parahippocampal cortex. Not surprisingly, no such difference was found in the right hemisphere, nor did we find that the parahippocampal cortex was more involved in the position task than either of the perirhinal cortices.

We additionally performed post-hoc tests of two kinds. First, we tested additional MTL regions during the encoding stage and found that the object encoding task appeared to evoke stronger activations than position encoding in a number of these regions, though there was evidence that the strength of these effects differed both by region and by hemisphere. With the present paradigm, we were therefore unable to produce truly dissociated effects of object vs. position encoding within the MTL. However, the results are still consistent with regional differences in this content effect. Further support for this view comes from our second post-hoc analysis, which focused on the effect of content during preparation and rehearsal stages. Here, we found that object preparation showed a higher activation than position preparation in the left entorhinal cortex and right hippocampus. During rehearsal, several MTL regions showed higher activation during object rehearsal than position rehearsal, including bilateral temporopolar cortex, right entorhinal cortex, right parahippocampal cortex, and right hippocampus. In contrast, the left entorhinal cortex was significantly more active during position rehearsal than object rehearsal.

4.3 Discussion

These findings strengthen the view that there is a functional dissociation within the MTL region, in particular between the perirhinal and parahippocampal cortex. The expected perirhinal involvement in object encoding, compared to position encoding, supports studies from the animal literature [86,88], human lesion data [141] as well as previous neuroimaging data [133135142]. The present analysis extended these studies not only providing a more robust anatomical determination of MTL activation, but also by the direct comparison of perirhinal and parahippocampal cortical involvement in object and position encoding. Through this, the present results are strongly supportive of a functional division

within the parahippocampal region, where anterior regions (perirhinal cortex) are more involved in object processing compared to spatial processing.

The lack of any significant involvement during position encoding in the parahippocampal cortex is somewhat surprising, given that previous studies [133135] have found significant involvement in this region during spatial tasks. However, two factors may explain these results. First, it is possible that the parahippocampal cortex is involved in the processing of complex spatial stimuli, such as images of scenery and contextual factors. In the present study, the use of a relatively simple spatial task the encoding of grid positions may be too simple to engage the parahippocampal cortex. Consequently, using more complex and demanding spatial stimuli may be a better way to obtain a full double dissociation within the MTL region for object and spatial processing.

Second, it has been suggested that the parahippocampal cortex should be subdivided into at least two regions; an anterior part that is preferentially involved in object processing, and a posterior part preferentially involved in spatial processing [143]. This view is partly supported by previous studies, and it is likely that the functional borders within the parahippocampal region do not follow tightly the anatomical borders between the perirhinal cortex and parahippocampal cortex. This implies that the use of the parahippocampal cortex as one region may lead to a reduced (or even erroneous) assessment of parahippocampal function.

In addition to these findings, additional content effects were found in other regions during encoding, preparation and rehearsal stages. The majority of these regions showed a preferential involvement during object processing, compared to position processing. However, it should be noted that the content effect showed different strengths during different processing steps.

Taken together, these results both (1) support and strengthen previous findings of involvement of the perirhinal cortex in object processing, and (2) provide unexpected involvement of adjacent MTL regions in intentional memory processes. Together, these unprecedented results may be an effect of a complex interplay of optimized image acquisition and analysis. The findings support the idea that visual processing of objects and positions is different in regions such as the left perirhinal and left parahippocampal cortex. It is further suggested that processing stage is a modulating factor for this content effect in different regions. Finally, the results may suggest that the involvement of posterior MTL regions in visuospatial processing requires complex visual stimuli (e.g., complex scenes) rather than the use of spatial location of a stimulus. Clearly, these results warrant further studies of content effects in the MTL region.

5.0 PAPER 2 – The effects of age on medial temporal lobe activation in intentional encoding of items and positions

Studies of the effect of age on brain activation have generally uncovered two phenomena. First, as stated by the HAROLD model, activation that is lateralized to one hemisphere in young subjects tends to become bilateral in old age. This has typically been interpreted as a compensatory mechanism. Second, other studies have shown that age can lead to regional reductions in activation, which has been interpreted as a general waning of neural specificity. Here, regions showing specific responses to specific stimuli in young adults show age-related reduction in such activation specificity. In particular, an age-related reduction in hemispheric asymmetry is most often found in the prefrontal cortex, while studies have also suggested similar mechanisms in other brain regions such as the parietal cortex, motor cortex and even in the MTL (hippocampus). Age-related reductions in neural specificity has been documented particularly in the temporal lobe, and it has been suggested that reduced asymmetry in the prefrontal cortex is a compensatory response to reduced neural efficiency in the temporal lobe. One may thus suggest that within the MTL, reduced neural specificity is the primary age-related change in neural activation.

5.1 Motivation for Paper 2

It is still unknown whether age is generally related to increased or decreased activations in the MTL region, or both. The main aim of this study was to elucidate the effect of age on MTL activation. In particular, since our previous study had documented that the left perirhinal cortex was more activated during object encoding than position encoding, our main hypothesis was that (according to the reduced neural specificity assumption) age would lead to reduced activation of the left perirhinal cortex during object encoding. The paradigm allowed further assessment of adjacent MTL regions. Although no prior hypotheses were formulated for these regions, it was generally assumed that regions displaying a neural specificity for content in young subjects would show reduced specificity with age. We focused this analysis on the encoding stage. Post-hoc analysis of the preparation and rehearsal stages were performed, but were not reported in this study.

5.2 Methods & results

As in the previous study, we used fMRI and a region of interest (ROI) approach to study the effects of age on neural specificity for content (object and position encoding) in the MTL region. Parameters for scanning were the same as in the first study. The study included subjects from the entire age range between 18 and 81 years, thus allowing an analysis of age as a continuous variable. In addition, we used MTL perfusion as a covariate in the GLM analysis.

Behavioural analysis showed that increasing age was related to a reduction in performance on both the object and position task, while the difference in performance between the two tasks did not change. Conversely, age had no effect on response time.

Our fMRI ROI analysis demonstrated an age-related increase in left perirhinal cortex activation during object encoding compared to position encoding. Age did not affect the relationship between the ratio of left perirhinal and parahippocampal cortex activation, suggesting that the effects observed in the left perirhinal cortex are confined to this structure. Post-hoc analysis of adjacent MTL regions demonstrated similar trends in the right temporopolar cortex and, at a lower statistical threshold (p=.06), the right parahippocampal cortex and left hippocampus.

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Finally, we found no significant relationship between these age-related changes in regional activation and performance.

5.3 Discussion

Contrary to our expectations, we found no age-related decline in the left perirhinal cortex activation. Our results suggested quite the opposite effect, i.e., that age leads to increased left perirhinal activation during object encoding compared to position encoding. Furthermore, since age did not affect the relationship between activations in the left perirhinal and left parahippocampal cortex, the changes observed do not suggest any change in the relationship between activations in these structures. Furthermore, similar results in the right temporopolar cortex, right parahippocampal cortex and left hippocampus suggest that age generally leads to increased activations in this region during object encoding.

As with other studies showing age-related increased activations, these results may be interpreted as a sign of compensatory mechanisms. That is, activation increases may represent compensatory responses to age-related reduced efficiency, and that this compensation occurs in order to meet the present task demands. However, two aspects suggest that this is not a viable explanation. First, age was generally associated with a reduced performance, and there was a trend for object memory to be more affected than position memory. This suggests that age-related changes are generally associated with reduced performance. Second, we found no relationship between regional changes in activation and performance, thus suggesting that the activation increases were not related to specific performance increase or preservation.

An alternative explanation is that increasing age leads to reduced regional performance, but this initially fits badly with the idea of regional activation *increase*. However, an alternative explanation may be that the observed activation increases may occur due to prolonged periods of activation. That is, while regional activation in young subjects only require a certain time for successful processing, this time is prolonged in old adults. If this is the case, the current analysis, where activation is measured as averaged blocks, would fail to distinguish between increased and prolonged activations. Thus, more studies into this effect are needed.

A possible confound in the present analysis is that the two tasks may be different with regard to task difficulty. Although task performance was not significantly different between the two tasks in young subjects, there was a tendency for the object memory task to become more difficult than the position memory task with increasing age. This suggests that at least some of the observed age-related differences in regional activation may be explained by changes in task demands.

Taken together, the present study provides unexpected results that warrant further investigation. In particular, the present study applied several factors in the assessment of age-related changes in regional brain activation that included optimized image acquisition for the MTL region, the inclusion of CBF as a covariate, and the use of a continuous age cohort from ages 18 to 81, thus modelling the entire age range. In addition, based on the results reported here, three improvements are suggested. First, there is a need to combine MTL-optimized assessment and whole-brain assessment, as this would have the potential to relate the changes observed in this study to changes occurring in, for example, the prefrontal cortex. Second, analysing regional activation other than averaged time blocks would hopefully shed light on the question of whether age leads to prolonged or increased activations per se. Finally, as discussed in the original paper (see appendix), an improved assessment of regional CBF would make it possible to correct the BOLD effects more directly, thus providing an even more robust estimate of the effects of age on regional CBF and hence upon on the BOLD signal.

6.0 General discussion

The aim of the present thesis has been to explore the effects of ageing on the function of MTL regions. Ageing is known to lead to a range of different changes in brain anatomy, activation and neurophysiology. Several studies have demonstrated age-related changes in brain activation in a range of brain regions and with the use of different approaches. Together, these approaches have documented both age-related increases and decreases in regional activation. Often, these results have been interpreted as either compensatory responses or reduced neural functions. That is, activation increases have been suggested as a sign of brain changes that enable a subject to maintain a certain level of performance. Conversely, reductions in regional activations have been interpreted as reduced neural efficiency, or even a dedifferentiation, suggesting that the specificity of neural responses to specific contents show an age-related waning. While these two interpretations have been suggested to explain the same phenomena, it is more likely that these processes represent processes at different stages or even different regions of the brain. For example, one scenario is that age-related reductions (or specificity) in brain activation in one region may lead to compensation in another region (typically the prefrontal cortex).

The present project set out from this diversity of findings and models. The MTL region was chosen as a model based on specific considerations. First, the MTL consists of regions that, despite their tightly interconnected anatomy, are involved in very different perceptual and cognitive functions, and yet make different contributions to episodic memory. Second, recent developments in the anatomical delineation of this region, the transfer of this knowledge to MR protocols, and the development of optimized image acquisition and analysis for this region, has allowed a more detailed analysis of regional activation and changes associated with ageing. Importantly, as recent studies had provided conflicting findings, using standard approaches for image acquisition and analysis for this region, it was expected that the methods applied here would provide a more robust assessment of MTL activation, and the effect of age on these processes.

6.1 The role of MTL regions in object encoding

In Paper 1, the aim was to test specifically whether the left perirhinal cortex was more involved in object encoding than position encoding. Conversely, we wanted to test whether the left parahippocampal cortex would show an opposite relationship, i.e., be more involved in spatial encoding than object encoding. Our findings supported our hypothesis about the role of the left perirhinal cortex in object encoding, lending further support to recent studies from both the human and non-human primate literature [144-146] showing that this structure is specifically involved in object perception and object memory. In contrast, our hypothesis of left parahippocampal cortex preferential involvement in position encoding was not supported, something that is inconsistent with other studies that have found such effects [91,92,135,143]. In addition, post-hoc analysis of both the encoding stage and other related stages demonstrated regional effects of content, and a modulation of processing stage. This suggests that task demands may influence MTL functioning, and that stimuli are not processed solely on the basis of their properties, but that this process may be modulated by attentional set-making, a function most likely involving a fronto-parietal network.

6.2 The effect of age on MTL function

With the establishment of regional differences in the encoding of different properties of stimuli, our next analysis set out to study the effect of ageing on this activation. Our results showing an age-related increase in the left perirhinal cortex during object encoding was unexpected, given that we expected reduced activation, which would suggest a reduced differentiation between object and position encoding activation. This may be a sign of a compensatory mechanism, but two facts argue

against this interpretation. First, there was no association between regional activation levels and performance. Rather, and contrary to this, the age-related activation increase was generally related to performance decrease. This makes the results harder to interpret both in terms of the dedifferentiation and compensation models.

An alternative model may be that there is indeed a reduced neural efficiency in the affected MTL regions. However, rather than leading to a reduction or waning of brain activation, it may be suggested that the left perirhinal cortex as a functional processing node attempts to solve its specific task in the processing and encoding of objects. In one sense, this would lead to a prolonged period of activation, or iterations, which would be measured as an increased (mean) signal in the BOLD fMRI analysis. However, before any firm conclusion can be made on this issue, studies focusing on single events, and analysing the signal over time, are needed.

6.3 Conclusion

Taken together, the two studies reported here demonstrate that specific MTL regions are differentially involved in episodic memory formation, and the effects of age on this process. The studies have led to significant improvements in all steps of the experimental procedures, including image acquisition, image preprocessing and analysis, and statistical analysis. Even more so, the studies may contribute to two specific discussions in cognitive neuroscience. First, they support the idea that MTL regions are functionally segregated, a view that stands in opposition to previous ideas of the MTL as a functionally homogeneous region. Second, they demonstrate that age has specific and unexpected effects on activation in the MTL region, which may contribute to both improved models in the cognitive neuroscience of ageing. Furthermore, these findings raises at least as many questions as it tries to solve. As a consequence, as is so often seen in science, these studies can only be tested against further studies that may inform, validate and improve the results and methods developed here.

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Appendix 1

Regional activation of the human medial temporal lobe during intentional encoding of objects and positions

Regional activation of the human medial temporal lobe during intentional encoding of objects and positions

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Abstract

The medial temporal lobe (MTL) is made up of several regions thought to be involved in memory encoding. However, the degree of functional specialization among these regions remains unclear. Some evidence exists for specialization related to memory content (e.g., object information vs. position information), but results from previous studies have been inconsistent. In this study, functional magnetic resonance imaging (fMRI) optimized for the MTL region was used to examine brain activation in 27 normal volunteers during intentional encoding of objects or positions. Results show that object encoding evoked stronger activation than position encoding in left perirhinal cortex, and the object/position contrast effects were significantly larger in left perirhinal than in left parahippocampal cortices. However, in left parahippocampal cortex we did not observe significantly stronger activation for positions than objects. Exploratory analyses showed that the object encoding task evoked stronger activation across a number of MTL regions, though there was evidence for regional differences in activation strength. Exploratory analysis of preparation and rehearsal stages showed additional MTL activation that was modulated by processing stage. The results provide additional evidence for functional specialization within the MTL, but were less clear regarding the specific nature of content specificity in these regions.

Word count: 199

Keywords: episodic memory, functional MRI, Region of Interest analysis, parahippocampal cortex, perirhinal cortex

The medial temporal lobe (MTL) region, which consists of extensively and reciprocally connected structures such as the temporopolar cortex, perirhinal cortex, entorhinal cortex, amygdala, hippocampus, and the posterior parahippocampal cortex, is thought to be central in declarative memory formation, through its widespread functional connections throughout the neocortex. Some theories have stressed the importance of the MTL in the formation of long-term declarative memory and have suggested that lesions in any part of the MTL produce memory deficits (Squire & Zola-Morgan 1991; Squire et al. 2004). Others have emphasized that different MTL structures play separate roles in declarative memory as well as in other cognitive functions such as higher-order visual perception (Murray et al. 2005; Graham & Gaffan 2005), working memory (Axmacher et al 2007, Piccioni et al. 2007, Olson et al. 2006a, b), and short-term memory (Piekema et al. 2006). It has been suggested that, during encoding, visual object features are processed in the perirhinal and lateral entorhinal cortex, while spatial context is processed in the adjacent medial entorhinal cortex and parahippocampal cortex (Eichenbaum, Yonelinas & Ranganath, 2007). Lesions to the perirhinal cortex alone, or together with the entorhinal cortex, can cause significant deficits in the ability of humans (Barense et al. 2005), monkeys (Baxter and Murray 2001) and rats (Prusky et al. 2004) to perform visual categorization and memory tasks, a deficit not found when lesions are restricted to the hippocampus. There is evidence that the memory functions of MTL structures are dissociated according to stimulus content, where the perirhinal cortex contributes more heavily to processing of visual object information (Bussey et al. 2006), while the hippocampus and parahippocampal cortex contribute more in the processing of spatial information (Epstein et al. 1999; Burgess et al. 2002).

Neuroimaging studies in humans have suggested a similar regional specialization within the MTL region related to memory content, although results have been inconsistent. In

one study, the perirhinal cortex was more strongly activated when processing novel visual items than novel spatial arrangements of familiar items, while the parahippocampal cortex showed the opposite relationship (Pihlajamäki et al. 2005). In another study, however, the perirhinal cortex was activated during both spatial and object memory encoding, while the anterior parahippocampal cortex was activated only during spatial encoding (Buffalo et al. 2006). While some of these differences may be attributed to differences in paradigm design and task demands, differences in scanning parameters and statistical analysis may also contribute to the inconsistency of the results.

*** PLEASE INSERT FIGURE 1 ABOUT HERE

The present study aimed to elucidate the roles of the perirhinal cortex and parahippocampal cortex in encoding of objects and positions. It has been suggested that encoding is predominantly associated with left hemispheric activity, while retrieval leads to an activation of right hemisphere regions including the prefrontal cortex and MTL region (Daselaar et al. 2003, Alkire et al. 1998, Montaldi et al. 1998, Desgranges, Baron & Eustache 1998). It was therefore assumed that content effects during encoding would be strongest in left hemisphere. In addition, we were interested in studying the role in encoding of objects and positions in adjacent MTL regions, including the temporopolar cortex, entorhinal cortex, hippocampus and amygdala (see Figure 1), as well as right perirhinal and parahippocampal cortex. To this end fMRI was performed during an object and spatial encoding task, using an imaging sequence optimized to measure blood-oxygen-level-dependent (BOLD) signal changes in the MTL region. A region of interest (ROI) approach was used to analyze the data, so that conventional anatomical criteria for defining MTL subregions could be applied, and to

avoid registration errors that might result from the application of spatial normalization methods.

We asked subjects to either encode (and rehearse and retrieve) object identities or object positions. Our hypotheses were that a) the left perirhinal cortex would be more activated by object encoding than position encoding; and that b) the left parahippocampal cortex would be more active during position encoding than object encoding. In addition to the hypothesis-specific tests of the effects in perirhinal and parahippocampal cortex, further post-hoc testing of the effect of content during encoding was performed on other MTL regions. In addition, our paradigm allowed us to study the effects of content during preparation and rehearsal stages, for which we had no prior hypotheses (see Figure 2).

*** PLEASE INSERT FIGURE 2 ABOUT HERE

Materials and Methods

Subjects

Twenty-seven subjects (12 female; mean age=25.85, std.=6.6, range 18-39, 20 right-handed, 6 left-handed and 1 ambidextrous, with normal or corrected to normal vision) were recruited through on-line advertisements (www.forsoegsperson.dk) from the region of Copenhagen, Denmark. All subjects first signed an informed consent, and were paid for their participation, then filled out a self-report questionnaire on medical history including neurological and psychiatric disorders. Subjects were also tested for estimated intelligence levels with DART (Danish Adult Reading Test) and WAIS vocabulary. For both tests a z-score was calculated based on Danish normative material.

Structural imaging protocol

Structural images, used for the drawing of ROIs, were acquired using a Siemens Magnetom Trio 3T MR scanner with an eight-channel head coil (Invivo, FL, USA) and included (1) a 3D whole brain MPRAGE (magnetization prepared rapid acquisition gradient echo) scan with a voxel dimension of 1x1x1 mm^3, field of view (FOV) 256 mm, matrix 256x256, repetition time (TR) / echo time (TE) / inversion time (TI) = 1540/3.93/800 ms, and a flip-angle of 9°; and (2) a 3D whole-head T2-weighted sequence with a voxel dimension of 1.1x1.1x1.1 mm^3, FOV 282 mm, matrix 256x256, TR/TE = 3000/354 ms, and a flip-angle of 28.5°.

Structural image post-processing

The N3 program (Sled, Zijdenbos, & Evans, 1998) was used to correct images for non-uniformity artifacts due to radio-frequency field inhomogeneities. Tissue classification was done using SPM2 (Wellcome Dept. of Imaging Neuroscience, London) on the N3 bias-corrected images (the SPM2 bias correction was turned off). Careful editing of the gray matter tissue images excluded voxels that were outside of the brain but adjacent to the MTL. Six ROIs (see Figure 1) in each hemisphere were drawn on the native space structural image using MNI Display (Montreal Neurological Institute, Montreal, Canada). An ROI drawing protocol for the temporopolar cortex, perirhinal cortex, entorhinal cortex, and parahippocampal cortex was adapted from the Insausti (Insausti et al. 1998) and Pruessner (Pruessner et al. 2002) protocols; neuroanatomic guidelines for hippocampus and amygdala were adapted from those of Watson et al. (1992), and the atlas of Duvernoy (1991) was consulted. The border between the perirhinal cortex and entorhinal cortex was set, in the coronal plane, at the top of the parahippocampal gyrus, making the perirhinal ROI cover the entire inferior bank of the collateral sulcus until the posterior border to the parahippocampal cortex. This practice differs from that of others (Insausti et al. 1998) who have applied a more

adaptive drawing protocol for the perirhinal-entorhinal border, based on the depth of the collateral sulcus. This deviation from previous criteria was made to reduce variability associated with subjective placement of the boundary within the collateral sulcus.

A test of ROI drawing reliability was performed on a different data set consisting of 13 subjects of healthy young subjects (9 female, age range 19-31). Here, all subject data including file headers were anonymized and an extra set for each subject was right-left flipped. Thus, ROIs in a total of 26 structural scans were drawn (by TZR) for intrarater comparison. An intraclass correlation test (Rousson, Gasser & Seifert 2002) on each ROI volume showed a mean r=.884 for all regions (range .615 to .916). The lowest values were for the bilateral temporopolar cortex, all other correlations exceeded .8.

Functional imaging protocol

At higher field strengths such as 3T, EPI susceptibility artifacts, which especially occur in areas close to the nasal cavities, ear cavities, and perforated bones (Bellgowan et al. 2006), are especially pronounced in the MTL region. Recent developments in image acquisition have reduced these artifacts (Cho & Ro 1992; Neufeld et al. 2005, Bellgowan et al. 2006), although in many neuroimaging studies of the MTL these methods have not been applied. For the functional scan we used a BOLD fMRI sequence optimized for the MTL structures based on the method suggested by Deichmann et al. (2003), adjusted to the MTL region.

We used EPI (Echo-Planar Imaging) with an 8-channel head coil, TR/TE = 2000/30 ms, 64 x 64 matrix. Initially, consistent placement and orientation of the participants' heads in the field of view was ensured by orienting subjects' heads to predefined orientation marks on the scanner head coil. The block of 33 slices was then oriented 20° oblique to the transverse plane so that the slices were roughly parallel to the long axis of the temporal lobe. The voxel

size was 3 x 3 x 2 mm with no interslice space. The 2 mm slice thickness was applied in order to further reduce susceptibility artifacts. The total scanning time was 741s. Pulse and respiration were recorded, and sampled at 50 Hz, using an MR-compatible pulse oximeter and a respiration belt.

