Combining Behaviour, fMRI and MR Spectroscopy to Study Selective Attention in Ageing

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A thesis submitted to the
University of Birmingham
for the degree of
DOCTOR OF PHILOSOPHY

School of Psychology
College of Life and Environment Sciences
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October 2012

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Abstract

Advancing age is associated with marked atrophy in prefrontal brain structures coupled with behavioural differences across multiple cognitive domains, and yet evidence from functional neuroimaging frequently indicates overactivation (mainly in the prefrontal cortex) for older relative to young adults. This rather paradoxical result, arising from the unimodal application of different techniques, has led to multiple theories of cognitive ageing, where each of the theories provides a unique interpretation of its technique-related findings. To overcome this we need to develop multimodal approaches where the relations between brain function and behaviour can be mapped at molecular, functional and behavioural levels. In particular, investigating the links between neuronal activity, the neurochemical environment and the effects of ageing on behaviour are essential to progress in understanding age-related deficits in cognition. Localizing neural activity with functional magnetic imaging (fMRI) provides good spatial resolution and is useful to the application of magnetic resonance spectroscopy (MRS), where *a priori* knowledge of the brain function is needed to investigate the concentration levels of neurotransmitters. One of the neurotransmitters measured by MRS is the main inhibitory neurotransmitter γ-Aminobyturic Acid (GABA), which has been suggested to play an important role in the modulation of cognition, neuronal activity and ageing. Therefore, one of the main aims of the thesis is to correlate GABA levels to neural activity and cognitive performance by combining GABA-edited MRS and BOLD (blood-oxygen-level-dependent) fMRI correlates in response to a specific cognitive task in young and older adults. Furthermore, existing cognitive theories in ageing (the inhibitory deficit theory and the capacity deficit theory) were extended with predictions about the multimodal data acquired in the thesis.

Two well-established cognitive paradigms were adopted as models to examine age-related cognitive deficits in (i) attentional selection by saliency and (ii) the interaction between working memory (WM) and attention. The latter model was further deployed to identify the effects of ageing on BOLD- and GABA-correlates associated to the neural mechanisms of WM-biased attention. The results demonstrated that decreased levels of resting GABA were correlated to increased BOLD signal change in the right anterior insula (AI) / inferior frontal gyrus (IFG) during encoding in older adults relative to young adults and this was associated with a weaker effect of WM on attentional guidance. The combination of GABA-edited MRS and fMRI revealed that age-associated decrease of levels of resting GABA might be related to deficits in modulation of attentional selection, resulting from the allocation of neuronal resources to support encoding. The findings were further discussed in terms of how they fit the extended predictions of the cognitive theories of ageing.

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Acknowledgements

I would like to acknowledge several people for their invaluable contribution to the production of this thesis. I wish to convey special thanks to Prof Glyn Humphreys for his enthusiastic supervision, endless patience and encouragement. Despite his move to a position of great responsibility in Oxford, Prof Humphreys continued providing great support and found the time to proof read all I asked for, as well as the usual supply of insightful guidance. My thanks go as well to Dr Pia Rotshtein, who was always ready to help with her door kept propped-open. She also passed to me an invaluable scientific knowledge about the whole world of neuroimaging, planning, analysing, interpreting and presenting imaging data. I would like to thank Dr Theodoros Arvanitis for being supportive throughout the whole thesis process and particularly in the spectroscopy study. Thank you very much, all of you, for teaching me so much! I could not have wished for better supervisors. I would also like to thank Dr Richard Edden and Dr. Martin Wilson, for their great contribution and practical support throughout the acquisition and analysis of my spectroscopy data. I would like to thank Prof Zoe Kourtzi for providing my first experience in neuroscience research and giving me the foundation skills for starting this thesis.

Steve Mayhew is another person to whom I would like to express my gratitude for understanding my Eastern-European personality, silly conversations, being a true friend, and yet providing me with professional spirit at different stages of my thesis. Shan Xu, Dimitrios Sampanis and Johnny King Lau are other comrades that I am thankful to for their friendship, help in data acquisition at any time of the day and a great time spent together. Along with all my thanks to all participants for being patient and always happy to help, I wish to thank Denise Clissett for being always there to help with participant recruitment and everyday practicalities. I would also like to thank other staff and students scattered around the Hills Building, Frankland Building, Aitkin Building and BUIC for their excellent support and training.