Activation paradigm

The task involved 19 blocks. Each block included a 1s instruction cue; a 3s preparation epoch; 6 stimuli presented serially 2s each for encoding; a 6s rehearsal epoch; and 6 stimuli presented serially 2.5s each for old/new recognition judgments (See Figure 2). Each encoding stimulus was a unique, colored Snodgrass and Vanderwart-like object (Rossion and Pourtois 2004) presented in a trial-unique location among 9 locations in a 3x3 spatial grid. During preparation and rehearsal, an empty grid was displayed. Just prior to the instruction cue and recognition phase a white cross appeared at the middle of the grid for 1 second, signaling the onset of the encoding or recognition phase. In the object memory blocks, recognition stimuli were 3 old and 3 novel stimuli in a fixed pseudorandom order. In the position memory blocks, recognition stimuli were grids in which an orange square appeared in 3 old and 3 novel positions in the grids, again in a pseudorandom order. The instruction cue indicated whether the subjects were to encode (and rehearse and recognize) the objects or the grid locations in the subsequent series of stimuli. Subjects were explicitly instructed to ignore positions if their task was to focus on objects, and, conversely, to ignore objects during the position task. During the rehearsal epoch, subjects were asked to try to keep the object identities or grid locations in mind. Ten object blocks and nine position blocks were presented in a fixed pseudorandom order. All subjects were trained outside the scanner for approximately 10 minutes using a different set of objects.

All stimuli were presented using IFIS and E-Prime software (http://www.pstnet.com/products/e-prime/) using a Canon LV 7545 LCD projector equipped with a Buhl optics lens with a refresh rate of 60 Hz (full brightness = 3700 ANSI lumens, setting = 10; Contrast = 800:1, setting = 32). The stimuli were back projected onto a screen and viewed via mirrors placed on the head coil above the subject's head. The full screen size corresponded to 24° x 18° visual angle and was presented in 800 x 600 pixel resolution.

BOLD analysis

The EPI data analysis was performed in native space using SPM2. Realignment with no smoothing was used. Each individual's EPI image series was coregistered to his/her AC-PC aligned structural image. For each content condition (object and position) preparation, encoding, rehearsal, and recognition were entered as separate regressors in the design matrix, leading to a total of 8 regressors of interest. Since the recognition stage used different visual stimuli, we did not analyze the effect of content during this stage, but included it as a regressor in the analysis. The regressors were convolved with a canonical haemodynamic response function. Nuisance regressors for respiration, heartbeat and motion were included in the analysis (Lund et al. 2006). For each ROI the average value for the contrasts of interest were fed into a second level analysis, where a one-sample t-test was made.

Our analysis first concentrated on the hypothesized effects in the left perirhinal and left parahippocampal cortex where we applied a one-tailed test, and then on the direct comparison of the left perirhinal cortex and left parahippocampal cortex using a paired-samples t-test. We then analyzed the effects of content in regions for which we had no prior hypotheses (i.e., temporopolar cortex, entorhinal cortex, hippocampus, and amygdala). Furthermore, we tested the effect of content during preparation and rehearsal stages. For all

post-hoc tests, we applied a correction for multiple comparisons (Bonferroni correction, n=12).

We also computed B_0 values in each region and examined the relationship of these values to contrast values. We did not find evidence for dependence between B_0 and BOLD contrast values. Consistent with this, residualizing the ROI contrast values for local B_0 values did not change these results.

Results

Based on a brief assessment (see Methods), subjects z-scores for DART (mean = .85, std. = 1.17) and WAIS vocabulary (mean = .40, std. = 1.28) were all within the normal range. Behavioral analysis for performance during the fMRI session was performed in SPSS 15.0; accuracy scores and reaction times during the recognition condition were computed for each subject applying a 2-tailed paired samples t-test for studying the effects of content on recognition accuracy and reaction times. Comparing reaction times and accuracy for object and position tasks, we found lower mean reaction time for object recognition (*objects*: mean/std.=1000/102 ms, *positions*: 1049/84; p=.050) but no difference in mean accuracy levels (*objects*: mean/std.=5.18/.40, *positions*: 5.30/.33 of six possible correct responses; p=. 193).

During encoding conditions, the left perirhinal cortex was significantly more active during object encoding than position encoding. The left parahippocampal cortex did not show any such effect of content (see Table 1). A test comparing the left perirhinal and left parahippocampal cortex showed a significant difference (t=2.21, p=.018, one-tailed), as illustrated in Figure 3.

*** PLEASE INSERT TABLE 1 ABOUT HERE

*** PLEASE INSERT FIGURE 3 ABOUT HERE

The post-hoc analyses then focused on the effect of content during encoding, in regions for which we did not have any prior hypotheses. Here, we found that the bilateral temporopolar cortex, bilateral entorhinal cortex, left hippocampus, and right amygdala showed significant effects of content during encoding (see Table 1 and Figure 3).

Our analysis of the preparation stage showed a significant effect of content in the left entorhinal cortex and right hippocampus, both structures showing increased activation during object preparation compared to position preparation. Finally, the analysis of the rehearsal stage produced significant content effects in the bilateral temporopolar cortex, bilateral entorhinal cortex, right parahippocampal cortex and right hippocampus. Notably, the left entorhinal cortex was significantly more activated during position rehearsal than object rehearsal, while all other significant regions were more active during the object task (see Table 2).

*** PLEASE INSERT TABLE 2 ABOUT HERE

Discussion

By studying the effects of intentional encoding of objects and positions, we found task induced changes in the MTL region. Our first analysis focused on the hypothesized effects of content during encoding in the left perirhinal cortex and left parahippocampal cortex. Here, we found that the left perirhinal cortex showed significantly higher activation during object

encoding than position encoding. This supports our hypothesis that the left perirhinal cortex is involved in object-specific encoding; and the finding corroborates similar results from both the human and non-human primate literature (Aggleton & Brown 2005, Wan et al. 1999, Bussey et al. 2002, 2005). Converging evidence from lesion studies of monkeys (Baxter & Murray 2001), rats (Prusky et al. 2004) and humans (Barense et al. 2005), as well as from neuroimaging studies (Pihlajamäki et al. 2005) have implicated the perirhinal cortex in object processing. Furthermore, studies using immediate early gene imaging in rats indicate that the perirhinal cortex, but not the hippocampus, is involved in processing novel, as opposed to familiar, visual objects. (Aggleton and Brown 2005, Wan et al. 1999, Zhu et al. 1995, 1996). Further support for this functional dissociation comes from anatomical studies showing that the perirhinal cortex receives the majority of its afferents from what has been termed the "ventral visual stream", a system involved in the identification and recognition of visual objects. In contrast, the parahippocampal cortex receives the majority of its input from areas that make up the "dorsal visual stream" that is thought to process location and spatial context of objects (Burwell & Amaral 1998; Witter et al. 1989).

Contrary to our expectations we did not find a significant effect of content in the left parahippocampal cortex; although a direct comparison showed that the left parahippocampal cortex differed significantly from the perirhinal cortex in its relative responses to the two conditions. The lack of a significant spatial processing effect in the parahippocampal cortex in this study is inconsistent with results of other studies that have documented such effects (Pihlajamäki et al. 2005, Epstein et al. 1999, Burgess et al. 2002). For example, Pihlajamäki et al. (2005) found increased activation in the perirhinal cortex during novel item processing, and increased activation in the parahippocampal cortex during processing of novel spatial arrangements. Buffalo et al. (2006) found that the perirhinal cortex was activated both during

item and spatial processing and there was only a trend toward greater object processing activation there; while the anterior parahippocampal cortex showed increased activation for spatial processing only.

On the other hand, demands for position processing have failed to activate parahippocampal cortex in other studies as well. Using a paradigm very similar to the present study, Mitchell et al. (2000) found that while object processing evoked more activity than position processing in limited parts of the MTL, no MTL regions exhibited greater activity during position processing.

There are several factors that may have contributed to discrepancies in results of these studies of object and position encoding. First, the object and position encoding tasks may have differed with respect to how much they taxed the encoding apparatus. In several studies, including the present study, the objects presented represented members of a virtually limitless set of possibilities, while the positions to be encoded represented a small set of possibilities; for example, in the present study only nine positions were possible. Thus, in one sense, objects were "low frequency" stimuli, while positions were "high frequency" stimuli. Low frequency stimuli are known to be more difficult to process at study phase, although they may be more easily recognized at the test phase (Glanzer & Adams 1985, Ostergaard 1998, Diana & Reder 2006). This may have resulted in the presence of object but not position effects in the present study and in the study by Mitchell et al. (2000). Interestingly, in the Buffalo et al. (2006) study, in which position effects in MTL structures were more prominent than object effects, the objects were drawn from a more restricted set of similar objects, and thus may have, from an encoding perspective, been more similar to higher frequency items.

Alternatively, the lack of position effects may relate to the low spatial complexity of the position stimuli. Recent studies suggest a role for the parahippocampal cortex region in

more complex spatial processing, such as scenes (Buffalo et al. 2006, Henderson, Larson & Zhu 2007, Epstein, Parker & Feiler 2007) and other contextual stimuli (Aminoff, Gronau & Bar 2007). Thus, although the current spatial task may be regarded as depending on a spatial processing system per se, it may not have involved sufficiently complex visual stimuli to engage the posterior MTL region. In addition, it has been suggested that the parahippocampal cortex is functionally divided into an anterior and posterior region, where the anterior parts are involved in object specific processing while the posterior parts are involved in position and place processing (Pihlajamäki et al. 2005). Consequently, the analysis of this region as one structure may have lead to superimposition of object and position specific activations.

In a post-hoc exploratory analysis of the encoding stage we studied the effect of content in other MTL regions, including the temporopolar cortex, entorhinal cortex, hippocampus, and amygdala. The object encoding task appeared to evoke stronger activations than positions in a number of these regions, though there was evidence that the strength of these effects differed both by region and by hemisphere. Thus, although the present paradigm was unable to produce truly dissociated effects of object vs. position encoding, the results are still consistent with regional differences in this content effect.

The paradigm allowed exploratory analysis of the effects of content during preparation and rehearsal. During preparation, we found that the left entorhinal cortex and right hippocampus showed significantly higher activation during object preparation than position preparation, but no region showed an opposite pattern. During rehearsal, several MTL regions showed higher activation during object rehearsal than position rehearsal, including bilateral temporopolar cortex, right entorhinal cortex, right parahippocampal cortex, and right hippocampus. Notably, the left entorhinal cortex was significantly more active during position rehearsal than object rehearsal. Compared to the results during preparation and encoding, this

suggests that the left entorhinal cortex exhibits a content-by-stage effect, in that it was significantly more active in object than position processing for preparation and encoding, but more active in position than object processing during rehearsal. These results are in line with studies showing that the entorhinal cortex is an important integrative processing node of both spatial and object information to the hippocampus (Fernández & Tendolkar 2006, Witter et al. 1989). The involvement of the bilateral temporopolar cortex in object encoding and object rehearsal, and the right amygdala in object encoding, are, to our knowledge, not previously documented in the literature. However, the results are related to studies suggesting a role for the temporopolar cortex in visual recognition (Nakamura & Kubota 1995), semantic memory (Davies et al. 2004), and recognition awareness (Sewards & Sewards 2002); and for the amygdala in emotional learning (e.g., Leppanen & Nelson 2006; Rosen & Donley 2006), and novelty processing (Fried et al. 2002, Moses et al 2002).

ROI analysis

In the present study, an optimized MRI sequence was used to test whether specific regions of the MTL are differentially activated during mnemonic processing of objects and positions. One caveat in the functional neuroimaging of MTL structures is the use of whole-brain image acquisition that may lead to significant loss of signal in this region due to susceptibility artifacts, especially at high-field fMRI scanners at and above 3T (Bellgowan et al. 2006). A second potential problem is the regular use of spatial normalization and smoothing of functional images (Ashburner and Friston 1999). Studies of such methods have shown that MTL regions are poorly registered across individuals with standard methods (Salmond et al. 2002), and systematically different in subjects with memory impairments (Krishnan et al. 2006). Thus, spatial normalization procedures may spatially displace MTL activations when

moving brains from native space into a standard frame of reference. In this paper we have tried to accommodate these difficulties by 1) using an image acquisition sequence that is optimized for the MTL region, and 2) analyzing regional brain activation in each subject's non-normalized brain by comparing the activation levels in anatomically predefined structures.

A few caveats should be considered for the present study. First, the perirhinal cortex and entorhinal cortex were defined using the collateral sulcus in its entire depth. The perirhinal ROI might therefore have partly included the lateral most part of the entorhinal cortex. This part of the entorhinal cortex has been shown to receive inputs from the perirhinal cortex as part of the ventral stream projections (Eichenbaum, Yonelinas & Ranganath 2007; Guarnieri et al. 2006). Distinctions observed here between perirhinal cortex and entorhinal cortex may have been mediated to some extent by this imprecision in boundary delineation.

Second, in order to maximize the signal to noise ratio in the MTL region the BOLD signal was not recorded at the whole-brain level. It is likely that at least some of the effects for content and processing steps that we have found in the MTL apply to other areas of the brain as well, such as the dorsolateral prefrontal cortex. This area is known to be involved in different functions that relate to the present paradigm, including preparation (Rowe et al. 2000) and both object and spatial working memory (Deco, Rolls & Horwitz 2004). Furthermore, one could expect that the parietal cortex may be involved in the attentional modulation produced by object or position instructions (Aso et al. 2007). Consequently, a combination of local (MTL) and global brain activation neuroimaging is needed to explore the complex interactions between the MTL regions and the regions with which they are connected.

Finally, the present analysis included considerations of the effect of regional B_0 inhomogeneities, and their relationship to differences in BOLD contrasts. B_0 in the MTL region is known to be heterogeneous, especially along the anterior-posterior axis, though B_0 values are more bilaterally symmetrical. Thus regional differences in the size of activation effects, particularly along the anterior-posterior axis, can result from poor sensitivity in anterior regions with low B_0 Our analysis revealed no evidence of a dependence of the object-position contrast values on B_0 and when regional B_0 values were used as covariates, the results of the analyses were unchanged. Thus it seems unlikely that the observed pattern of content effects across the regions we examined is strongly influenced by B_0 inhomogeneity.

There may be other factors that make direct comparison of BOLD effects within MTL regions difficult. For example, it has been argued that systematic differences in the microvascular structure and especially the relative alignment of draining veins to B_0 may have an impact on the BOLD signal (Sheth et al. 2004, Roberts et al. 2007, Prinster et al. 1997, Hall et al. 2002). In the MTL, regions such as the hippocampus, amygdala and the parahippocampal region have significantly different microvasculature, and it may be possible that this has an impact on regional differences in the BOLD signal, although the difference within the parahippocampal region is expected to be less pronounced, thus allowing comparison between perirhinal and parahippocampal cortex. Other factors include differences in partial volume effects (due to different ROI sizes), differences in coil sensitivity, and differences in physiological noise (e.g., the proximity to large arteries). Many of these factors can be accounted for, at least partially, as we have done with the use of cardiac and respiratory recordings as nuisance regressors in the model. The collective effect of these factors on regional differences in BOLD signal, as well as methods to control for these factors, requires more attention.

Conclusion

The present study provides further evidence for a significant role of several MTL regions in the processing of object information; and, as predicted, there was evidence that objects were more strongly activating in the left perirhinal than in the left parahippocampal cortex. Unfortunately, perhaps because the encoding demands in the position condition were lower, the results are less conclusive regarding the relative roles of the MTL structures in encoding of spatial information. The post-hoc analyses of content effects in other regions,and during other processing stages, produced evidence for object activation in several MTL regions where this was not expected, including temporopolar cortex and amygdala, as well as at stages, such as preparation and rehearsal when it was not expected. In entorhinal cortex, the content effects appeared to reverse from an object effect in the encoding stage to a position effect in the rehearsal stage. These unexpected observations warrant further examination for confirmation in prospective studies.

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Table 1 - Statistical values content effects during encoding. Asterisk indicates significant vales after Bonferroni correction.

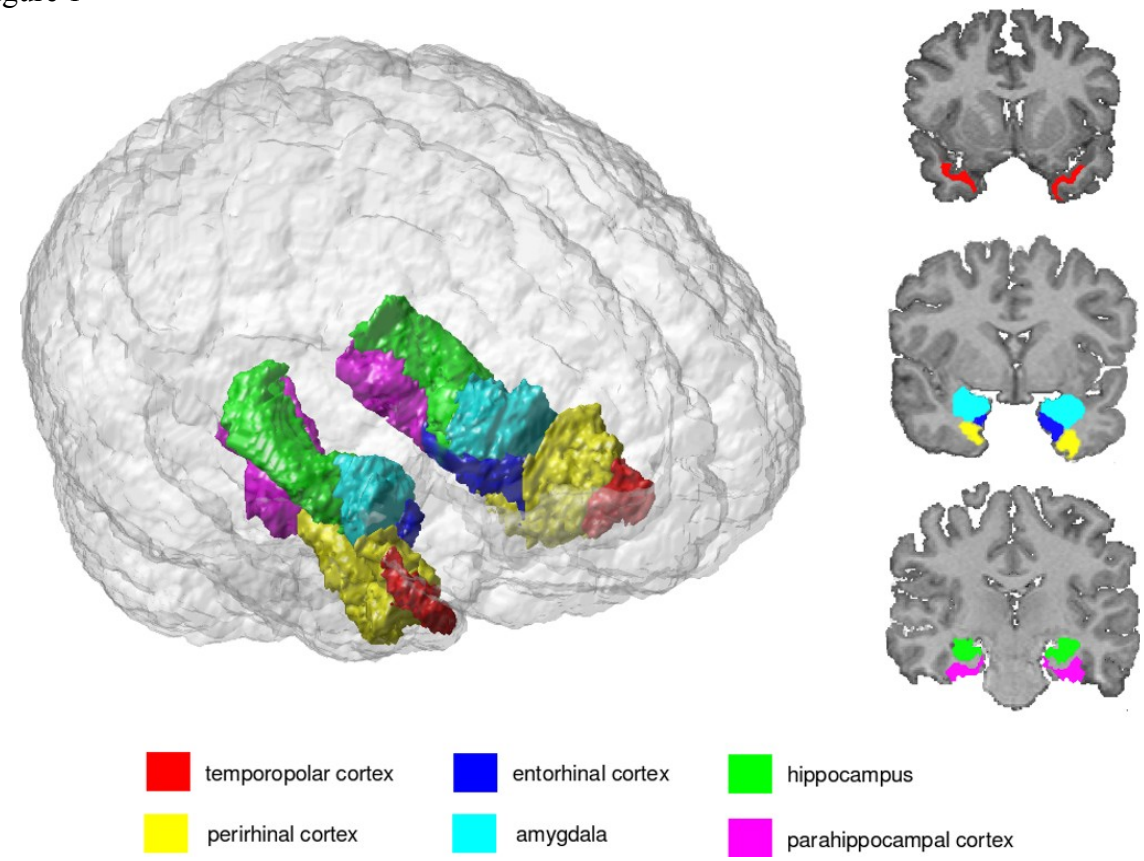
Region	**one-sample t-test**	
	t	**p**
temporopolar left	6.60	<.001*
temporopolar right	5.50	<.001*
entorhinal left	6.64	<.001*
entorhinal right	5.56	<.001*
perirhinal left	4.70	<.001*
perirhinal right	-.14	.892
parahippocampal left	1.47	.154
parahippocampal right	-.18	.862
hippocampus left	5.77	<.001*
hippocampus right	2.06	.05
amygdala left	2.27	.032
amygdala right	4.67	<.001*

Table 2 – Statistical values for post-hoc tests of preparation and rehearsal stages. Asterisk indicates significant values after Bonferroni correction.

Region	Preparation one-sample t-test		Rehearsal one-sample t-test	
	t	p	t	p
temporopolar left	1.87	.075	4.23	<.001*
temporopolar right	.65	.580	5.68	<.001*
entorhinal left	3.69	.001*	-3.50	.002*
entorhinal right	.92	.366	3.78	<.001*
perirhinal left	2.64	.015	2.46	.022
perirhinal right	.89	.381	-1.49	.149
parahippocampal left	-.63	.537	.53	.599
parahippocampal right	1.21	.241	3.42	.003*
hippocampus left	-1.48	.153	-.79	.439
hippocampus right	3.19	.004*	4.81	<.001*
amygdala left	-1.12	.275	-1.82	.082
amygdala right	-.88	.386	.82	.420