In addition, I gratefully acknowledge the Biotechnology and Biological Sciences Research Council for funding my MPhil/PhD course.

Finally, I especially want to thank my wonderful wife, Agata, for standing beside me throughout my studies and writing this thesis. Her patience, understanding, and sacrifice provided continuous support, while keeping our home full of love and joy. In the course of the PhD programme, she also made me a proud father after giving birth and taking a great care of our beautiful son, Adam..His presence has been a well of positive energy and his smile a blessing, particularly during the last months of my thesis. Last but not least thanks to my family for being always there.

Contents

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LIST OF FIGURES

Glossary of common acronyms

Term	Definition [Unit]
3D	Three dimensional
B_0	Main magnetic field [T]
B_1	Magnetic field created by RF transmit coil (B1 $\perp$ B0) [T]
Cho	Choline
CNS	Central Nervous System
Ci	Citrate
Cr	Creatine
CSI	Chemical shift imaging
FFT	Fast-Fourier transformation
fMRI	Functional Magnetic Resonance Imaging
fROI	Functional region of interest
FID	Free induction decay
FOV	Field of view
FWE	Family-wise Error
FWHM	Full-width of half maximum [Hz]
GABA	γ – Aminobutyric Acid
GLM	General Linear Model
M_0	Equilibrium magnetization
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MRSI	Magnetic resonance spectroscopy imaging
NMR	Nuclear magnetic resonance
PRESS	Point-resolved spectroscopy
ROI	Region of interest
SE	Spin echo
SNR	Signal-to-noise ratio
SPM	Statistic Parametric Mapping
SVS	Single voxel spectroscopy
T_1	Longitudinal (spin-lattice) relaxation time [s]
T_2	Transversal (spin-spin) relaxation time [s]
T_2*	Transversal relaxation time including static magnetic
TE	Echo time [s]
TI	Inversion time [s]
T_M	Mixing time [s]
TMS	Transcranial Magnetic Stimulation
TR	Repetition time [s]
TSE	Turbo spin echo
VOI	Volume of interest
WM	Working memory

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CHAPTER 1. Introduction

1.1. Cognitive Neuroscience of Ageing

Over the last 100 years human life expectancy has increased dramatically following medical progress and improved sanitation. The global demographics of the world population show that the increase of life expectancy might result in a tri-fold increase of today's number of citizens in their third stage of life (above 60 years) to about 2 billion by 2050 (Zlotnik, 2007). It is likely that the number of older adults developing neurological disease associated with aging, such as stroke, dementia or Alzheimer's disease will increase as well. Indeed, nearly 50% of older adults over 85 years of age have some form of dementia (Hebert et al., 2003), exerting a burden to the individuals, their families and the health system. This has drawn the interest of scientists from different research areas including molecular biology, genetics, psychology, social sciences, epidemiology, economy and law to study the natural process of aging (Ballesteros et al., 2009).

A vast number of theories of aging have been proposed and all are consistent in arguing that ageing is a complex interaction of cultural, social, genetic, neurophysiological, neuropsychological, and biochemical processes at micro- and macromolecular levels (Conn, 2006). With advancing age, these complex interactions

cause a disturbance of homeostasis due to the inability of repair mechanisms to cope with the stress conditions, resulting in alteration of organs, system functions and cognition. With respect to the ageing central nervous system (CNS) including the brain, it is well established that there is a significant age-related brain shrinkage, an expansion of the ventricles and sulci due to cerebral atrophy, synaptic loss (Raz, 2005; Raz et al., 2007) and hypometabolism (Boumezbeur et al., 2010). Moreover, it has been shown that neuronal loss, vascular changes, glial and dendritic alterations are associated with the age-related decline in cognitive performance (Raz et al., 2007; Dickstein et al., 2007; Meltzer et al., 2003; Kochunov et al., 2008; Tisserand et al., 2004). However, our knowledge is scarce about the link between age-related biological alterations and any associated deterioration of cognitive function.