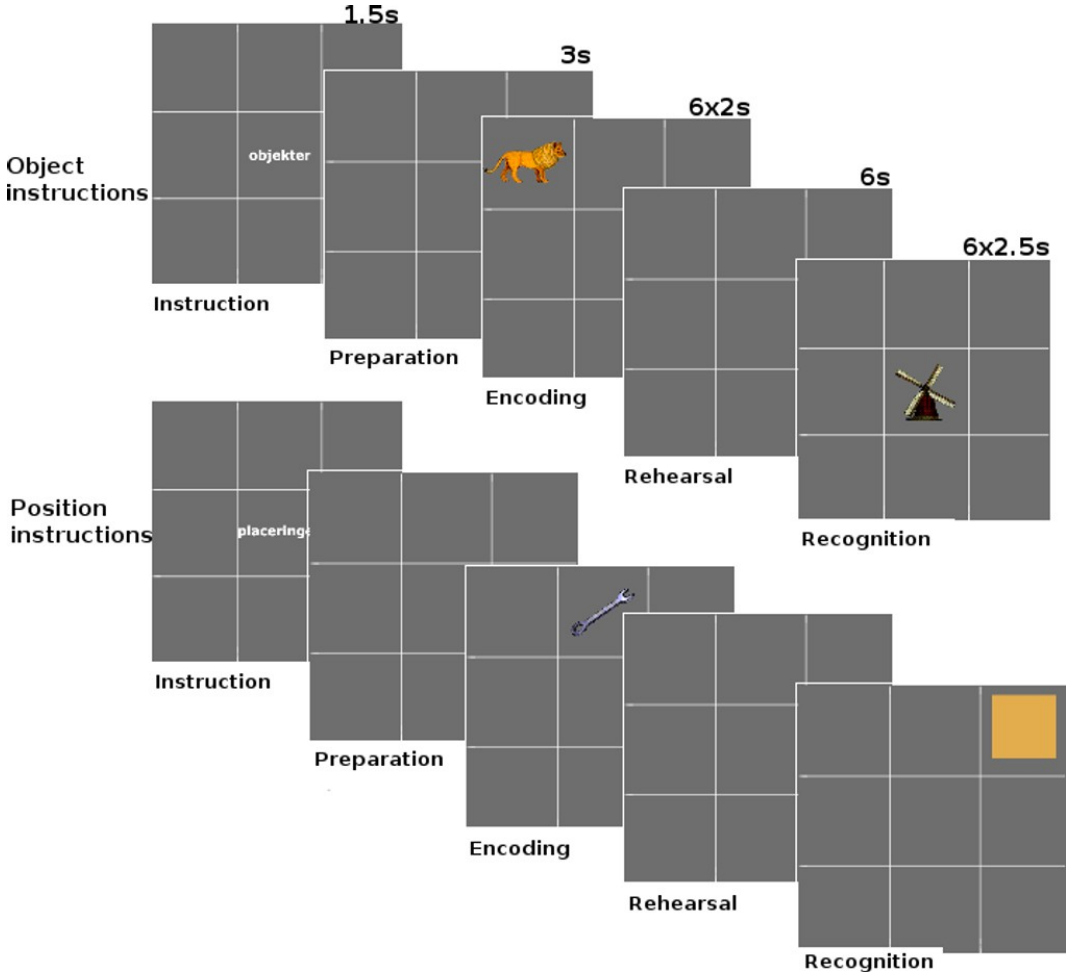
CAPTIONS

Figure 1



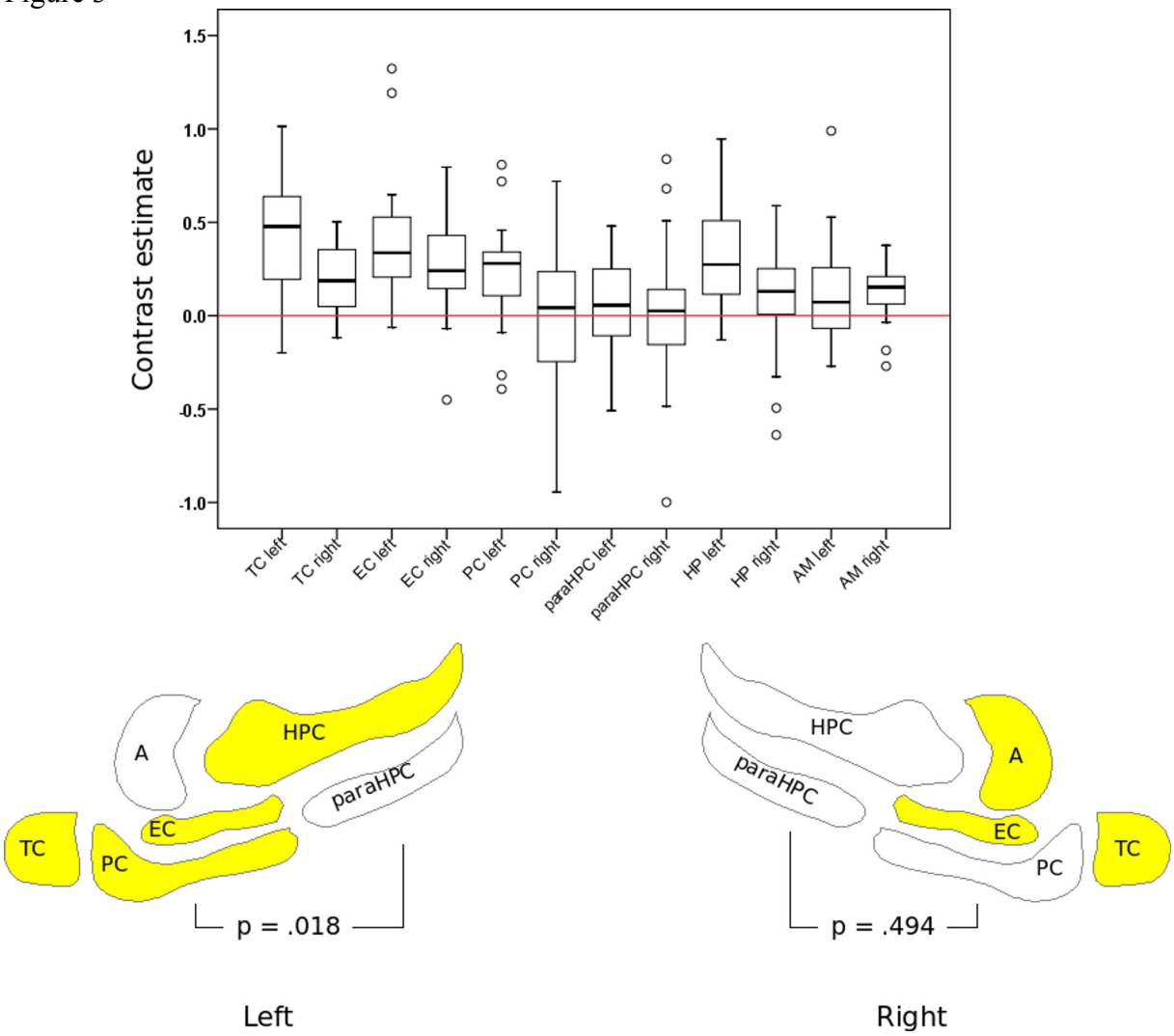
The medial temporal lobe regions of interest studied. Left: 3D reconstruction of ROIs drawn on one of the subjects in the study, and positioned within a transparent view of the native brain. Right: three coronal slices showing the original ROI drawing in the same subject. See the Experimental section for further details.

Figure 2



The memory paradigm shown according to the two instruction versions. Both runs consisted of an instruction cue; a preparation phase; an encoding phase with six trial-unique items and positions; a rehearsal phase; and a recognition phase with old-new judgments. Only the instruction cue and recognition phases were visually different between the conditions. Numbers above top image row indicate block duration in seconds. See the Experimental methods section for a more detailed explanation.

Figure 3



Regional contrast effects during encoding. Top: Boxplot showing the effect of content in all MTL regions of interest. Each plot shows the differential value of each contrast for each MTL region. Each box indicates upper, median and lower quartiles, whiskers indicate smallest and largest non-outlier observation, circles indicate outliers. Red line indicates null effect. Bottom: Color scaled model of regional differences (at $p<.001$) during encoding, where bottom lines and numbers indicate (one-tailed) p-values for the comparison between perirhinal and parahippocampal cortex in each hemisphere. Abbreviations are: TC = temporopolar cortex, EC = entorhinal cortex, PC = perirhinal cortex, paraHPC = parahippocampal cortex, HP = hippocampus, and AM = amygdala.

Appendix 2

The effects of age on medial temporal lobe activation during intentional encoding

The effects of age on medial temporal lobe activation during intentional encoding

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Abstract

Ageing leads to multiple changes in brain morphology, neurophysiology and cognitive functions. However, studies of the effect of age on brain activation have produced conflicting findings, especially in regions such as the medial temporal lobe (MTL). Some studies have found age-related activation decreases in specific regions, while others report regional increases in activation. Here, we studied the effect of age on regional MTL activation during intentional encoding of objects and positions, applied a region of interest analysis and corrected for individual differences in cerebral blood flow. In particular, we focused on the left perirhinal cortex, which has been shown to be more involved in object encoding than position encoding. We hypothesized that increasing age would be associated with decreased left perirhinal activation during object encoding. Contrary to this, we found an age-related increase in object encoding activation compared to position encoding. Post-hoc analysis of other MTL regions showed a similar effect in the right temporopolar cortex, and tentatively in the right parahippocampal cortex and left hippocampus. These findings are discussed in light of theories about the cognitive neuroscience of ageing.

Words: 184

Keywords: episodic memory; object memory; spatial memory; preparation; rehearsal; neural specificity; compensatory mechanism

1. Introduction

Episodic memory, the memory of unique personal experiences (Tulving 2002), is one of the cognitive functions that are most affected by ageing (Mitchell et al. 2000). A number of studies have demonstrated that the medial temporal lobe (MTL) region is crucial for normal episodic memory function (Yonelinas et al. 2007, Hayes et al. 2004). Furthermore, MTL subregions have been shown to make different contributions to object and spatial memory processes (Pihlajamäki et al. 2003, 2004, 2005), in the binding of object and spatial information (Mitchell et al. 2000), in encoding and retrieval (de Zubicaray et al. 2001, Tsukiura et al. 2005, Kirwan et al. 2004), and in familiarity and recognition judgements (Henson 2005, Henson et al. 2005, Daselaar et al. 2006, Gonsalves et al. 2005).

Studies of the effects of ageing on MTL function have provided mixed results. Some studies have found age-related activation decreases in the MTL region and adjacent ventral temporal regions during visual perception and encoding (Park et al. 2004), in working memory function (Vandenbroucke et al. 2004, Mencl et al. 2000), and in the binding of visuo-spatial information (Mitchell et al. 2000). These results have generally been interpreted as an age-related regional loss of function. Other studies have found age-related activation increases, such as increased bilateral activation in temporal, prefrontal and parietal regions (Grady et al. 2000, Cabeza 2002, Dolcos et al. 2002, Cabeza et al. 2004, Raz et al. 2004, Townsend et al. 2006, Ward et al. 2006). Furthermore, studies have found a relationship between an age-related bilateral activation and improved performance, suggesting that reduced hemispheric asymmetry in older adults may be a sign of compensatory mechanisms (Cabeza 2002).

Some factors may explain these diverging results. First, increasing age is known to lead to changes in cerebral blood flow (CBF) (Parkes et al. 2004, Biagi et al. 2007), which may in part explain changes in BOLD fMRI activations. Indeed, it has recently been demonstrated that age-

related alterations in CBF influence BOLD fMRI results (Restom et al. 2007), in particular that age-related increases in the BOLD signal are related to a reduction in CBF. The failure to include CBF as a covariate may therefore lead to erroneous estimates of age-related changes in BOLD fMRI. Second, the complex anatomy of the MTL region has been shown to be poorly registered to a brain template using standard spatial normalization procedures (Kirwan et al. 2006). Together, diverging results may be caused by poor correction of both functional and spatial aspects of neural activation.

The left perirhinal cortex has recently been shown to be more active during object encoding compared to position encoding (Ramsøy et al., unpublished data). If, as suggested, ageing leads to reduced activation in regions involved in particular functions, one would expect a similar process to occur in the MTL. In particular, we hypothesized that the left perirhinal cortex would show an age-related activation reduction during object encoding compared to position encoding. In order to place this age-related alteration in the left perirhinal cortex in context, the age-effects upon activation in adjacent MTL regions were also studied (see Figure 1).

To this end, MTL activation was assessed during intentional encoding, using a region of interest (ROI) fMRI approach and with an image acquisition optimized for the MTL region. As recommended by recent studies, age-related changes in CBF were included as a covariate in the analysis.

2. Materials and methods

2.1 Study population

Subjects were recruited through online advertisements (www.forsoegsperson.dk) from the region of Copenhagen, Denmark. All subjects filled out a self-report questionnaire on medical history. Subjects were rejected in the case of self-reported claustrophobia, a history of neurological or psychiatric disorders or a family history thereof, or hypertension. All subjects enrolled in the study signed an informed consent following the guidelines of the declaration of Helsinki, and were paid

for their participation. The study protocol was approved by the local ethics committee (KF 01 131/03). In all, 64 subjects underwent a comprehensive neuropsychological assessment (lasting approximately 1 hour), followed by a morphology scanning session (45 minutes). During a break subjects trained outside the scanner on three cognitive paradigms (the memory task reported here, a categorization task, and an emotion task), after which they performed the three tasks in the same order during functional MRI scans. The second scanning session, which also included perfusion imaging, lasted for approximately 1 hour. After visual inspection, three subjects were excluded from the analysis due to unexpected signs of neuropathology and were referred to further clinical assessment, and another subject was excluded from analysis due to a benign metal artefact. One subject aborted due to unexpected claustrophobia, three subjects were excluded from the analysis due to errors with the fMRI encoding paradigm, and another two subjects were excluded due to image preprocessing problems. In all, 54 subjects (age range 18 to 81 years, 31 male, 46 right handed, 7 left handed, 1 ambidextrous) were analysed in the present study.

2.2 Neuropsychological testing

A comprehensive battery of neuropsychological tests was administered (by TZR) to all subjects, including tests of attention, working memory, long-term memory and executive functions. The scores on the Danish Adult Reading Test (DART, a Danish version of the National Adult Reading Test) and WAIS vocabulary were first normalized into z-scores based on Danish norms. In order to test whether there were any age-related differences in estimated intelligence, the z-scores were analysed with a multivariate general linear model (GLM) with each z-score as a dependent variable, and age and gender as independent variables. The z-scores were also used to assess general cognitive function in each subject. The full neuropsychological analysis will be reported elsewhere.

2.3 Structural imaging protocol

Consistent head placement within the scanner was ensured by orienting and fixating the head to

predefined reference marks on the scanner head coil. Structural images, used for the ROI drawing, were acquired using a Siemens Magnetom Trio 3T MR scanner with an eight-channel head coil (Invivo, FL, USA) and included (1) a 3D whole brain MPRAGE (Magnetization Prepared Rapid Acquisition Gradient Echo) scan with a voxel dimension of 1x1x1 mm^3, field of view (FOV) 256 mm, matrix 192x256x256, repetition time (TR) / echo time (TE) / inversion time (TI) = 1540/3.93/800 ms, and a flip-angle of 9°; and (2) a 3D whole-head T2-weighted sequence with a voxel dimension of 1.1x1.1x1.1 mm^3, FOV 282 mm, matrix 256x256, TR/TE = 3000/354 ms, and a flip-angle of 28.5°.

2.4 Regions of interest

The N3 program (Sled et al. 1998) was used to correct images for non-uniformity artefacts due to radio-frequency field inhomogeneities. Tissue classification was done using SPM2 (Wellcome Dept. of Imaging Neuroscience, London) on the N3 bias-corrected images, with the SPM2 bias correction turned off. Careful editing of the grey matter tissue images classified voxels that were outside of the brain but adjacent to the MTL. Six ROIs in each hemisphere were drawn on the native space structural image using MNI Display (Montreal Neurological Institute, Montreal, Canada). An ROI drawing protocol for the temporopolar cortex, perirhinal cortex, entorhinal cortex and parahippocampal cortex was adapted from the Insausti (1998a) protocol; and neuroanatomic guidelines for hippocampus and amygdala were adapted from those of Pruessner et al. (2000), and the atlas of Duvernoy (1991) was consulted (see Figure 1). The border between the perirhinal cortex and entorhinal cortex was set, in the coronal plane, at the top of the parahippocampal gyrus, making the perirhinal ROI cover the entire collateral sulcus up to the posterior border to the parahippocampal cortex. This differs from that of others (Insausti et al. 1998a) who have applied a more adaptive drawing protocol for the perirhinal-entorhinal border, based on the depth of the collateral sulcus. This deviation was made to reduce variability associated with subjective placement of the boundary within the collateral sulcus.

*** PLEASE INSERT FIGURE 1 ABOUT HERE

2.5 Perfusion MRI

For CBF assessment with Arterial Spin Labelling, we used a PICORE (Proximal Inversion with a Control for Off-Resonance Effects) sequence (Wong et al. 1997) with GE EPI readout and pre-saturation, a 64x64 matrix, 14 slices with 5 mm slice thickness. Slice orientation was 20° T→C, with a 3x3 mm in-plane resolution, and with echo time (TE)/ repetition time (TR) of 24/2600 and inversion time (TI) of 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 ms in a fixed pseudorandom order. ΔTI=50 ms (time between subsequent slices), and slices were acquired in ascending order with 72 repetitions (36 pairs) at each TI. We used a flow crusher gradient with b=5 s/mm2 and a bandwidth = 2604 Hz/Px.

For the analysis of the ASL data the first image of each TI series was coregistered to the first image in the TI=200 ms series using SPM2 with normalized mutual information. Then, all subsequent images in each TI series were realigned to the first image in series using SPM2 with least squares. The 3D T1-weighted structural image was then coregistered to the ASL image. The ROIs drawn on the 3D T1-weighted structural images were resliced to the ASL image orientation and position, and tag and control values were averaged for each ROI at each TI. T1 and M0 were estimated for each ROI by fitting a T1 relaxation curve to tag and control values at multiple TI values. A general kinetic model (Buxton 1998) was fitted to difference magnetization (control-tag) signals at multiple TI values giving estimates of perfusion, transit delay and bolus width. Assumptions in the model: T1 of blood = 1600 ms and blood-brain partition coefficient = .9.

Regions in individual subjects where either the T1 relaxation curve or the kinetic model gave an non-physiological fit were excluded. Only T1 values fulfilling 600ms<T1<2500ms and model fits giving positive transit delays were considered physiological. 35% of the model fits gave non-physiological parameters, but these were not systematically related to specific regions. We then

tested whether there were significant differences in perfusion between regions, by applying a one-way ANOVA, and found no significant effect of region on perfusion. Thus, we chose to calculate the median MTL perfusion value for each subject, in order to produce a more robust estimate of perfusion. We first tested whether age had an effect on this perfusion value. We then included MTL baseline perfusion as a covariate in the multivariate analysis of the fMRI data.

2.6 Functional imaging protocol

For the functional scan we used a BOLD fMRI sequence optimized for the MTL region based on the method suggested by Deichmann et al. (2003). We used an EPI (Echo-Planar Imaging) sequence with an 8-channel head coil, TR/TE = 2000/30 ms, 64 x 64 matrix. The block of 33 slices was oriented 20° oblique to the transverse plane so that the slices were roughly parallel to the long axis of the temporal lobe. The voxel size was 3 x 3 x 2 mm with no interslice space. The 2 mm slice thickness was applied in order to further reduce susceptibility artefacts. The total scanning time was 703 s. Pulse and respiration were recorded using an MR-compatible pulse oximeter and a respiration belt, sampled at 50 Hz.

2.7 Activation paradigm

The task involved 19 blocks. Each block included a 1s instruction cue; a 3s preparation epoch; 6 stimuli presented serially 2s each for encoding; a 6s rehearsal epoch; and 6 stimuli presented serially 2.5s each for old/new recognition judgements (see Figure 2). Each encoding stimulus was a unique, coloured Snodgrass and Vanderwart-like drawing of a living or non-living object (Rossion and Pourtois 2004) presented in a trial-unique location among 9 positions in a 3x3 spatial grid. During preparation and rehearsal, an empty grid was displayed. Just prior to the instruction cue and recognition phase a white cross appeared at the middle of the grid for 1 second, signalling the onset of the encoding or recognition phase. In the object memory trials, recognition stimuli were 3 old and 3 novel objects presented in a fixed pseudorandom order. In position memory trials, recognition

stimuli consisted of an orange square that appeared in 3 old and 3 novel positions within the grid, presented in a pseudorandom order. The verbal instruction cue at the beginning of each trial indicated whether the subjects were to encode (and rehearse and recognize) objects or grid positions in the subsequent series of stimuli. During the rehearsal epoch, subjects were asked to try to keep the objects or grid positions in mind. Ten object blocks and nine position blocks were presented in a fixed pseudorandom order. Behavioural responses were recorded during the recognition phase of the paradigm, including reaction time and response accuracy. Training outside the scanner was performed using a different set of objects.

Stimuli were presented using E-prime (www.pstnet.com) and IFIS-SA System software (MRI Devices Corp., Wisconsin), in a Windows 98 environment (Microsoft Corporation) using a Canon LV 7545 LCD projector equipped with a Buhl optics lens with a refresh rate of 60 Hz (full brightness = 3700 ANSI lumens, setting = 10; Contrast=800:1, setting = 32). The stimuli were back projected onto a screen and viewed via mirrors placed on the head coil above the subject s head. The full screen size corresponded to 24° x 18° visual angle and was presented in 800 x 600 pixel resolution.

*** PLEASE INSERT FIGURE 2 ABOUT HERE

2.8 Analysis of behavioural data

For the analysis of the effects of age on response accuracy (how many correct responses each subject made) we used a multivariate GLM with object accuracy (ACC-o) and position accuracy (ACC-p) as dependent variables, and age as the independent variable. In order to study whether either of the two tests was significantly more affected by age than the other, we further analysed the ratio between the two scores. The effect of age on this contrast value (ACC) was performed with a univariate regression analysis with age as the independent variable.

2.9 BOLD analysis

EPI data analysis was performed in native space using SPM2. Realignment with no smoothing was used. EPI image series was coregistered to each individual's AC-PC aligned structural image. For each content condition (object and position) preparation, encoding, rehearsal, and recognition were entered as separate regressors in the design matrix, leading to a total of 8 regressors of interest. The regressors were convolved with a canonical haemodynamic response function. Nuisance regressors for respiration, heartbeat and motion were included in the analysis (Lund et al. 2006). For each ROI the average value for the contrasts of interest (object encoding minus position encoding) were fed into the second level analysis. Here, in order to study the effects of age on regional content effects, we analyzed the data using a multivariate GLM with encoding contrast values for each region as dependent variables, and with age and perfusion as covariates. In the present analysis, we focused on the encoding stage.

We further studied the effect of age on the relative activation difference between the left perirhinal cortex and left parahippocampal cortex. This was done by subtracting the left parahippocampal contrast value from the left perirhinal contrast value for each person, and enter this value into a GLM as a dependent variable, with age and perfusion as independent variables.

To establish whether regions showed significant content effects across the age cohort, we compared our results with those from a previous analysis of the youngest sample of this cohort (n=27, 12 female; mean age=25.85, std.=6.6, range 18-39, 20 right-handed, 6 left-handed and 1 ambidexterous, with normal or corrected to normal vision) (Ramsøy et al., unpublished data). All 2nd level statistical analyses were performed in Statistica 7.0 (www.statsoft.com).

3. Results

3.1 Population data

There were no effects of age on estimated IQ levels, as measured by the DART (t=1.10, p=.276)

and WAIS vocabulary (t=-.08, p=.941), as shown in Figure 3. As these scores are highly correlated to general intelligence estimates, this result suggests that the general cognitive level was comparable across the cohort.

*** PLEASE INSERT FIGURE 3 ABOUT HERE.

3.2 Cerebral blood flow

The analysis of the effects of age on MTL perfusion showed no significant effect (t=.625, p=.535), as shown in Figure 3. It should be noted that despite the lack of age effect on CBF, there were large individual differences in MTL perfusion.

3.3 Behavioural results

We then studied the effect of age on behaviour during the encoding task, as measured by reaction time and accuracy of responses that were recorded during the retrieval stage. Our GLM analysis showed no age-effect on reaction time for either object (t=.67, p=.504) or position (t=.003, p=.997) responses. Age was associated with a reduction in both performance scores (ACC-o: t=-3.91, p<. 001; ACC-p: t=-3.31, p<.001). The ratio between ACC-o and ACC-p did not show any significant effect of age (t=1.46, p=.151), suggesting that the relative task performance between the two tasks was stable across the age cohort.

3.4 fMRI results

Our main analysis was concerned with the left perirhinal cortex. Here, age was associated with an increased activation during object encoding compared to position encoding (See Table 1 and Figure 4). We further tested for age by region interaction by comparing the left perirhinal and left parahippocampal cortex activation on the object > position encoding contrast, and found no significant result (t=.596, p=.554).

Post-hoc analysis of the encoding stage furthermore showed that age was associated with similar age-related increases in activation during object compared to position encoding in the right

temporopolar cortex and, at a lower statistical threshold, the right parahippocampal cortex and left hippocampus.

By comparing the ageing results to regions activated in the young group, significant activations across the age cohort were found for object encoding compared to position encoding in left temporopolar cortex, bilateral entorhinal cortex and right amygdala (see Table 1 and Figure 4).

*** PLEASE INSERT TABLE 1 ABOUT HERE

*** PLEASE INSERT FIGURE 4 ABOUT HERE

Age was not associated with change in the activity differences in the left perirhinal and left parahippocampal cortices, suggesting that the task-related dissociation between these two regions was unaffected by age.

Analysing the effect of CBF on regional contrast values, we found significant effects in the left perirhinal cortex ($t=-3.09$, $p=.003$), and at lower levels of significance, in the right entorhinal cortex ($t=1.88$, $p=.065$) and left hippocampus ($t=-1.75$, $p=.086$).

4. Discussion

The main aim of this study was to study the effects of age on regional activation in the MTL, in particular the left perirhinal cortex. Previous studies have documented that structures within the MTL are differentially involved in the processing of objects and positions (Pihlajamäki et al. 2003, 2005, Buffalo et al. 2006). In particular, we have recently found that the left perirhinal cortex is more involved in object encoding than position encoding (Ramsøy et al., unpublished data). Here, we extend these findings by showing age-related alterations in MTL function during intentional encoding. Our analysis was primarily interested in the effects of age upon activation in the left perirhinal cortex during intentional encoding of objects and positions. In addition, our paradigm

allowed additional probing of the effects of age on activation in adjacent MTL regions during encoding. Importantly, baseline perfusion (CBF) scores were used as a covarite in the analysis, as recent studies have shown that age-related variance may have an impact on the BOLD fMRI signal (Restom et al. 2007).

4.1 The effect of age on memory performance

Several studies have shown that working memory and episodic memory are significantly reduced in healthy ageing (West et al. 2005, Waters et al. 2005, Cook et al. 2007, Wingfield et al. 2002, Henderson et al. 2007). In the present study we asked subjects in ages 18-81 to encode and retrieve objects or positions. By studying the effects of age on performance during this task, we found that both object memory and position memory were significantly reduced with age, while the internal relationship between these two performances did not change.

4.2 The effect of age on regional activation in the MTL

Our analysis demonstrated an age-related activation increase in the left perirhinal and right temporopolar cortex during object encoding compared to position encoding. Furthermore, at a lower level of statistical significance, a similar effect was found in the right parahippocampal cortex and left hippocampus. Previous studies have shown that the anatomical and functional integrity of the MTL declines with age. Volumetric decline with age has been shown in the hippocampus (Sowell et al. 2002, Jernigan et al. 2005, Geinisman et al. 1995), and in the entorhinal, perirhinal and parahippocampal cortex (Insausti et al. 1998b). Importantly, age-related MTL atrophy has been correlated with reduction in memory performance (Raz et al. 2000, 2004). Thus, studies of brain morphology have suggested a link between age-related changes in episodic memory and neuroanatomy.

In the present study, a central question was whether age-related changes would show a general decrease within the left perirhinal cortex. Previous studies have shown that this region is critically involved in object memory compared to position encoding (Buffalo et al. 2006,

Pihlajamäki et al. 2005). In a recent study using the same paradigm as in the present study, we found that a young subset of the present cohort showed significant higher activation in the left perirhinal corex during object encoding than position encoding, and that this effect was significantly stronger than in the left parahippocampal cortex.

We hypothesized that increasing age would be associated with activation decrease in the left perirhinal cortex, and that the difference between left perirhinal and left parahippocampal cortex activation would show an age-related decrease. Contrary to this, our result suggests that age was associated with increased left perirhinal activation during object encoding compared to position encoding. Furthermore, we find that the difference between perirhinal and parahippocampal activations was unaffected by age. This rejects our hypothesis of an age-related activation derease in the perirhinal cortex. One explanation of this finding may be that, while age may initially lead to reduced function in the left perirhinal cortex, this effect may be countered by a prolonged response in this region. Put differently, although activation is still confined within the same region, age-related reductions in neural efficiency in the left perirhinal cortex is alleviated through an increase in computational iterations.

In a recent study by Mattay et al. (2006) it was found that at comparable performance levels during a low load working memory task, older subjects showed a bilateral engagement of the PfC, compared to young subjects. When working memory load increased, older subjects performed worse than young subjects, and showed a reduction in the bilateral recruitment of the PfC. This suggests that, within capacity, compensatory mechanisms are called upon to maintain proficiency in task performance. However, as cognitive demand increases older adults are pushed past a threshold beyond which physiological compensation cannot be made and a decline in performance occurs. A similar mechanism might explain the results seen here. That is, the age-related increased activation seen in the left perirhinal cortex may be indicative of within-capacity compensation. It could thus be suggested that as the effect of ageing increased, or in the case of disease or stress, this activation

increase in the left perirhinal cortex during object encoding would reduce.

Our post-hoc analysis focused on other MTL regions during encoding, for which we had no apriori hypothesis. In our recent study of young subjects, the bilateral temporopolar cortex, bilateral entorhinal cortex, and left hippocampus (in addition to the left perirhinal cortex) showed a significantly higher activation during object encoding compared to position encoding. Studying the effects of age on this activation, we found that the right temporopolar cortex showed an activation increase during object encoding compared to position encoding. A similar, non-significant, trend was found in the right parahippocampal cortex and left hippocampus. These results were comparable to what we found in the left perirhinal cortex, further suggesting an age-related increase in activation during object encoding compared to position encoding in these regions. Comparing these age-effects to the results found in young subjects, the increased activation in the left temporopolar cortex and bilateral entorhinal cortex during object encoding did not change with age.

Previous studies have implied the MTL region in encoding and have shown that MTL activation during encoding predicts subsequent retrieval success. It has been found that the hippocampus shows increased activation for subsequently remembered face-name associations compared to pairs that are forgotten (Chua et al. 2007), and results suggest that the MTL is part of a large-scale network for object memory, together with fusiform cortex and prefrontal cortex (Dickerson et al. 2007). Similarly, Staresina and colleagues (2006) found that activation in the left inferior frontal gyrus and hippocampus correlated with later relational memory, and that the left perirhinal cortex mediated successful word-colour binding. Finally, studies by Ranganath et al. (2005, 2006) have found that successful memory formation is the result of transient cortico-hippocampal interaction that includes the PfC and MTL regions such as the perirhinal cortex. Together, these studies suggest that normal performance of episodic memory is the result of increased interaction between MTL subregions such as the hippocampus and perirhinal cortex and the PfC. The present results during the encoding stage suggest an age-related increase in regional

activation for the encoding of object information compared to spatial information. As older subjects performed significantly lower at retrieval, these age-related changes do not fit a view suggesting that age is related to reduced neural specificity. On the contrary, the age-related changes observed here suggest that age is related to increased neural specificity during encoding. Furthermore, the observed activation increase could be interpreted as a compensatory response in the MTL, ensuring preserved performance. However, as the age-related changes were related to a general decrease in performance, this explanation is unsupported.

4.3 Dedifferentiation or compensation?

Among theories of the effects of ageing on brain function, two prominent ideas relate to the present study. According to the dedifferentiation view of ageing, increasing age leads to a loss of neural specificity, in that functions that are neurally segregated in young adults show an age-related loss of neural differentiation (Grady et al. 2002, Park et al. 2002). Contrary to this, the compensation view suggests that the detrimental effects of healthy ageing induces neural compensatory mechanisms that ensure a high level of performance, as exemplified by studies showing reduced hemispheric asymmetry in PfC activation in older subjects (Cabeza 2002, Cabeza et al. 2002, Tessitore et al. 2005). The compensation view and dedifferentiation view should not be seen as mutually exclusive, and it has been suggested that increased activations in some regions act as compensatory responses to dedifferentiation in other regions (Cabeza 2002, Persson et al. 2006, Lorenzo-López et al. 2007).

The age-related activation increase in certain MTL regions found in this study, are inconsistent with a strict dedifferentiation view of ageing, which presumes a general waning of neural specialization. Rather, the present results may be indicative of a compensatory mechanism, although the changes observed here were generally indicative of age-related reduction in behavioural performance, and not, as a compensatory model suggests, that increased activations are associated with preserved performance. As noted, an alternative explanation may be that age does indeed lead to altered neural efficiency in the affected regions, and that this is compensated through

increased or prolonged activation. This further suggests that additional effects of ageing, disease or stress may lead to a failure in this compensatory mechanisms, leading to further decline in task performance. The present findings thus warrant further study of the effect of age and disease upon regional activations in the MTL.

The behavioural results suggest that, although not significant, the object memory task tends to be more affected by age than the position memory task. It is possible that the object and position encoding tasks differed with respect to how much they taxed the encoding apparatus. The objects presented represented members of a virtually limitless set of possibilities, while the positions to be encoded represented a small set of possibilities; only nine positions were possible. Thus, in one sense, objects were low frequency stimuli, while positions were high frequency stimuli. Low frequency stimuli are known to be more difficult to process at study phase, although they may be more easily recognized at the test phase (Glanzer & Adams 1985, Ostergaard 1998, Diana & Reder 2006). Although, in this study, the effect of age on the ratio between object and position memory did not reach statistical significance, there was a trend for the object memory task to be relatively more affected by age. This suggests that some of the age-related changes in MTL activation observed in this study may be influenced by the difference in difficulty between the two tasks.

4.4 ROI analysis

In the present study we used an optimized image acquisition for the study of MTL activation. The use of whole-brain fMRI is known to lead to significant loss of signal in this region due to susceptibility artifacts, especially at high-field fMRI scanners at and above 3T (Bellgowan et al. 2006). Furthermore, the use of spatial normalization of functional images, a method in which individual brains are structurally deformed in order to make different locations in each subject's brain correspond to the same locations in another subject's brain (Ashburner and Friston 1999) has been shown to be poorly registered across individuals with standard methods (Salmond et al. 2002), and systematically different in subjects with memory impairments (Krishnan et al. 2006). These,

and related problems, are overcome by the present use of optimized image acquisition, and the analysis of regional brain activation using non-normalized methods such as ROI analysis.

A few caveats should be considered for the present study. First, as the drawing of the perirhinal-entorhinal border was defined at the top of the collateral sulcus, the perirhinal ROI might have partially included the lateral most part of the entorhinal cortex. The lateral entorhinal cortex has been shown to receive inputs from the perirhinal cortex as part of the ventral stream projections (Eichenbaum 2007). Distinctions observed here between perirhinal cortex and entorhinal cortex may have been mediated to some extent by this imprecision in boundary delineation.

Second, in order to maximize the signal to noise ratio in the MTL region, the BOLD signal was not recorded at the whole-brain level. It is possible that many of the observed age-related changes in regional activation are related to, or even the product of, changes in brain activation in other regions of the brain, such as the prefrontal-parietal attentional network. This network has been shown to be involved in preparatory and anticipatory activation (Rowe et al. 2000, 2002, 2007) and both object and spatial working memory (Deco et al. 2004, Aso et al. 2007), and other studies using similar tasks have indeed demonstrated age-related changes in those regions (Mitchell et al. 2000). As the present study did not include the assessment of these regions, studies comparing age-effects on MTL activation and other global brain changes are needed.

Third, we included CBF as a regressor in the BOLD fMRI analysis. As the BOLD fMRI signal is influenced by CBF, age-related alterations and individual differences in baseline CBF levels may lead to differences in the BOLD signal (Fernández-Seara et al. 2007, Federspiel et al. 2006). Consequently, conclusions about age-related changes based on uncorrected BOLD fMRI may be significantly biased by non-neural age-related changes in the cerebrovasculature. In the present study, we found that baseline CBF did not show any general age-related changes in the MTL region. In order to include CBF as a covariate in the BOLD fMRI analysis, we used an optimized imaging sequence for assessing CBF in the MTL region, and analyzed the results using

an ROI based approach. However, the ROI based results showed large variance in the signal to noise ratio, although there were no systematic regional effects. In order to produce a robust estimate of MTL perfusion, we chose to estimate CBF using the median MTL value. Further development of image acquisition and analysis of CBF in this region is needed in order to extract more specific and robust estimates of regional values in MTL structures. Thus, although the current inclusion of CBF as a covariate in the present analysis did not show any age-related changes, we did find that it influenced the estimated contrast between object and position encoding in certain MTL regions, in particular the left perirhinal cortex. This suggests that CBF may serve as a correction for individual differences in the BOLD signal that are caused by low or high levels of regional perfusion in the MTL. However, since we were unable to produce robust estimates of CBF in individual MTL regions, these results should be interpreted with caution, and further studies on regional CBF values and their effect upon regional BOLD effects are needed.

Conclusion

Taken together, the present study provides unexpected findings of age-related changes in regional activation in the MTL. Contrary to our hypothesis, the left perirhinal cortex, along with adjacent regions in the MTL, showed an age-related activation increase. This finding is neither directly consistent with interpretations of reduced neural efficiency or compensatory responses. Here, we offer an alternative explanation. The observed activation increases may occur due to prolonged periods of activation. That is, while regional activation in young subjects only require a certain time for successful processing, this time is prolonged with increasing age. If this is the case, the current analysis, where activation is measured as averaged blocks, would fail to distinguish between increased and prolonged activations. Consequently, studies using single events in similar settings are needed. The present study applied several factors in the assessment of age-related changes in regional brain activation that included optimized image acquisition for the MTL region, the

inclusion of baseline CBF as a covariate, and the use of a continuous age cohort from ages 18 to 81, thus modelling the entire age range. In addition, based on the results reported here, three improvements are suggested. First, there is a need to combine MTL-optimized assessment and whole-brain assessment, as this would have the potential to relate the changes observed in this study to changes occurring in, e.g., the prefrontal cortex. Second, analysing regional activation other than by averaged time blocks may shed light on the question of whether age leads to prolonged or increased activations per se. Finally, an improved assessment of regional CBF would make it possible to correct the BOLD effects in a more region-specific manner, thus providing an even more robust estimate of the effects of age on regional CBF and its relation to the BOLD signal.

Notes

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Disclosure statement

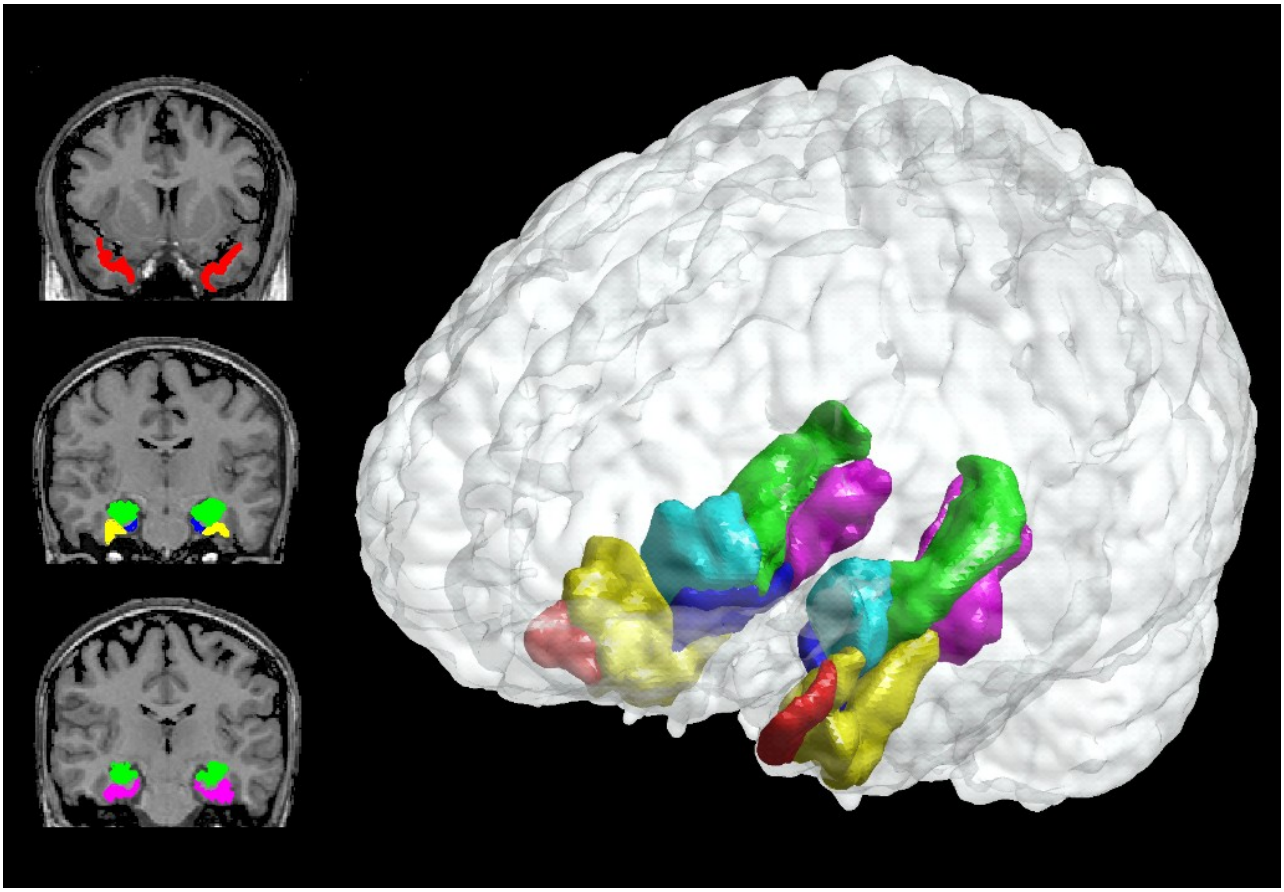
All authors acknowledge that there are no actual or potential conflicts of interest with the work presented here.

Table 1 Activations of the MTL during intentional encoding, and age-effects on regional activation in the MTL, with CBF as a covariate. '#' indicates regions found to show significant effect of content in young group (Ramsøy et al., unpublished data), significant age-effects marked by asterisk.

Region	Age effect	
	T	**p**
left temporopolar cortex #	-1.23	.226
right temporopolar cortex #	2.85	.006 *
left entorhinal cortex #	-1.00	.324
right entorhinal cortex #	-1.54	.130
left perirhinal cortex #	2.73	.009 *
right perirhinal cortex	1.59	.118
left parahippocampal cortex	1.44	.157
right parahippocampal cortex	1.89	.065
left hippocampus #	1.89	.065
right hippocampus	.20	.845
left amygdala	.57	.571
right amygdala #	-.23	.784

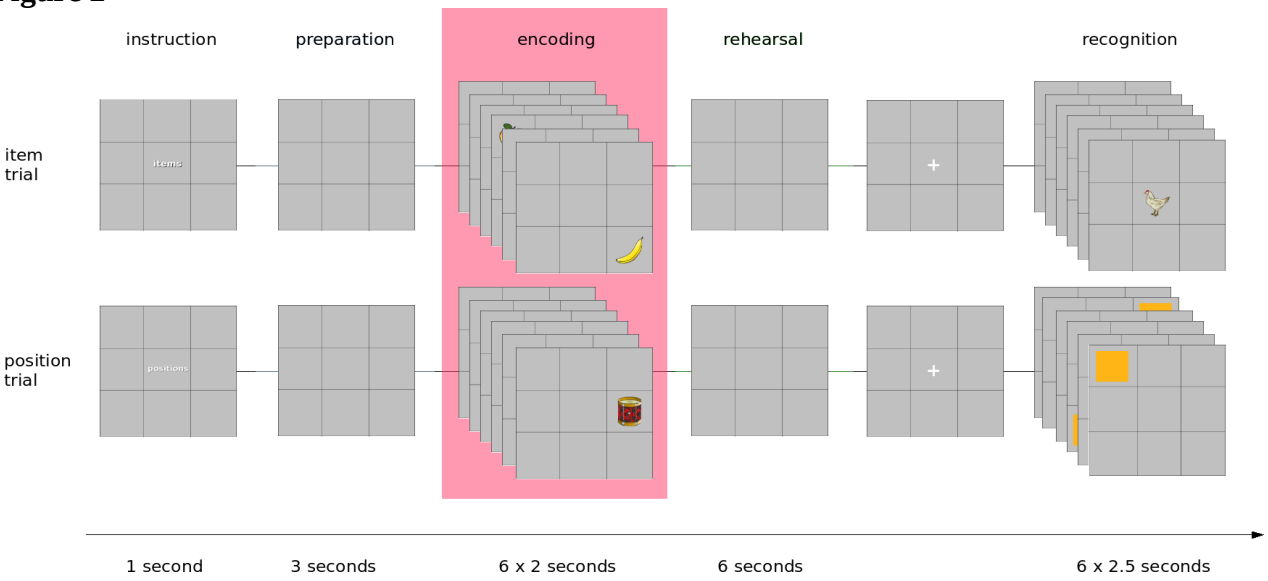
Figures

Figure 1



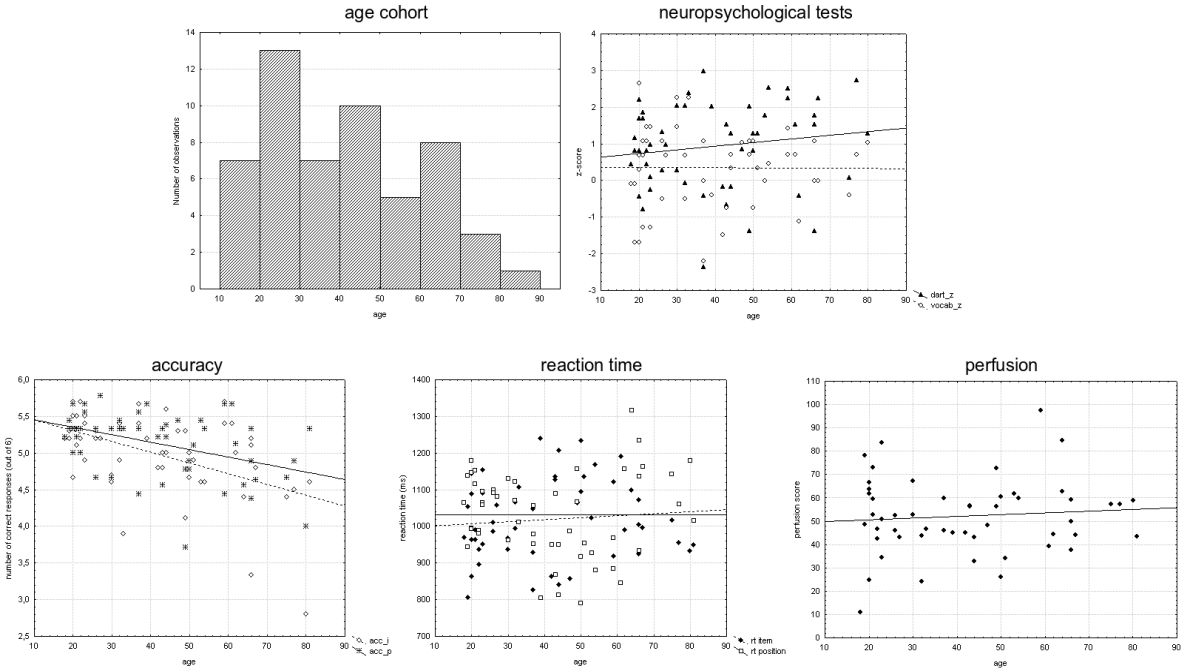
CAPTION:
The medial temporal lobe regions, illustrated by region drawings from one subject. *Left*: Coronal slices showing original ROI drawings including temporopolar cortex (red), entorhinal cortex (blue), perirhinal cortex (yellow), hippocampus (green), parahippocampal cortex (pink), and amygdala (cyan, shown only in glass brain). *Right*: 3D reconstruction of the same ROIs, displayed within a transparent view of the native brain.