For example, research into cognitive ageing has been based predominantly on behavioural measures where differences in performance between different cognitive conditions for young and older adults are attributed to age-related deficits. Multiple studies observe consistently an age-related decline in a number of cognitive domains including perception, long term memory, working memory (WM), language, attention, speed processing (for a review Dennis & Cabeza, 2008). A number of cognitive theories have been put forward in an attempt to capture findings related to age-related cognitive deficits. Theories of cognitive ageing include the sensory deficit theory (SDT), the inhibitory deficit theory (IDT) and the capacity deficit theory (CDT). However, all these cognitive theories (which will be discussed below in detail) have no direct link with the

underlying neural mechanisms related to specific cognitive function and how these neural mechanisms relate to biological ageing.

More recently neuroscientists have used neuroimaging techniques to identify the underlying neural mechanisms related to specific cognitive functions. The combination of behavioural and multimodal neuroimaging approaches to studying the ageing brain (Cabeza et al., 2005; Grady, 2008) provides the opportunity to explain cognitive theories of ageing in relation to the findings from neuroimaging (Dennis & Cabeza, 2008). A substantial amount of evidence regarding the link between brain structure and function emerges from the contribution of positron emission tomography (PET) and magnetic resonance imaging (MRI).

MRI is non-invasive and provides high spatial visualization of morphological, chemical and functional changes within the brain. In the past decade, the advancement of MRI technology helped establish the technique successfully as a sensitive quantitative measure in neuroscientific research (Minati et al., 2007; Raz, 2005). There are a number of MR techniques implemented into brain imaging research specific to ageing, some of them include conventional MRI (T1-weighted images), Diffusion Tensor Imaging (DTI), functional Magnetic Resonance Imaging (fMRI), Magnetic Resonance Spectroscopy (MRS), resting state analysis (RS) and Arterial Spin Labelling (ASL). By exploiting the scanner in different ways, based on the information from the hydrogen protons of water molecules, these techniques measure different properties of brain tissue. Each methodology has its advantages and disadvantages, and their combination complements

each other. I consider the contribution of each technique to the research of ageing in separate sections below.

1.2. Neuroimaging in Ageing

This section aims at synthesizing the vast number of neuroimaging findings on cognitive ageing. At first I present literature from structural imaging including morphometry measures of grey matter (GM) and integrity measures of white matter. The following subsection presents neuroimaging evidence from functional imaging and a number of theories/models based on these measures. The last, third, subsection focuses on neurotransmitter measures, more particularly the most abundant inhibitory transmitter, γ-Aminobutyric acid (GABA) and its potential role in neurocognitive ageing.

1.2.1. Structural Measures

1.2.1.1. Gray Matter (evidence from VBM)

The conventional MRI experiment (most commonly T1- and T2-weighted images) provides information about the macroscopic environment of the tissue of interest based on the measurement of hydrogen molecules in water. There are two major applications of morphological MR images in the field of ageing: i) to deliver high spatial resolution in combination with other MR techniques (fMRI, DTI, spectroscopy); and ii) to investigate potential changes in the regional gray matter (GM) and white matter

volume as a function of age (Ashburner & Friston, 2000; Good et al., 2001) – a method called Voxel Based Morphometry (VBM).

VBM is a statistical approach for image analysis based on the comparison of brain voxel intensities between two groups of subjects (Wright et al., 1995; Ashburner & Friston, 2000). With the VBM analysis approach, scientists have been able to establish - *in-vivo* age-associated brain atrophy by looking at the global gray and white matter volume in different age groups of people. However, it should be noted that VBM has its inherent limitations and extra care should be taken when interpreting results (Thacker, 2008). Probably the two most concerning points related to the use of VBM are the segmentation and alignment steps, which might become less problematic once a better solution is taken than the currently used age-biased templates. For example, the alignment of all participants in one study to a common age-biased template cannot guarantee that each voxel in each image corresponds to the same stereotaxic point across all subjects. Another criticism is the choice of the smoothing extent. It has been suggested that the choice of the smoothing range should match the spatial extent of the tissue of interest (Rosenfeld & Kak, 1982). However, there is no overall agreement for the correct spatial extent when *a posteriori* information about the size of the volume of interest is lacking. Indeed independent VBM studies demonstrate that different choices of smoothing filter (from 0 to 16mm FWHM) highlight different significant areas (Jones et al., 2005; Park et al., 2004b).