Figure 2



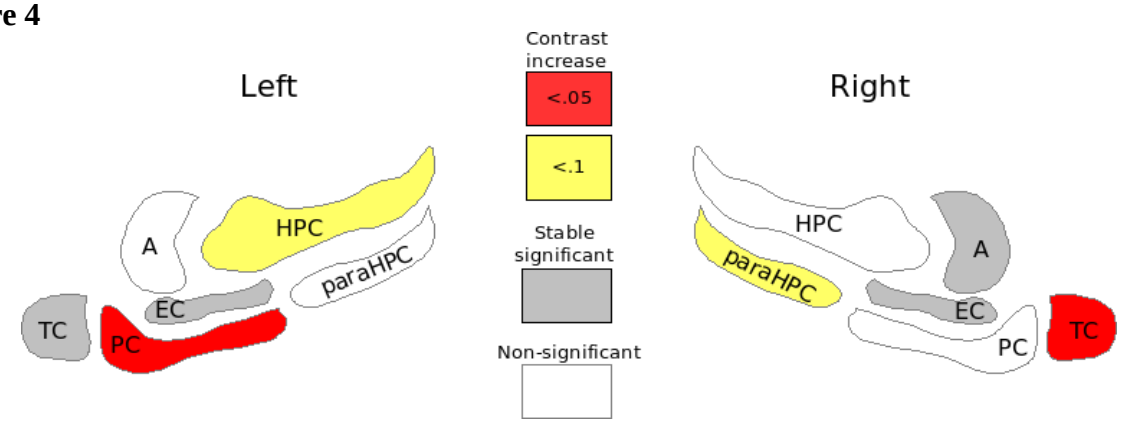
CAPTION:
The memory paradigm. Object and position trials consisted of an instruction cue; a preparation phase; an encoding phase with six trial-unique objects and positions; a rehearsal phase; and a recognition phase with old-new judgements. Only the instruction cue and recognition phases were visually different between the conditions. Numbers at the bottom indicate block duration. The red square highlights the encoding stage that was analysed in the present study.

Figure 3



CAPTION: Descriptive figures of age cohort, and plots of age-effects on verbal skills, behavioural parameters (accuracy and reaction time), and MTL perfusion. See text for further details.

Figure 4



CAPTION:
Illustration of age-related changes in neural activation during encoding. The left perirhinal cortex and right temporopolar cortex (in red) show significant age-related content effect increases in the object vs. position encoding comparison. At a lower statistical threshold (p<.1, in yellow) the right parahippocampal cortex and left hippocampus show a similar trend. Grey indicates activations identified in young subjects that were not affected by age. White represents regions that neither show general or age-related content effects.

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Appendix 3

Functional Magnetic Resonance Imaging

The following is a description of fMRI as a method to study MTL function and the effect of age. In order to understand the principles of this method, it is necessary to briefly review the basics of functional Magnetic Resonance Imaging in general. This review is necessary in order to understand the actions taken in this project to optimize signal acquisition of MTL regions, and minimize the effect of both scanner-related and biologically based artefacts.

Table of Contents

The physiological basis of functional brain mapping[1]

The physiological basis of the functional neuroimaging signal is the coupling between regional cerebral activation and blood flow, and further the uncoupling between flow and oxygen consumption during activation [2,3]. Put simple, an increase in activity in the cerebral tissue leads to a blood flow increase that can be measured with PET. The increase in flow markedly exceeds the increase in oxygen consumption so that the concentration of deoxyhemoglobin decreases. This local decrease in deoxyhemoglobin gives rise to the fMRI signal.

The epileptic seizure can be considered as an extreme non-physiological activation where all neurons fire at maximal rate. In this condition blood flow is markedly increased and oxygen consumption is also increased but much less resulting in an increase in oxygen content in the cerebral venous blood . This corresponds to the observation by Cooper and co-workers [4] of an increased oxygen tension in cerebral tissue. Fox and Raichle [5] demonstrated, in an elegant PET study, that during normal physiological activation brain blood flow, as measured by $^{15}O_2$, and glucose phosphorylation, as measured by the ^{18}F-FDG, increase in parallel whereas oxygen consumption only increases to a minor extent.

Theoretical calculations suggest that a large share of the metabolic energy is spent on action-potential propagation along axon collaterals. However, empirical studies using simultaneous electrophysiological and haemodynamic recordings by Lauritzen and co-workers [6] and by Logothetis and colleagues [7] have demonstrated that the increase in flow and BOLD-signal reflects the increased synaptic activity and local field potentials in the dendrites, rather than a higher firing activity in the postsynaptic neurons. Thus, a release of stimulating as well as of inhibiting neurotransmitters will result in an increased metabolic turn-over that increases blood flow and the BOLD-signal.

Magnetic Resonance Imaging

MRI is based on the principles of nuclear magnetic resonance, which is a spectroscopic technique used to obtain information about the chemical and physical properties of molecules. As such, MRI started out as a so-called tomographic imaging technique a method for obtaining pictures of the interior of the body. Today, MRI has advanced far beyond this and now represents a battery of different approaches that can measure the structure, function, connectivity, and chemistry of any part of the body.

MRI is based on the absorption and emission of energy in the radio frequency range of the electromagnetic spectrum. The human body is mostly made of fat and water body tissues that have many hydrogen atoms. As such, the human body consists of about 65% hydrogen atoms. These hydrogen nuclei form the very basis for the signal in MRI.

1 The following descriptions are, unless otherwise stated, taken from Ramsøy, Balslev & Paulson (2007), in [1]

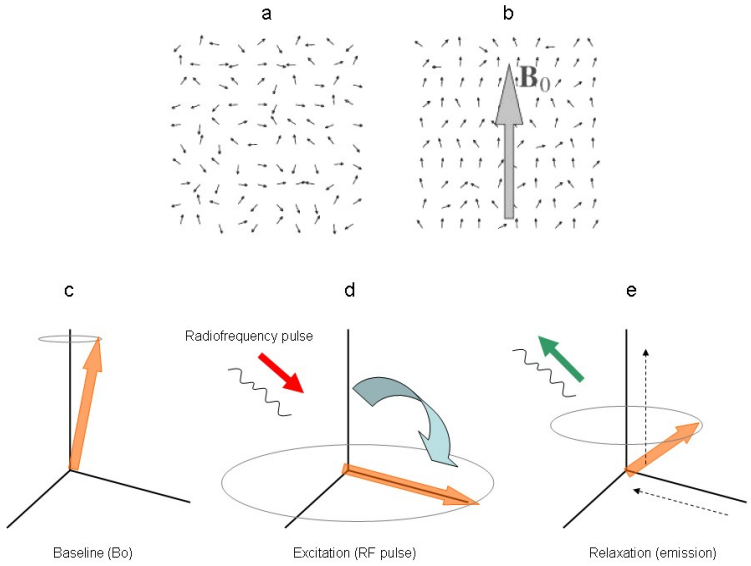


Illustration 1: The signal that makes up the MRI. Outside the scanner the atoms are oriented at random in the brain (a). When a subject is put into the scanner, the spins align to the magnetic field of the scanner (B_0). However, the alignment is not perfect, since neighboring spins influence each other (b). At such baseline, the spins circles along the y axis (vertical axis in figure), i.e. the B_0 field (c). When a radiofrequency (RF) pulse is applied the spin is influenced, and rotated down (d). This is a state of disequilibrium, and during equilibration towards the B_0 field the spin releases energy that it received from the RF pulse. The local milieu of the atom i.e. whether it is in grey matter, white matter, bone or cerebrospinal fluid determines the speed of this relaxation. This is the basis of contrast in the MR image, and thus what makes it possible to visualize the different tissues of the body.

In a magnetic field such as the MR scanner, the magnetic orientation of hydrogen proton is aligned to the magnetic field and spins around this orientation (see Illustration 1b and 1c), also called the Larmor frequency. If a brief electromagnetic (radio-frequency) pulse is applied, it temporarily distorts the proton spin (Illustration 1d). When the radiofrequency pulse ends, the spins return to equilibrium, a process called relaxation (Figure 4e). The relaxation can be described with two time constants: Time-1 and Time-2. The regrowth of the magnetization along the magnetic field is termed longitudinal relaxation, and the time in milliseconds required for a certain percentage of the tissue nuclei to realign is termed "Time 1" or T1. Both relaxation processes follow an exponential time course. When returning to equilibrium, radio waves at the Larmor frequency are emitted from the tissue and sampled by a receiver. This is the basis of so-called T1-weighted imaging, which produces the most well-known structural images in MRI. T2-weighted imaging relies upon local dephasing of spins following the application of a transverse energy pulse; this transverse relaxation time is termed "Time 2" or T2.

The T1 and T2 constants provide the basis for most medical imaging. In different parts of the body, such as the brain, different tissues alter the speed in which T1 and T2 relaxation occurs. The three most typical tissues of the brain are grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). The influence of these tissues produces different signal intensities contrast that makes it easier to distinguish between them. By varying different parameters during scanning, such as the rate and amplitude of the radiofrequency pulse, or the time from excitation to recording, it is possible to highlight different properties of the tissues and their differences.

Acquisition of an MR image requires spatial encoding in three dimensions. To obtain slice selection, a radiofrequency pulse with a narrow frequency range is applied in the presence of a spatial magnetic field gradient along the slice direction. Spatial encoding in the readout direction is obtained by applying a field gradient across the excited space, and spatial encoding in the second in-plane dimension is created by applying a gradient in the phase-encoding direction before each readout line. The reconstruction of images then occurs through Fourier-transformation of the raw data.

The contrast in an MR image is determined by the sequence and the parameters of the sequence, among others the echo time (TE) and the repetition time (TR). TE is the time from excitation of the nuclei to sampling of the centre of each readout line, and TR is the time elapsed between two excitations. With a short TR and a short TE the T1 contrast is maximized, and with a long TR and a long TE the T2 contrast is maximized [8].

T1 and T2 change with the strength of the applied field. With increasing strength T1 increases, although not proportionally for white matter and grey matter, which leads to a decreased T1-contrast between white and grey matter at high field. With increasing field strength T2 decreases and, due to disproportionate changes in T2 between grey and white matter, the T2 contrast between the two tissue types increases [11]. Due to the changes in T1 and T2 with increasing field strength, some adjustments of sequence parameters are required to obtain the same or better image contrast in structural imaging when advancing from low field to high field. In addition, different strategies to obtain good image contrast at high field are often employed, e.g., in T1-weighted sequences at high field, an inversion pulse can be added to improve T1 contrast (this also applies to low field).

One of the advantages of high field strength is an increased signal to noise ratio (SNR), which is calculated as the ratio between signal from the tissue and the standard deviation of the noise image. As the signal is proportional to the square of the applied field, $(B_0)^2$, and the noise is proportional to the applied field, (B_0), SNR increases linearly with field strength. The higher SNR at high field strength also allows sampling of thinner sections and higher image resolution within similar imaging times. However, for fMRI the relationship between signal and noise is not as straightforward, due to the use of Echo-Planar Imaging (EPI), which will be discussed in the following sections.

Functional MRI

While T1 and T2-weighted images are superior at imaging the structure of the brain, MRI also offers ways to measure different functions of the brain. In general, there are two main approaches: Blood Oxygen Level Dependent (BOLD) fMRI and perfusion MRI. While the BOLD approach relies on a complex series of events that couple brain activation to vascular changes and the relative level of regional oxygenated blood, perfusion MRI measures the cerebral blood flow (CBF).

BOLD fMRI is the most used and well known way to assess brain activation with MRI. Brain activation changes the relative concentration of oxygenated and deoxygenated hemoglobin blood with or without oxygen, respectively in the local blood supply. While oxygenated blood is diamagnetic and does not change the MRI signal, deoxygenated blood is paramagnetic and leads to a drop in the MRI signal. If there is more deoxygenated blood in a region it therefore leads to a drop in the BOLD signal, and more oxygenated blood in the region leads to a higher signal. The BOLD response to a stimulus can be thought of as the combination of four processes (see Illustration 2):

(1) **An initial decrease** (dip) in signal caused by a combination of a negative metabolic and non-metabolic BOLD effects. In other words, when groups of neurons fire they consume more oxygen. When this happens, the local level of oxygenated blood drops, and there is relatively more deoxygenated blood in that area. In addition, there is also a dilation of the blood vessels, which further increases the negative BOLD effect.
(2) **A sustained signal increase** or positive BOLD effect due to an increased blood flow and a corresponding shift in the deoxy/oxy hemoglobin ratio. When the neurons go back to a lower level of activation, their increased consumption of oxygenated blood drops. At the same time, influx of new oxygenated blood is still increased due to the previous demand. As

the blood oxygenation level increases, the signal continues to increase. This drop in demand of oxygenated blood, combined with a delayed supply of oxygenated blood, leads to a dramatic overshoot of the relative amount of oxygenated blood. This abundance leads to the main effect of the BOLD signal.

(3) **A sustained signal decrease** which is caused by the return to normal flow and normal deoxy/oxy hemoglobin ratios.

(4) **A post-stimulus undershoot** caused by a slow recovery in cerebral blood volume.

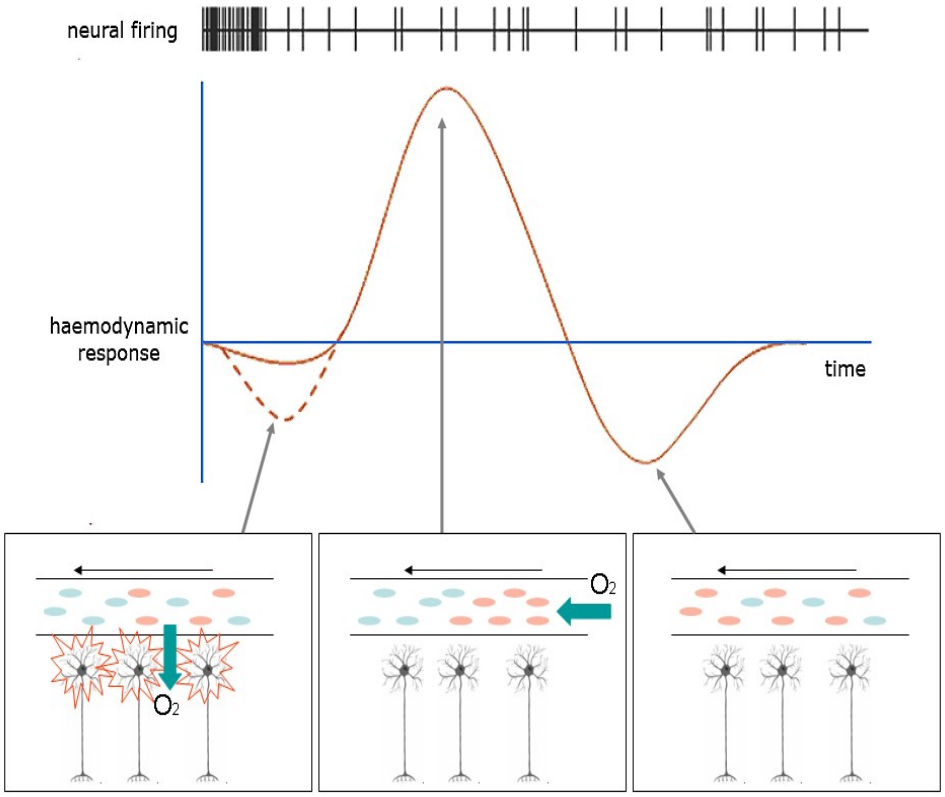


Illustration 2: The BOLD signal simplified in four steps. Step 1: higher neural activation leads to an increase in the consumption of oxygen from the blood, leading to a lower level of oxygenated blood, and more deoxygenated blood, wich cuses a drop in the BOLD signal. Step 2: the vascular response to increased oxygen consumption leads to a dramatic increase in new, oxygenated blood at the same time as the oxygen consumption drops due to decreased levels of neuronal activation. Step 3: a normalizing of flow and deoxy /oxyhemoglobin levels (not shown). Step 4: a post-stimulus undershoot caused by the slow recovery of blood volume.

The initial dip is thought to be the measure closest related to the neural activation, since it relates to the first drop in signal intensity due to consumption of oxygenated blood. However, the signal changes at this stage are so small that they are mostly detectable by the use of extra strong magnetic fields and therefore MRI scanners. It is therefore the second phase the sustained signal increase that is used in most BOLD fMRI studies. This signal is an even more indirect measure of neural activation, as it is the result of a delayed vascular overshoot of oxygenated blood, as a response of demand for oxygenated blood in a region. Although the BOLD response is an indirect and delayed measure of neural activation, it has been shown to have a time resolution at the millisecond scale [9]. In addition, the method has a very high spatial resolution. With recent technical advances the resolution has been brought down to the sub-millimeter scale.

Echo planar imaging

Echo-planar imaging (EPI) is a fast MRI technique that allows acquisition of single images in as little as 20 msec, and performance of multiple-image studies in as little as 20 seconds. EPI achieves its speed by obtaining all spatial-encoding information after a single radio-frequency (RF) excitation. Conventional imaging requires multiple RF excitations, separated by the repetition time (TR), to acquire this information. (Standard pulse sequences are used to obtain echo-planar images, which have diagnostic utility similar to that of conventional MR images.) In addition, EPI is less sensitive to motion than is conventional MR imaging and allows imaging of rapidly changing physiologic processes such as blood flow and kinetic activity.

In an ideal homogenous field the trasverse relaxation follows an exponential signal decay constant, the T2. However, in physiological tissue the transverse relaxation happens faster due to local field inhomogeneities. When these are present the decay constant is called T2*. The amount of local field inhomogeneities depends on numerous factors, including the physiological state and the composition of the local blood supply. Therefore, local field inhomogeneities that are due to regional differences in the deoxy-/oxyhemoglobine ratio are measurable with the use of gradient echo- or spin-echo based sequences. The gradient-echo measurement is T2* dependent, while the spin-echo sequence is T2 dependent. In current fMRI research, gradient-echo is the most commonly used sequence.

The very fast acquisition of EPI MR images is possible by the use of an oscillating read-out gradient which creates a rapid back and forth traversal along the read-out axis. Following each line a brief pulse (so-called 'blip') is given by the phase encoding gradient in order to move to the next line [1011].

For *spatial resolution*, most fMRI studies use a slice thickness of 3-5 mm and an in-plane resolution of 3-5 mm. However, even higher spatial resolution is possible provided by the use of an appropriate strategy that may include a combination of scanner technology and functional paradigm design. The use of such opimized approaches have demonstrated functional units at the scale of ocular dominance columns in the primary visual cortex [12]. With respect to *temporal* resolution, the minimal time between two stimuli activating the same brain region that can be detected by BOLD fMRI is around 2 seconds [13], but the minimal inter-stimulus interval for stimuli that activate different brain regions can be much smaller. For example, Menon et al. [14] studied the temporal resolution of the BOLD response with a visual paradigm that sifted between stimulation of the right and left hemifields at different intervals (0, 125, 250, 500 and 1000 ms). This study demonstrated that the haemodynamic responses in the right and left visual cortex closely mirrored those of the stimulus onsets, with only a small temporal offset (~5 ms). Taken together, through the use of appropriate technical and design related parameters, BOLD fMRI may achieve a high spatial and temporal resolution.

Image preprocessing

Before statistical analysis can be performed on functional MR images, a number of operations must be performed in order to minimize the influence of artefacts and to generally prepare the dataset for analysis both at the individual level and in a group study. We now move closer to specific considerations taken in the present project. Issues concerning spatial normalization and smoothing are vital in this discussion[Ŧ]. Here, we first briefly review some of the most common steps in the preprocessing (see Illustration 3).

Ŧ We will not review slice-timing. Images are acquired as separate slices over some time (e.g., around 2 seconds), leading to a temporal delay between the first and last image. Slice-timing is a method for correcting this effect.

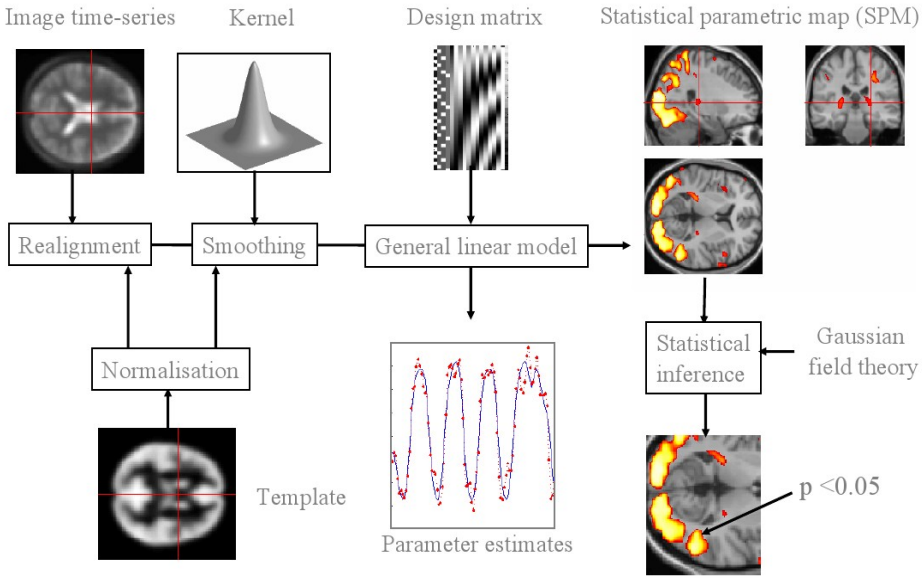


Illustration 3: Schematic of typical image transformations that start with an imaging data sequence and end with a statistical parametric map (SPM). Voxel-based analyses require the data to be in the same anatomical space: This is effected by realigning the data (and removing movement-related signal components that persist after realignment). After realignment the images may be subjected to non-linear warping so that they match a template that already conforms to a standard anatomical space. After smoothing, the general linear model is employed to (i) estimate the parameters of the model and (ii) derive the appropriate univariate test statistic at every voxel. The test statistics that ensue (usually T or F statistics) constitute the SPM. The final stage is to make statistical inferences on the basis of the SPM and Random Field theory and characterize the responses observed using the fitted responses or parameter estimates. From Friston (2007). Compared to this standard approach, the present project did not apply normalization or smoothing steps.

Motion correction

Minor movements are corrected for by applying spatial transformations to the images and aligning all images to the first image (i.e., a reference image). To align images to a reference image one commonly uses a six parameter rigid body transformation with three rotations (around the x, y and z axes), and three translations (in the x, y, and z directions). However, residual motion effects have been demonstrated even after such correction, and it has been suggested that further analysis should include these parameters in pre- or post-processing stages [15,16]. In the present study, residual motion is used as a nuisance regressor at the 1st level analysis, as shown in Illustration 5 (see the section on statistical testing for further details).

Co-registration

One of the most basic requirements for multimodal imaging is that we are able to define the spatial relationships between measures. To obtain registration between a series of images and a reference image, as seen in motion correction, determination of the transformation parameters can be estimated by minimizing the sum of squares intensity difference between the images [17]. However, this approach can not be used when one needs to register images of different modalities to each

other (such as T1 and EPI images). This is because the relation between tissue types and signal intensity is different between modalities. In this case, a different method, called 'mutual information' is used.

Spatial normalization

In addition to these procedures, a large proportion of fMRI studies apply spatial normalization and smoothing in order to make EPI images from individuals comparable, i.e. in order to allow for statistical testing of effects at the group level. However, there are problems associated with these methods that make them less desireable when studying effects in the MTL region. Spatial normalization is performed by structurally deforming individual brains in order to make different locations in each subject's brain correspond to the same locations in another subject's brain [18]. Studies of standard spatial normalization methods have found that MTL regions are poorly registered across individuals with standard methods [19], and systematically different in subjects with memory impairments [20]. Thus, spatial normalization procedures may spatially displace MTL activations when moving brains from native space into a standard frame of reference. This was also found in a pilot study in the present project, and presented at international conferences (see illustration 4). While this method used a standard procedure for spatial normalization in SPM2 (12 parameter affine registration in combination with non-linear basis functions), as has been traditional to previous research in this region, more recent approaches suggest that spatial normalization in this region can be improved [21-23].

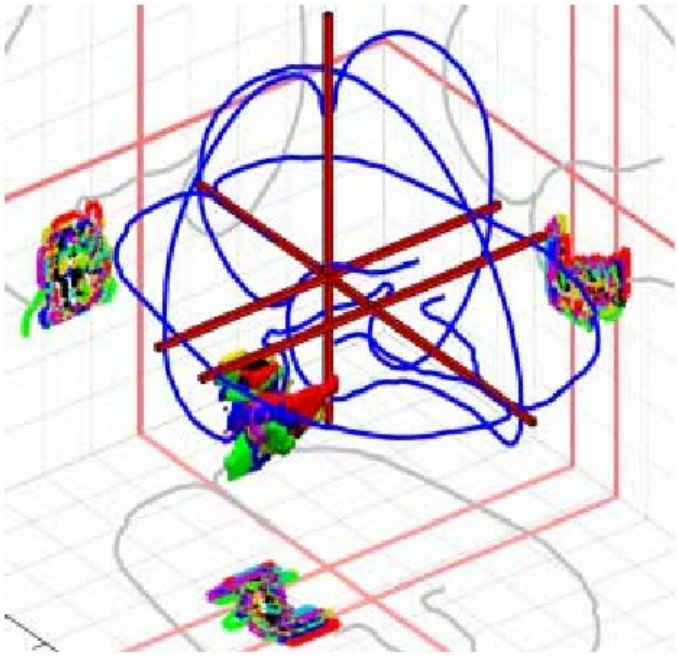


Illustration 4: Cornercube visualization (using the Brede toolbox) of the result of spatial normalization of the left PrC of 6 subjects (limited number for clarity). Normalization was a 12 parameter affine transformation to MNI space performed in SPM2. Note the large variation in shape, size and location of the different ROIs when mapped to the common MNI space. A perfect fit for all subjects would be displayed as one colour.

Smoothing

Smoothing, on the other hand, is a method for spatially blurring the images in order to reduce noise and remove imperfections in the spatial normalization. The smoothing procedure averages signals from neighbouring voxels and is often done by applying a Gaussian filter with a full width at half maximum of 6-8 mm. Normally, the filter should not exceed the size of the expected activation cluster. However, smoothing may lead to unwanted partial volume effects, as described in the next section. Looking at small regions, such as the perirhinal cortex, smoothing may lead to an undesired inclusion of information from neighbouring regions such as the hippocampus and entorhinal cortex, or white matter signal. By drawing regions of interest (ROI), spatial smoothing and these associated problems is usually avoided. In addition, the use of tissue classification and through this the analysis of grey matter voxels only, the problem with partial volume effects is thought to be minimal.

Statistical analysis: the general linear model

After successful pre-processing of images, statistical analysis is now possible. The motive for this analysis is to determine which voxels are activated by the stimulation paradigm, compared to a second brain state (typically a baseline fixation condition, but see later for discussion). The most commonly used approach is the general linear model (GLM). In the GLM, imaging data are compared to a model of the expected data, and the model is fitted to the time-course of each voxel. It may be written as

$$\mathbf{Y = X\beta + U}$$

where ***Y*** is a matrix with series of univariate or multivariate measurements, ***X*** is the (design) matrix, ***ß*** (beta) is a matrix containing parameters that are usually to be estimated and ***U*** is a matrix containing errors or noise. The residual is usually assumed to follow a multivariate normal distribution. If the residual is not a multivariate normal distribution, generalized linear models[Ŧ] may be used to relax assumptions about Y and U.

The general linear model incorporates a number of different statistical models of analysis of variance (ANOVA), linear regression, t-test and F-test. If there is only one column in Y (i.e., one dependent variable) then the model can also be referred to as the multiple regression model (multiple linear regression). Hypothesis tests with the general linear model can be made in two ways: multivariate and mass-univariate. In relation to the analysis of fMRI data, the analysis is typically performed for each voxel, resulting in so-called beta-values for each voxel.

The GLM allows modelling of effects other than the predictive variables that may modulate the response variable. Such factors include thermal noise, residual movements that are not corrected for with the rigid body transformation, and physiological noise related to cardiac pulsation and respiratory effects. The cardiac pulsation induces movements in the brain, especially in regions around arteries where the pulse-wave induces motion. The MTL region is particularly exposed to cardiac noise due to its location close to larger arteries of the brain. In addition, there may be differences between regions along the anterior-posterior axis in the proximity to these arteries, thus inducing an unwanted bias in the analysis, especially if these regions are compared directly (see next section).

A *first level* design matrix for time series analysis typically has one one row for every scan or sample, and one column for every explanatory variable. These variables include both effects of interest (e.g., cognitive task) and may also include confounding varibales (e.g., physiological noise). The modelling og residual artefacts at the 1st level is not often done in fMRI studies, although it has been shown that this may improve the estimate of neural activation relative to confounding biological and physical variables [16]. An example of a 1st level matrix is shown in Illustration 5, which is taken from the present encoding study. From this level of analysis, one can extract contrast values, which is a linear combination of beta values of choice, such as the beta values for object encoding and position encoding.

Ŧ The generalized linear model is a useful generalization of ordinary least squares regression. It relates the random distribution of the measured variable of the experiment (the distribution function) to the systematic (non-random) portion of the experiment (the linear predictor) through a function called the link function. A possible point of confusion has to do with the distinction between generalized linear models and the general linear model, two broad statistical models. The general linear model may be viewed as a case of the generalized linear model with identity link. As most exact results of interest are obtained only for the general linear model, the development of the general linear model has undergone a somewhat longer historical development. Results for the generalized linear model with non-identity link are asymptotic (tending to work well with large samples).

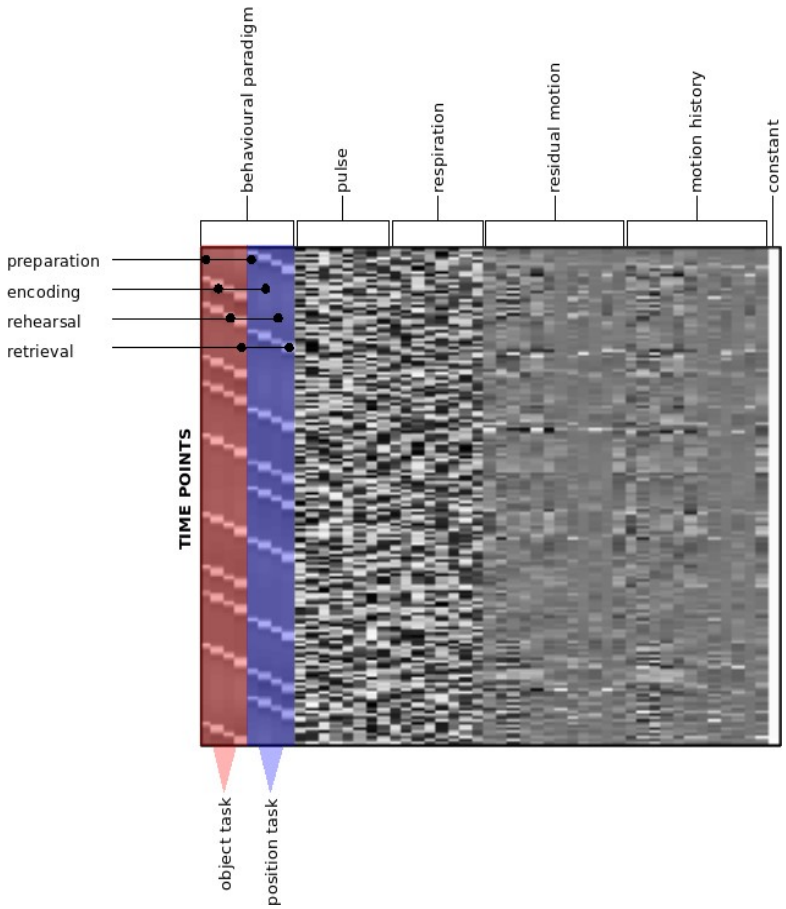


Illustration 5: Design matrix of first level (single subject) analysis with columns specifying predictors and rows specifying scans (time points). The first four columns (red) specifies object preparation, encoding, rehearsal and recognition stages from the episodic memory paradigm. The next four columns (blue) specifies the same stages for the position processing task. The next columns model pulse, respiration, residual motion and motion history. Gray scale (to white) levels refers to the values of the predictors. For example, in the first column high intensities correspond to the occurrence of object preparation, column two intensities correspond to object encoding ocurrences.

The *second level* (group) analysis in the present project did not apply the use of SPM, but used the calculated mean contrast values (the linear combination of beta values) for each MTL ROI. A first level, we did not model any baseline stage, but compared object and position processing directly for the preparation, encoding and rehearsal stages. Since the recognition stage consisted of significantly different visual stimuli, this stage was modelled but not included in the analysis. From each subject, we therefore used the contrast values for each ROI during each processing stage, and analyzed group effects at the 2nd level using statistical software such as Statistica (www.statsoft.com). The analyses are detailed in the methods sections of each manuscript. Basically, two approaches were used: for the young group, a one-sample t-test was used to test whether any region showed an effect of content, and Bonferroni correction for multiple omparisons was applied. For the effects of age, a multivariate GLM was applied to study the age-effects on these contrast scores, with gender and perfusion as covariates.

Artefacts in fMRI studies

BOLD fMRI has many advantages compared to other modalities for studying brain function (such as EEG and PET). However, there are several problems associated with this method. Some of these problems are directly relevant for the present project, and have been taken into account in the study designs. The treatment of some of these artefacts are discussed next.

In general, an image artefact is any feature which appears (or disappears) in an image which is not representing the original imaged object. An image artefact is sometime the result of improper images processing, and other times a consequence of natural processes or properties of the human body. It is important to be familiar with the appearance of artefacts because they can obscure, and be mistaken for, pathology, and they can influence regional signal values in an undesired way.

EEG = electroencephalography, PET = Positron Emission Tomography

Although image artefacts have many different sources, those that are considered to be most relevant to the present project are treated here, including susceptibility (including B_0 inhomogeneity), motion, microvascular structure, and partial volume effects.

Susceptibility

A magnetic susceptibility artefact is caused by the presence of an object in the field of view (FOV) with a higher or lower magnetic susceptibility. The magnetic susceptibility of a material is a measure of whether an applied magnetic field creates a larger or smaller field within the material. Materials that are diamagnetic have a slightly lesser field than in a vacuum, while paramagnetic materials have a slightly greater field. Ferromagnetic materials have a much higher field. As a result, the magnetic field lines bend into the object. Consequently, the fields change rapidly at various locations around the object. Often, this artefact is caused by metal, such as a titanium or stainless steel object inside the body, but they also occur in oxygen filled regions, e.g., the nasal cavities.

All magnetic resonance imaging studies assume a homogeneous B_0 magnetic field, especially when comparing activation in two or more brain regions. An inhomogeneous B_0 magnetic field causes distorted images. The distortions can be either spatial, intensity, or both. Intensity distortions result from the field homogeneity in a location being greater or less than that in the rest of the imaged object. The T2* in this region is different, and therefore the signal will tend to be different. For example, if the homogeneity is less, the T2* will be smaller and the signal will be reduced (or distorted). Spatial distortions result from long-range field gradients in B_0 that are constant in time. They cause spins to resonate at Larmor frequencies other than that prescribed by an imaging sequence. Ideally, spins at a single x position should experience a single magnetic field and resonate at a single frequency. With a distorted gradient, there is no linear relationship between position x and frequency n. Because linearity is assumed in the imaging process, the resultant image is distorted.

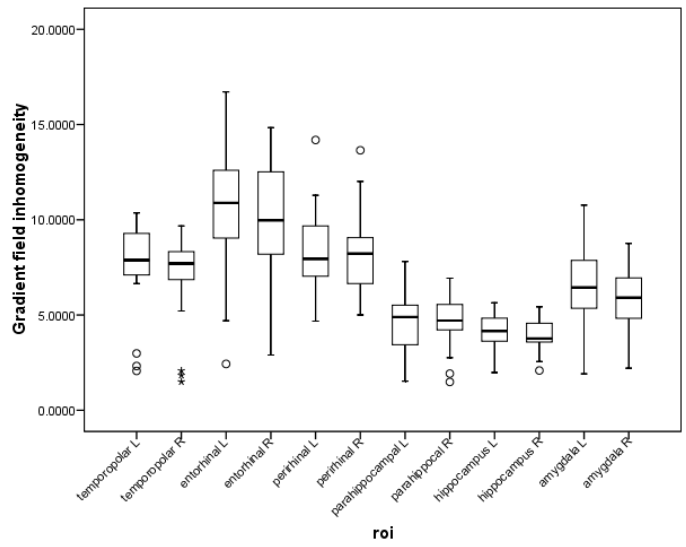


Illustration 6: Regional differences in gradient field inhomogeneity in the MTL. Boxes indicate upper, median and lower quartiles, whiskers indicate smallest and largest non-outlier observation, circles indicate outliers, stars indicate extreme values. Y axis uses arbitrary units.

In our first study, we were interested in direct comparison of the BOLD signal in ROIs within the same hemisphere. One possible problem may be the differences in B_0 inhomogeneity between MTL regions, thus producing unwanted systematic differences in regional BOLD fMRI values. In the MTL region, structures are unevenly positioned to areas with large magnetic vectors, and this may lead to systematic differences in BOLD estimates. In the present project, gradient field maps, used for the estimation of B_0 inhomogeneity, was acquired with a voxel size of 3x3x2 mm^3, FOV 192 mm, 33 slices, TR/TE(1)/TE(2) = 488/6.16/8.62 ms, and a flip-angle of 60°. The images were first coregistered to the AC-PC aligned structural image. Phase unwrapping was performed using the

SPM FieldMap toolbox. For each ROI, we calculated the mean magnitude of the gradient for all voxels within the ROI. This was used as an expression of how much the B_0 field changes within each ROI, which in turn, is known to influence T2* and thus BOLD signal. In order to test for this effect, we first applied a one-way ANOVA on the ROI values. Second, while there might be a difference between regions along the anterior-posterior axis, it is reasonable to assume that the B_0 inhomogeneity effect within bilateral ROI pairs are not significantly different. In order to test this, we applied a paired-samples t-test for each ROI pair. Finally, we tested whether B_0 value was related to BOLD contrast value within each ROI by running a correlation analysis, testing for both linear (Pearson) and non-linear (Spearman) correlations.

Our one-way ANOVA showed that there are significant regional differences in B_0 inhomogeneity ($F=13.566$, $p<.001$, $df=11$). Second, our comparison of ROI pairs showed that no ROI showed a significant difference between hemispheres. These results suggest that ROI values can be compared as hemispheric pairs (e.g., right and left perirhinal cortex). Finally, we performed a correlation analysis to test whether there were significant relationships between gradient values and BOLD regional contrast values and found that there were no significant relationships in the perirhinal cortex or parahippocampal cortex ROIs. This suggests that comparison between the perirhinal and parahippocampal cortex for each hemisphere are possible, even when there are differences in B_0 inhomogeneity.

Motion artefacts

Motion occurs in the imaged object or a part of the imaged object during the imaging sequence. This generally results in a blurring of the entire image with ghost images in the phase encoding direction. Movement of a small portion of the imaged object results in a blurring of that small portion of the object across the image. Small head movements are common in fMRI even if the subject is carefully fixated with cushions in the head coil and instructed not to move their heads. Other sources include respiration- and cardiac-induced movements. Illustration 6 demonstrates motion artefacts corrected for in this project.

Microvascular structure

It has been argued that systematic differences in microvascular structure and especially the relative alignment of draining veins to the static magnetic field (B_0 may have an impact on the BOLD signal [24,25,26,27]. In the MTL, regions such as the hippocampus, amygdala and the parahippocampal region have significantly different structural properties and microvasculature, and it is possible that this has an impact on regional differences in the BOLD signal.

Partial volume effects

In general, the term partial-volume artefact describes any artefact that occurs when the size of the image voxel is larger than the size of the feature to be imaged. For example, if a small voxel contains only fat or water signal, and a larger voxel might contain a combination of the two, the large voxel possess a signal intensity equal to the weighted average of the quantity of water and fat present in the voxel. Another manifestation of this type of artefact is a loss of resolution caused by multiple features present in the image voxel. For example, a small blood vessel passing diagonally through a slice may appear sharp in a 3 mm thick slice, but distorted and blurred in a 5 mm or 10 mm slice. In region based analyses, different region sizes are likely to be affected by partial volume effects differently. However, with the use of image segmentation and no smoothing of the EPI images, this effect can be argued to be minimal.

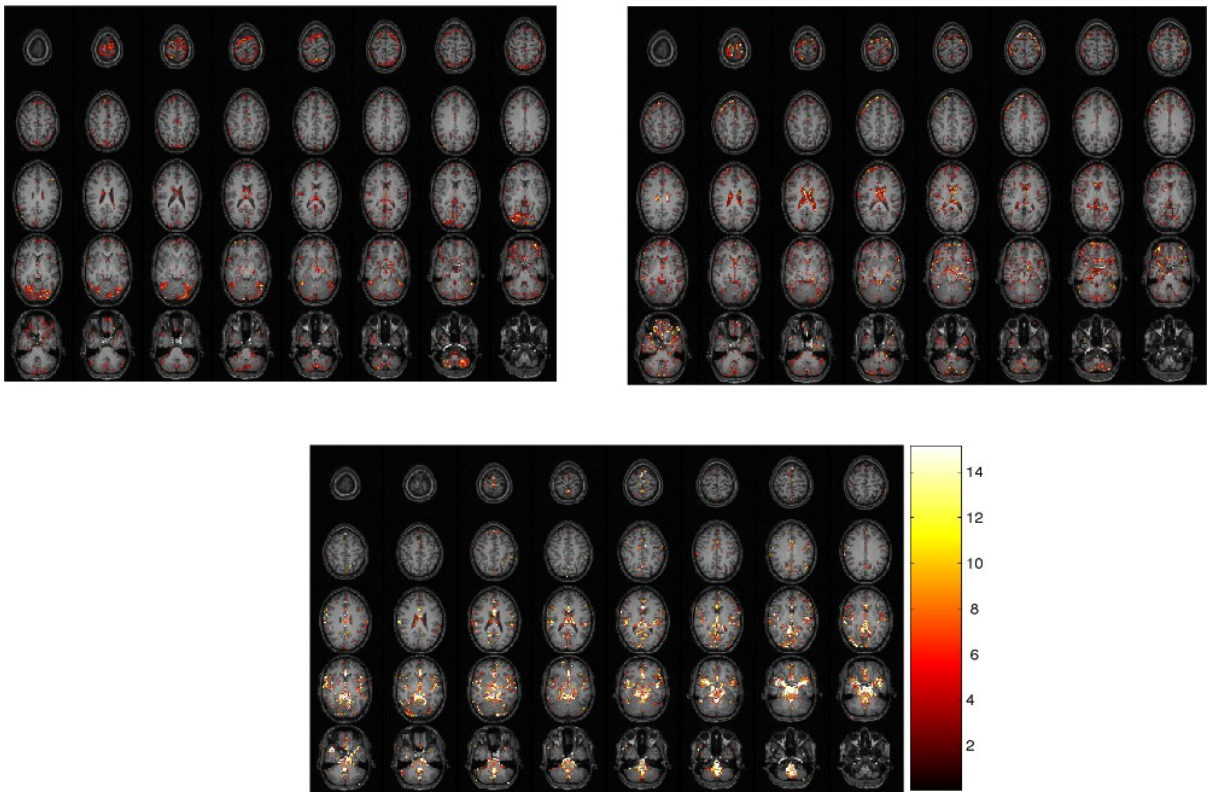


Illustration 7: The effect of noise on fMRI images. Top left = residual movement effects, top right = respiratory induced noise, bottom = cardiac-induced noise. The figures show (in colour) F-test values of the voxels showing significant (p=0.05 corrected using GRF) effect of a linear combination of the regressors describing each residual movement effect (F-test). It is seen that the residual movement effects are dominant near the edges of the brain; the respiratory induced noise is dominant near the edges of the brain as well as near in the larger veins and in the ventricles, and the cardiac-induced noise is dominant near larger vessels (e.g. medial cerebral artery and Circle of Willis). From reference 14.

Cerebral Blood Flow

The present study included an estimate of individual differences in cerebral blood flow (CBF). Previous studies have demonstrated an age-related reduction in CBF, and that this has an influence on changes in the BOLD fMRI signal. We assessed CBF by applying Arterial Spin Labelling MRI. Furthermore, our approach took into consideration that age-related changes in CBF may be mediated in a delayed, rather than a reduced, vascular response. Thus, the present ASL estimate used the individually optimized estimate, taking potential delays into account.

We used a narrow field of view for the ASL sequence, covering only the temporal lobe, and analyzed regional differences in CBF using the same ROIs as in the BOLD fMRI analysis. However, there were two causes for not using each ROI CBF value in the BOLD fMRI analysis. First, the perfusion calculation failed in many ROIs (~35%), meaning that individual ROIs often had too poor model fits to be consider valid values. These values were not systematically related to any region, so that they could be excluded by region. Second, a one-way ANOVA showed that there were no significant difference between regions (that were not excluded) on CBF value (F=1.3, p=.2241; see also Illustration 8)[Ŧ]. Consequently, we chose to use the median CBF ROI value as representative for CBF in all MTL regions. Thus, we were unable to use a region-specific correction during our analysis. It is therefore possible that the success of assessing regional differences may provide slightly different results. However, as there did not seem to be any major differences in CBF between regions (or even between hemispheres), one can assume that the calculation of a median CBF value is representative for both individual regions as well as the MTL as a whole.

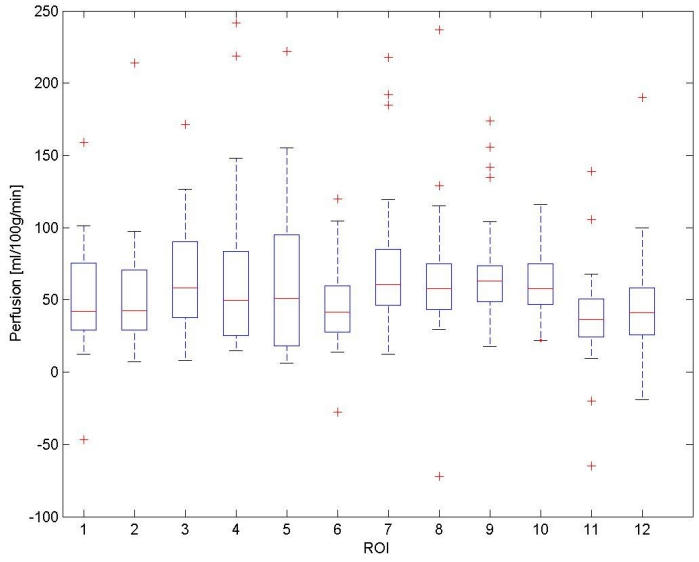


Illustration 8: Boxplot of regional differences in CBF values. Each plot shows the CBF value for each MTL region. Each box indicates upper, median and lower quartiles, whiskers indicate smallest and largest non-outlier observation, crosses indicate outliers.

In the analysis, we showed that some contrast values were affected by CBF. In particular, the contrast values in the left perirhinal cortex, right entorhinal cortex and left hippocampus showed significant effects of CBF. This suggests that although there were no effects of age on CBF in the MTL region, individual variance may have an impact on BOLD estimates of neural activation. Consequently, it is suggested that the inclusion of baseline CBF should be considered as a covariate when performing BOLD fMRI studies, even in healthy individuals, in order to reduce unwanted non-neural variance. However, these results also suggest that more work is needed in the estimation of regional CBF in the MTL region.

Ŧ In this analysis, we assumed that the data were normal distributed. However, if this criterion is not met, one would have to use a more advanced analysis approach, such as non-linear methods.

Regions of interest

In this project, we have applied a region of interest (ROI) approach, by using hand-drawn regions of the MTL on each individual brain. The following section briefly presents the protocol for each ROI. In the current project we have used MNI Display, a part of the MNI Register software package. This software allows the simultaneous viewing of all three slice orientations. This has proved essential to the correct drawing of the MTL region.

The ROI protocol has been created from the Insautsi[28] and Pruessner[29] protocols, and the atlas of Duvernoy[30] was consulted. In the following, we review some of the criteria for the MTL regions. We start out by outlining the entire (extended) MTL region as a whole, and then subdivide into smaller regions.

The entire MTL

The anterior border of the MTL is determined by the temporopolar region (TC). This is made by first finding the Gyrus of Schwalbe and the infero-temporal sulcus (see figure 3A).

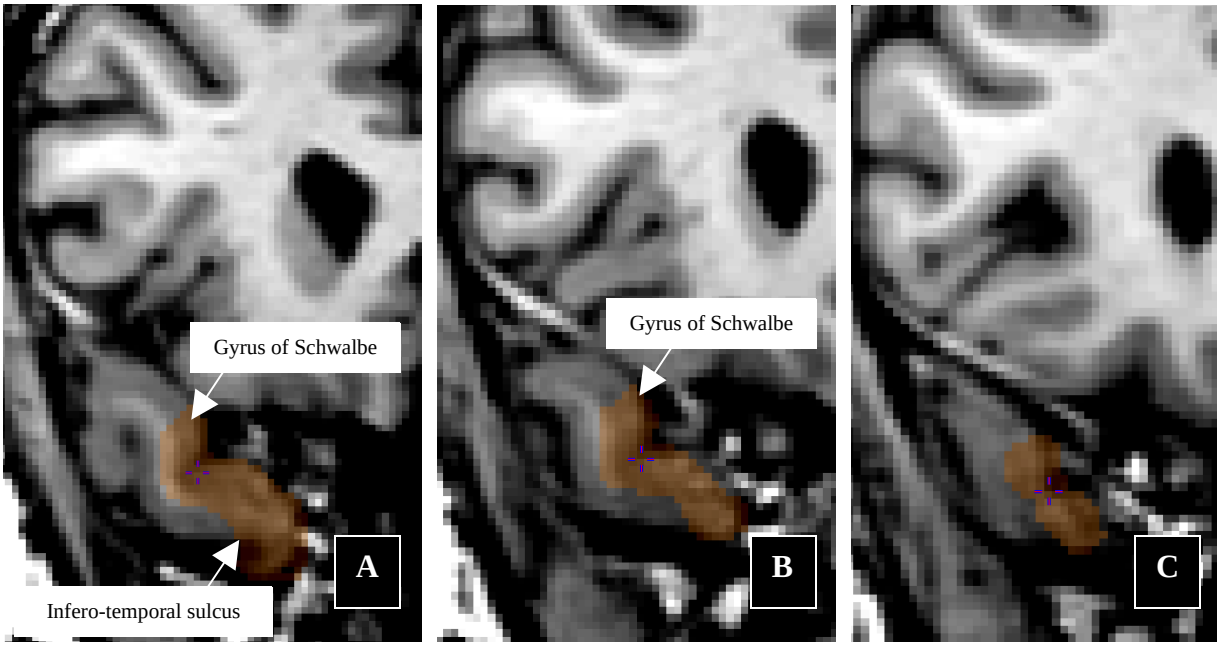


Figure 1 – Landmarks for the anterior borders of the MTL. Moving anteriorly through A – B – C

When these landmarks are found, we apply the localizations to the more anterior parts of the TC where either one or none of these landmarks are visible (see figure 1B & 1C).

Moving posteriorly, the ventrolateral border is determined by the lateral most part of the collateral sulcus. The dorsolateral border is the Gyrus of Schwalbe (see figure 2B). If the collateral sulcus appears late, the dorsolateral border is chosen as the dorsal part of the limen insula (see figure 2A).

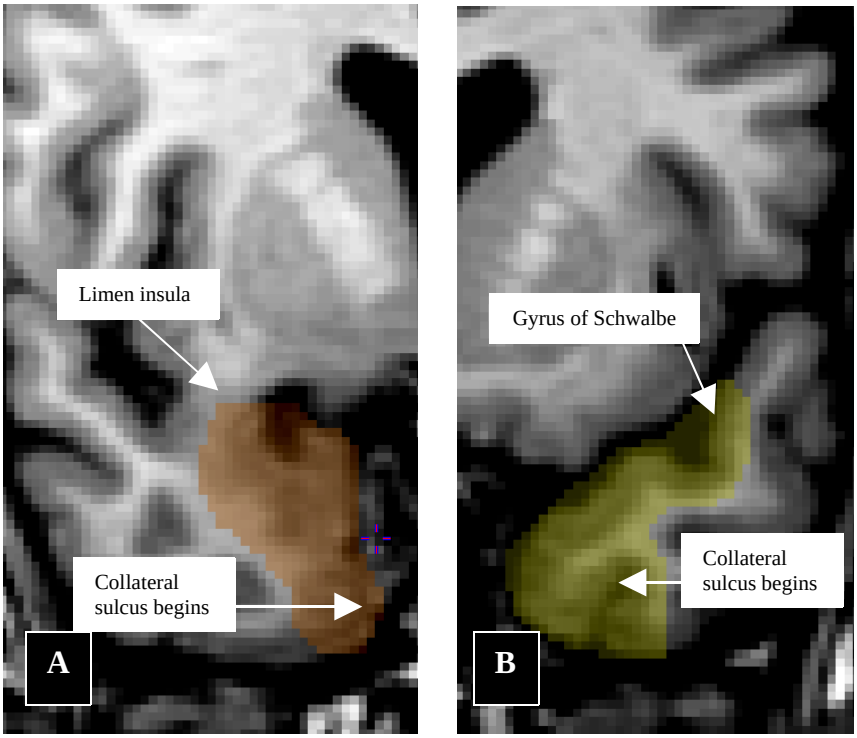


Figure 2 – Variations in MTL borders, the Gyrus of Schwalbe, limen insula and the collateral sulcus. Note: both images are from the same subject

At the level of the amygdala, the dorsolateral border includes the limen insula and the ventrolateral border is the lateral end of the collateral sulcus. This latter border follows the orientation of the collateral sulcus (see Figure 3 & 4, red line).

Further posterior, the amygdala and the collateral sulcus make out the borders of the MTL. The dorsolateral border is the amygdala, spanning the limen insula. The ventrolateral border is, as in the previous slices, the lateral end of the collateral sulcus (see figure 4).

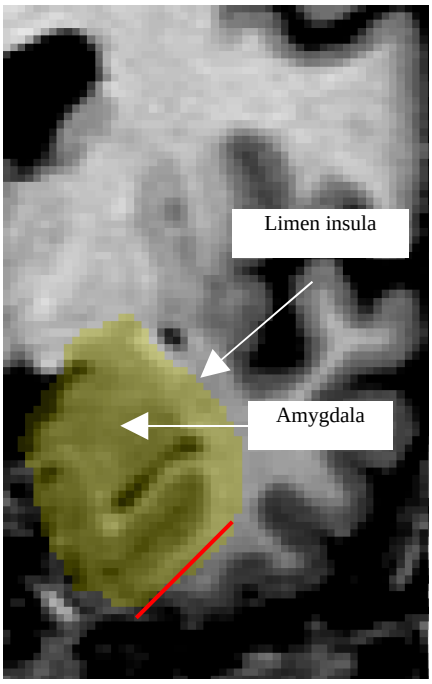


Figure 3 Borders determined by the amygdala / limen insula and the perirhinal region / collateral sulcus

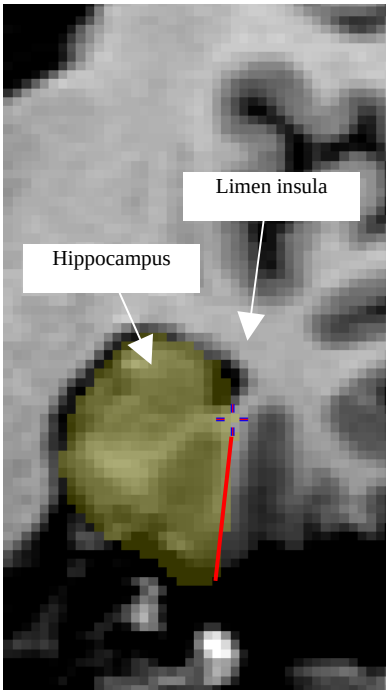


Figure 4 Borders determined by the hippocampus / limen insula and the parahippocampal region / collateral sulcus

At the level of the hippocampus we apply the same rule as with the level of amygdala; the dorsolateral border is determined by the hippocampus / limen insula, and the ventrolateral border by the lateral end of the collateral sulcus (see figure 5).

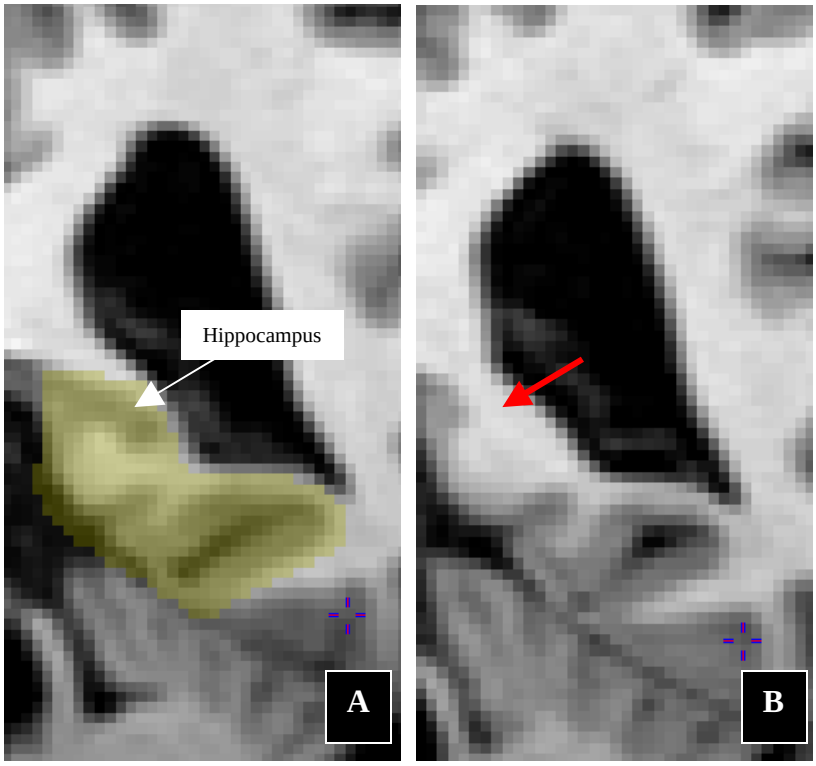


Figure 5 Borders determined by the hippocampal tail and the parahippocampal region / collateral sulcus (A). Note that although the end of the paraHPC is determined by the end of the hippocampal tail (B, red arrow)

At the posterior-most end of the MTL we use the borders of the hippocampus tail and the parahippocampal region / collateral sulcus (see figure 5A). The parahippocampal region ends at the same slice as the hippocampal tail disappears (figure 5B).

The amygdala (A)

Once the entire MTL is drawn as one structure, we start to extract the amygdala of each hemisphere. The anterior border is given as the first bulge of the dorsomedial part of the MTL, demonstrated in the figure below (B, red arrow). Before this bulge appears the region has the same appearance as the rest of the MTL (A).

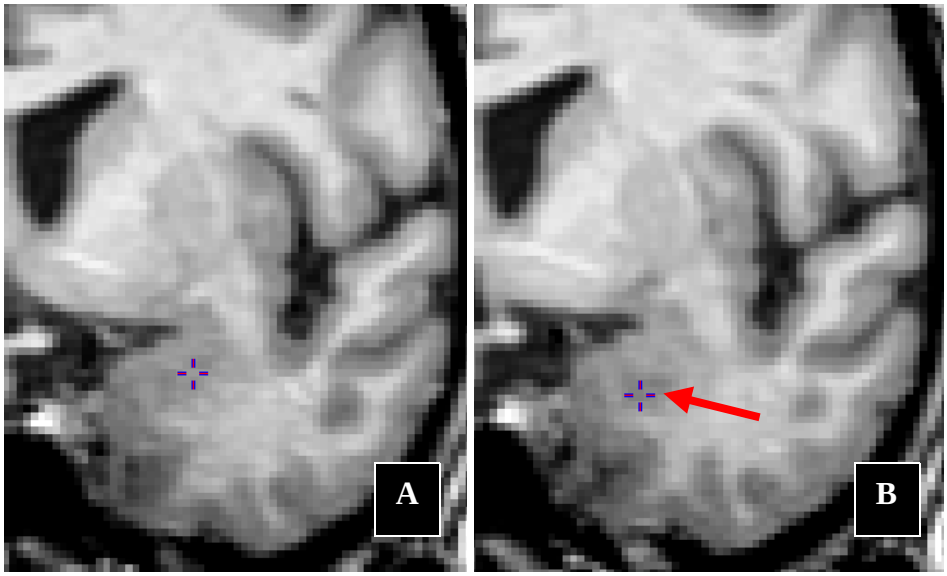


Figure 6 The appearance of the amygdala, occurring as a bulge (B, red arrow), while pre-amygdala slices do not show any similar structure (A)

We use the whole MTL ROI as an outset (see figure 7A) and draw the medial border of the amygdala (red lines) and fill it out (figure 7B).

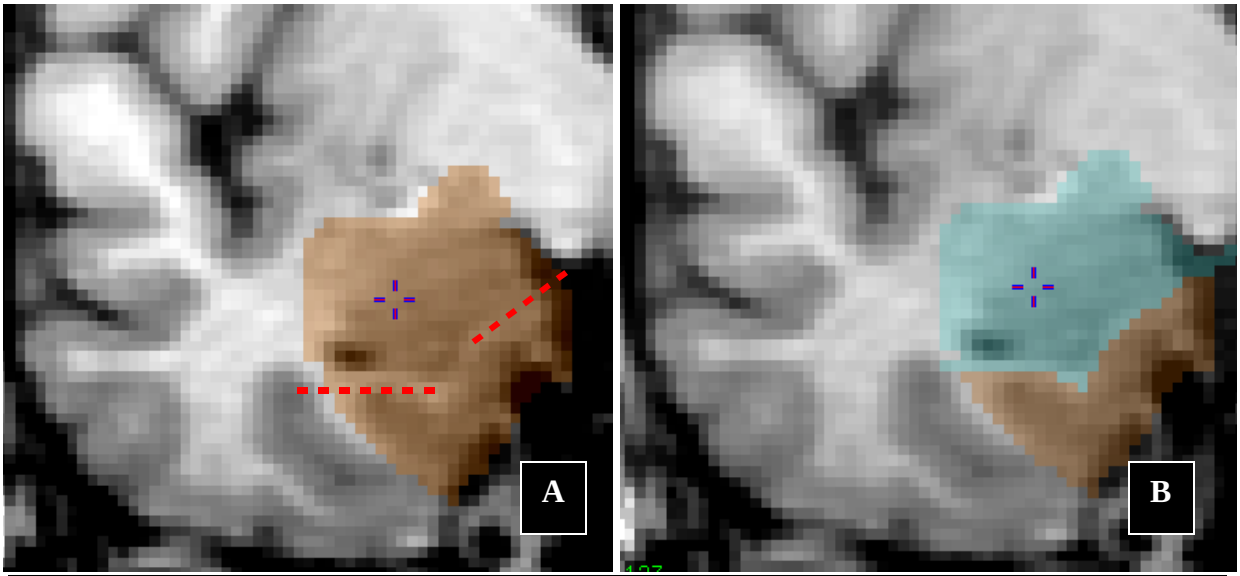


Figure 7 The amygdala is made out of the already existing MTL ROI. This is done by drawing a line (A, red dotted lines) and filling it out (B)

The caudal end of the amygdala is marked by the increase to full size of the hippocampus. The amygdala is seen dorsal to the hippocampus (figure 8A & B), and finally disappears dorsally (figure 8C).

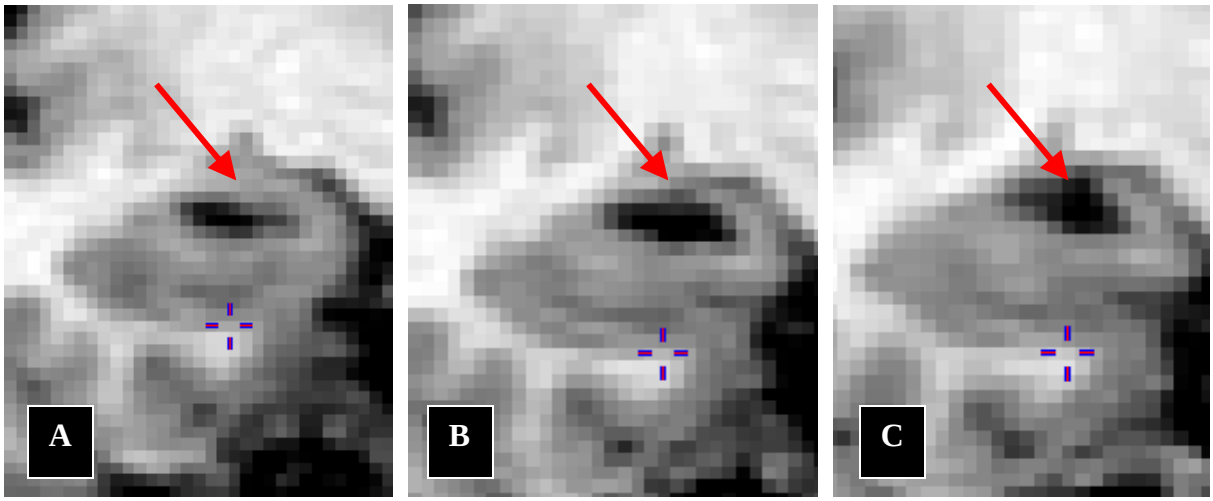


Figure 8 The caudal end of the amygdala.

The hippocampal region (HP) in 5 steps

The majority of the hippocampus is relatively easy to make out on saggital slices, although one needs first to draw the borders of the structure on other views. This is done in the following way:

1. The *anterior border* (the hippocampal head) is seen as a protrusion appearing inferior to the amygdala, as shown in Figure 9A, white arrow.
2. The *posterior border* (the hippocampal tail) is the disappearance of grey matter caudally, as shown in Figure 5.
3. The *medial border* is drawn on coronal views. The hippocampal medial border is the CSF medially to the region, as shown in Figure 9B, red line.
4. The lateral border is drawn on both coronal and saggital views. The lateral border is the white matter laterally to the grey matter, shown in Figure 9B, green line.
5. The rest of the hippocampus is drawn on saggital slices within the borders made out in steps 1-4, as shown in Figure 9C.

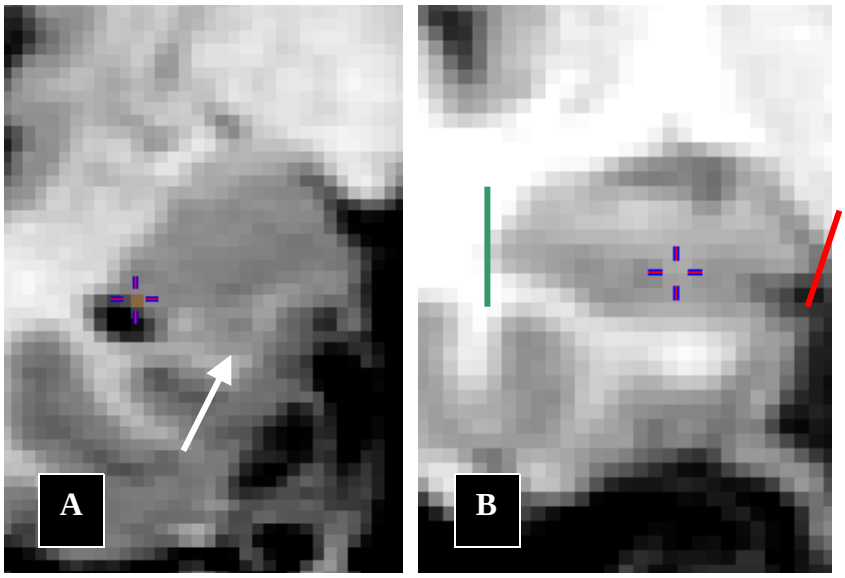


Figure 9 The hippocampal anterior (A), medial and lateral (B) borders.

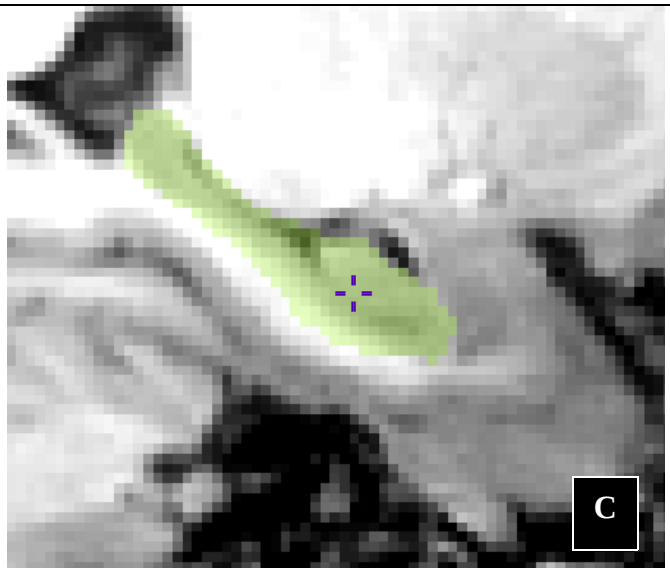


Figure 9 (cont.) The hippocampus proper (C)

The temporopolar region (TC)

The borders of the temporopolar region follow the outline in the first section:

- *The anterior border* is the temporal pole, and where the TC at its anterior most end inherits the dorsolateral and ventrolateral borders from previous sections, where the Gyrus of Schwalbe and inferotemporal sulcus are visible (see Figure 2)
- *The posterior border* is the same as the anterior border of the perirhinal region: the appearance of the collateral sulcus, as shown in Figure 10

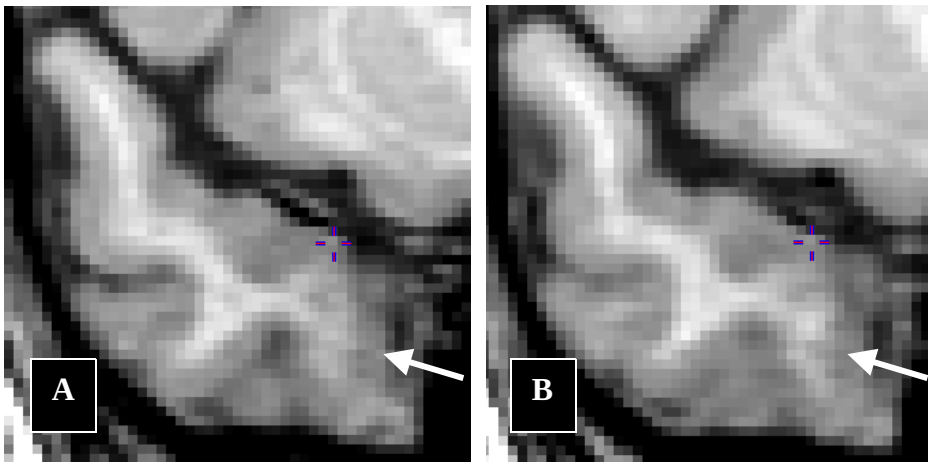


Figure 10 The appearance of the collateral sulcus (B), not visible in the more anterior slice (A)

The perirhinal region (PC)

As with the temporopolar region, the perirhinal region (PC) starts anteriorly at the appearance of the collateral sulcus. Then the following rules apply:

- The posterior border of the PC is given by the disappearance of the *gyrus intralimbicus* (Figure 11 A & B, white arrow)
- The lateral and medial borders are given by the orientation of the collateral sulcus (Figure 12)

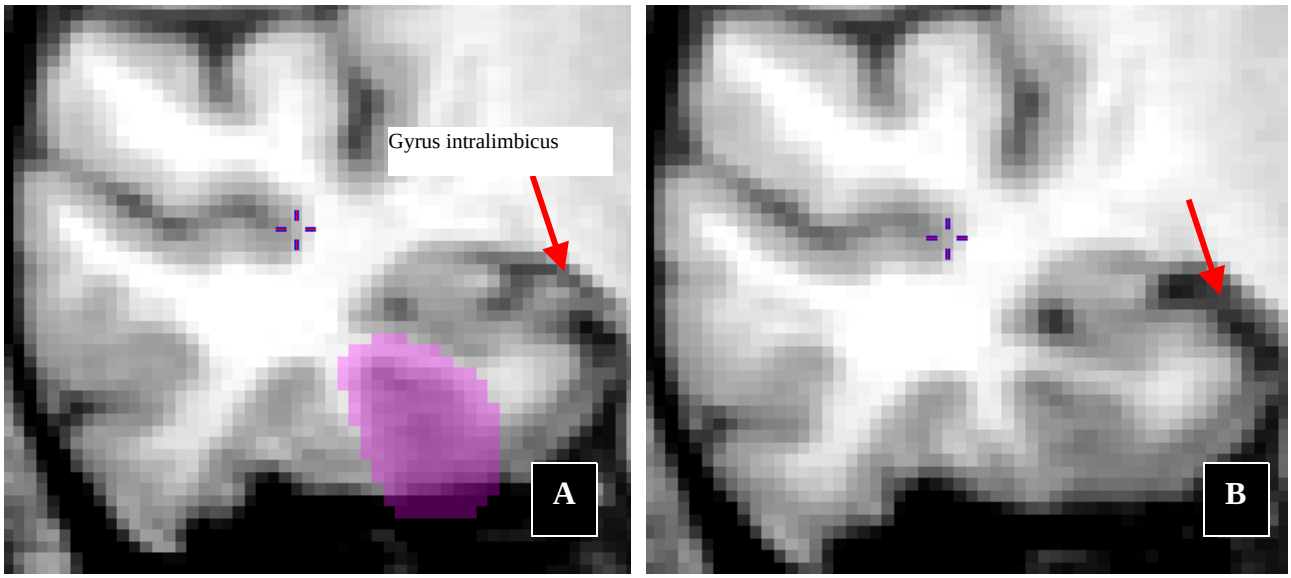


Figure 11 The disappearance of the gyrus intralimbicus (A) is the posterior border of the perirhinal region (B)

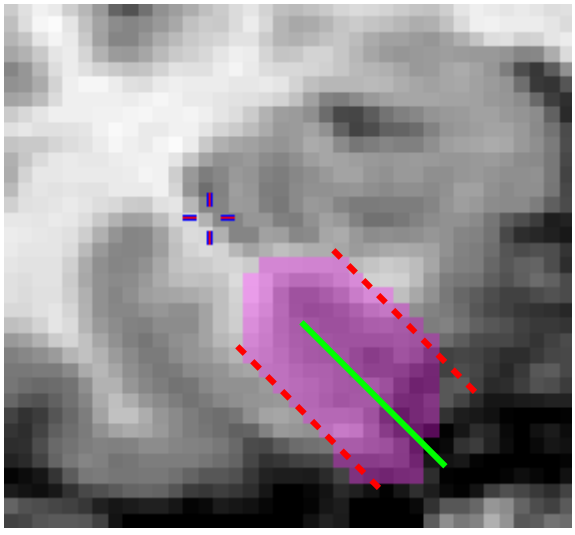


Figure 12 The medial and lateral borders (red dotted lines) of the perirhinal region are determined by the orientation of the collateral sulcus (green line)

The entorhinal region (EC)

The borders of the entorhinal region are given as follows:

- *The anterior border* is determined by the appearance of the sulcus semiannularis (Figure 13 B)
- *The posterior border* is determined by the disappearance of the gyrus intralimbicus, as with the perirhinal region (see Figure 11)
- *The medial border* are determined by the sulcus semiannularis (Figure 14)
- *The lateral border* is determined by the border of the perirhinal region, i.e. the collateral sulcus (Figure 14)

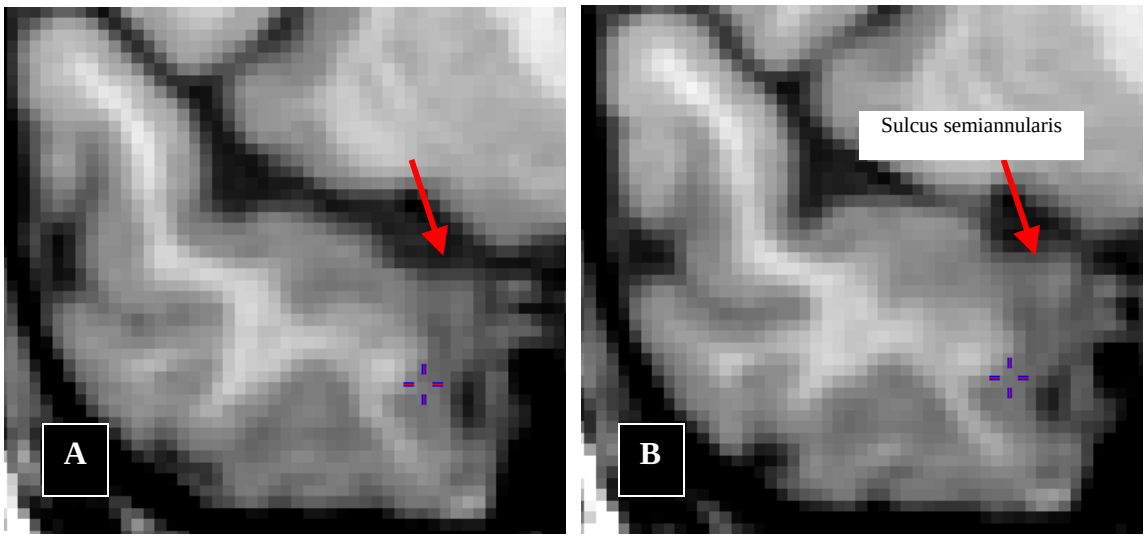


Figure 13 The appearance of the sulcus semiannularis (B) represents the anterior border of the entorhinal region

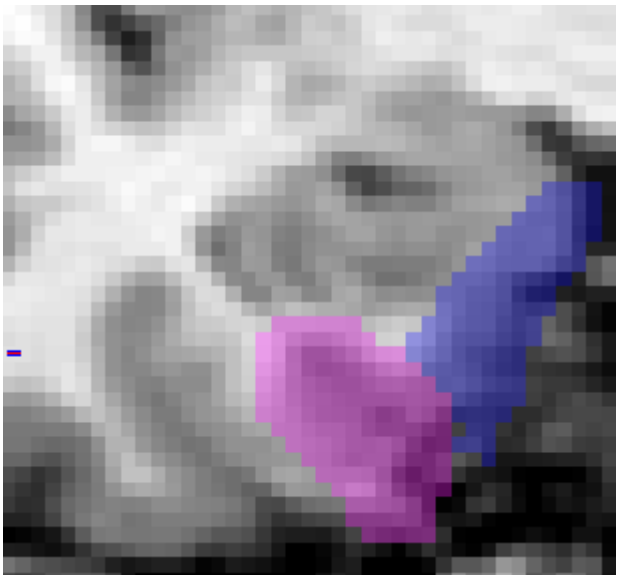


Figure 14 Medial and lateral borders of the EC (blue)

The parahippocampal region (paraHPC)

The borders of the paraHPC are now relatively easy to make out, given the previous constraints for other structures:

- *The anterior border* is at the disappearance of the gyrus intralimbicus, where the paraHPC overtakes the region of both PC and EC (Figure 15)
- *The posterior border* is given by the end of the hippocampus (tail), as seen in Figure 5
- *Medially and laterally* the paraHPC is constrained in the same way as the PC and EC.

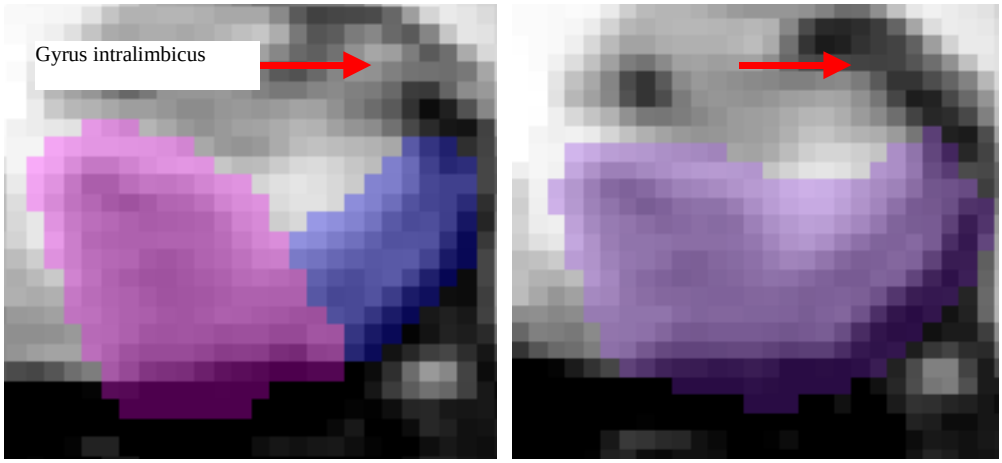


Figure 15 Posterior border between the PC, EC and paraHPC

Important notes

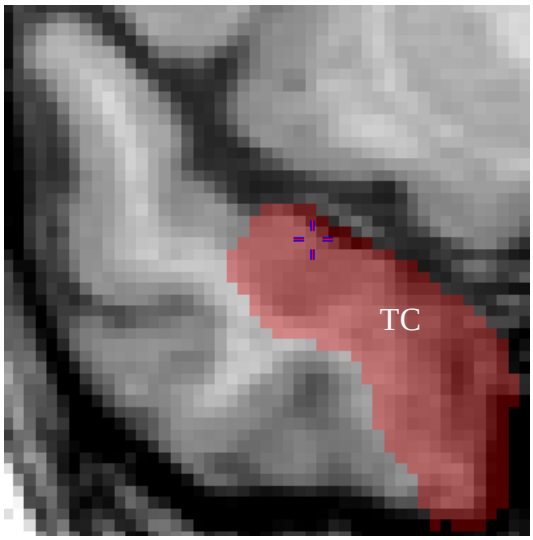
The decisions about these ROIs have been made from the outset of a specific problem. Since we do not have the cytoarchitectonic data (i.e. golden standard) available per subject our ROI drawings are *estimates* of the true regions. Optimally, we should adapt the ROI drawing to specific cytoarchitectonic landmarks such as the thinning of layer II and III when we go from the EC to the PC, or the part of the EC lying dorsally on top of the amygdala in some brains. However, such decisions rely heavily on what we can see in each brain. It is likely that there are systematic differences in the brains of healthy young and healthy old subjects, as well as MCI and AD patients. Such systematic difference means that a ROI drawing protocol that seeks to adapt to the brains features as much as possible is likely to be biased according to the brain type (e.g. MCI and AD relative to healthy old) that is evaluated.

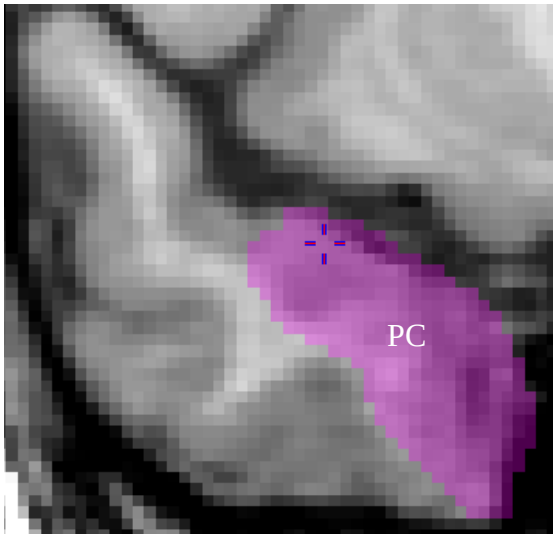
Therefore, this protocol seeks to find systematic landmarks that are available in all subject categories. As a consequence several differences from an "optimal" ROI are seen:

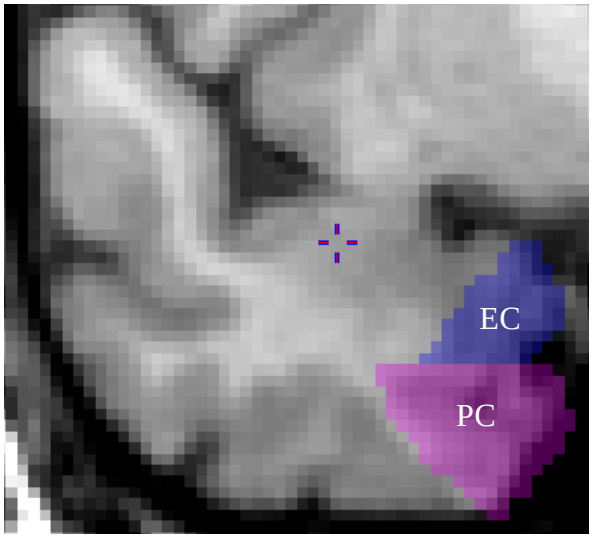
- *The temporopolar region* is smaller than that suggested by Insausti et al.[28], as we only draw the medial parts of the anterior most parts of the TC.
- *The perirhinal region* is larger than usual in the majority of the structure. This is due to the rigorous drawing of the PC according to the entirety of the collateral sulcus, where one often sees that the ventromedial parts of the sulcus are entorhinal. Including this area is probably relevant to the testing of MCI and AD, since it has been suggested that it is in exactly this structure that the neuropathology initiates. That being said, the posterior border of the PC determined by the sulcus semiannularis is earlier than a standard (adaptive) protocol, thus making the PC tail significantly smaller.
- As a consequence of the PC border determined by the collateral sulcus, *the entorhinal region* will be smaller than one would see according to an adaptive protocol.
- *The hippocampal and amygdalar regions* may be imprecise in the anterior and posterior ends. However, there is does not seem to be any reason to assume that this cause any systematic difference between the groups.
- *The parahippocampal region* may be evaluated as larger and longer with the use of an adaptive protocol, due to the anterior border being determined by the sulcus semiannularis.

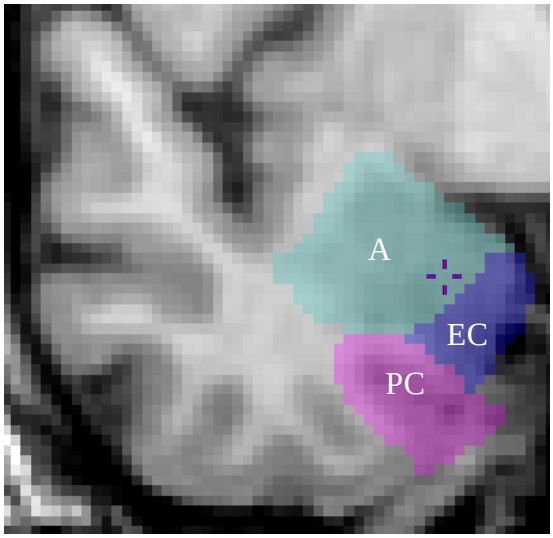
Summary: all ROIs

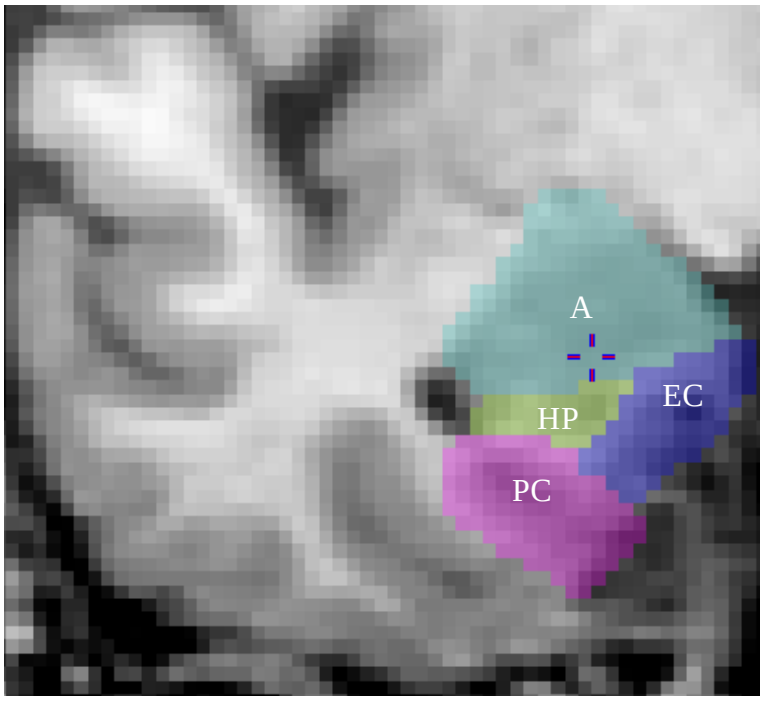
Here are the end examples of each different ROI:

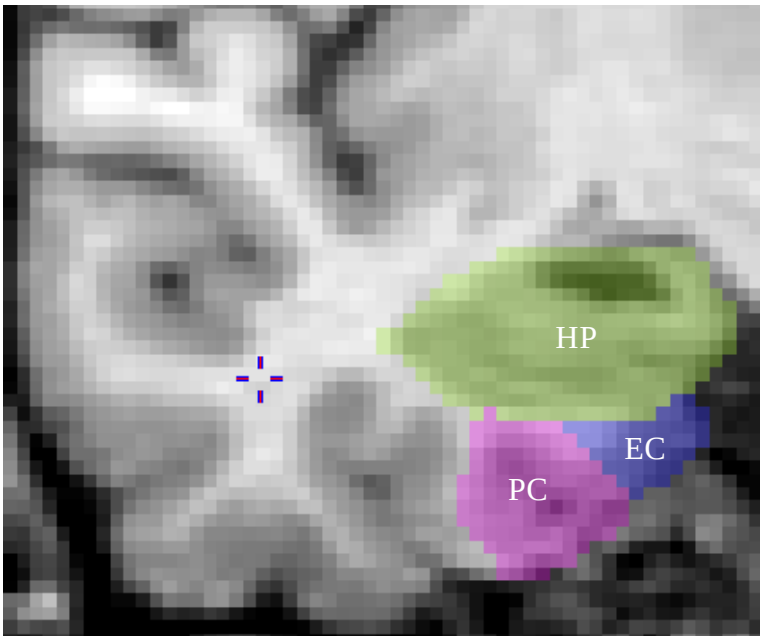


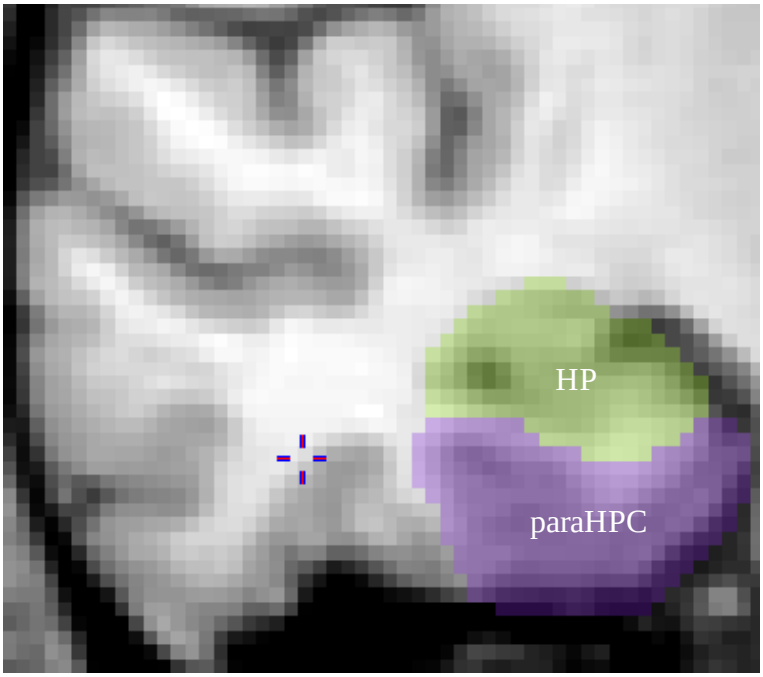







A
EC
HP
PC


HP
EC
PC


HP
paraHPC

Conclusion

The aim of this review has been to present the methodological background and considerations used in the present study. During the planning and pilot study phase, several discoveries were made that demonstrated the need for special attention to factors during image acquisition, image preprocessing and statistical analysis. These issues have been presented here. The methods deviced and implemented in this project including optimized imaging parameters and ROI based analysis are believed to provide a more robust, reliable and anatomically specific assessment of MTL activations. In addition, the inclusion of CBF as a covariate in the study of age-related effects on MTL activation may reduce inwanted individual variance. Taken together, the approach presented in this project is suggested as a model for future research using fMRI to study the MTL region.

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