Despite the current drawbacks of VBM, neuroimaging studies agree on a significant decrease in global gray matter (GM) volume and cortical thickness in healthy young adults at a rate 0.12% per year, which triples (0.35% per year) in adults over 52 years of age, suggesting a non-linear trend with age (Raz et al., 2005).

More interestingly, the decline in GM is not uniform across the whole brain; rather it varies across brain areas. For example, while frontal and temporal regions show a steep decline of GM volume (Giorgio et al., 2008; Kalpouzos et al., 2009; Good et al., 2001; Smith et al., 2007; Resnick et al., 2003), sensory specific brain areas remain preserved throughout the lifespan (Fallon et al., 2006; Tyler et al., 2010). This suggests that age-related decline in cognitive functions might be linked to specific brain areas sensitive to ageing which are also believed to support specific cognitive functions.

1.2.1.2. White Matter

Along with conventional MRI, diffusion weighted MR and diffusion tensor imaging have contributed to better understanding the microstructure of white matter in the ageing brain (Mori & Zhang, 2006). Similarly to VBM studies on grey matter, DTI studies have reported a non-linear trend for global loss of white matter integrity throughout the lifespan (Giorgio et al., 2008). Studies show that while there is an increased integrity in white matter throughout adolescence, white matter volume and integrity decrease linearly with advancing age (Abe et al., 2002; Pfefferbaum et al., 2000; Vernooij et al., 2008; Nusbaum et al., 2001; Pfefferbaum et al., 2000; Abe et al., 2008).

This suggests a nonlinear, 'inverted U-shape', age-associated trend of the global white mater volume, which reaches its peak in the second decade of life (Giedd et al., 1999; Snook et al., 2005; Giorgio et al., 2008) and afterwards decreases linearly with age (Pfefferbaum et al., 2000; Hasan et al., 2008). The rate of decrease in white matter throughout senescence is reported to be slower than for grey matter (Hsu et al., 2008).

Although there are a large number of studies examining age-related degeneration of white matter, supporting the idea for a global decrease of white matter with advancing age, there is still an inconsistency regarding the distribution and the extent of white matter microstructural alterations. The only region where changes have been reported consistently is the corpus callosum, particularly the genu of the corpus callosum (Sullivan et al., 2006; Hasan et al., 2008). Other common areas of white matter affected by ageing are the anterior corona radiata and the internal capsule (Hsu et al., 2008; Sullivan et al., 2008; Abe et al., 2008). In addition to these areas two recent studies have suggested the fornix and the hippocampal region to be age affected as well (Pagani et al., 2008; Vernooij et al., 2008).

There is also some evidence that the decline of white matter integrity in frontal areas is associated with cognitive performance in older adults. A number of large-scale studies have correlated DTI measures with cognitive performance and demonstrated that integrity in the anterior brain regions is associated with changes in a number of cognitive domains, including executive function, information processing and memory (Klingberg et al., 2000; Madden et al., 2004b; Forstmann et al., 2008; Sasson et al., 2012). The above

studies suggest that there is significant age-associated atrophy in grey matter and white matter in the frontal areas, and these alterations are associated with age-related deficits in a number of cognitive domains.

1.2.2. Functional measures

Since the beginning of the nineties, fMRI has increasingly become a powerful tool among psychologists to study cognitive functions. The signal acquired in fMRI is based on the difference of venous blood oxygen levels (Blood-Oxygen Level Dependent – BOLD) within a specific brain region during different conditions/tasks (for deeper understanding of the technique, refer to Logothetis, 2008). In research on ageing, the comparison of BOLD signal differences between younger adults and older adults in particular cognitive tasks can be attributed to age-related task-specific cognitive decline (Davis et al., 2008).

It is important to note that while the BOLD signal can be shown to be strongly coupled with synaptic activity indicated by local field potentials (Logothetis et al., 2001), it is also the indirect product of cerebral blood flow (CBF), the cerebral blood volume (CBV) and the cerebral blood oxygen consumption ($CMRO_2$) (D'Esposito et al., 2003). Thus, vasculature changes with age, such as stenosis, curtosis and elasticity (Rother et al., 2002; Derdeyn et al., 1999), might lead to alterations in the neurovascular coupling (D'Esposito et al., 1999a; D'Esposito et al., 1999b), though more research in this field is required.

In the previous sections, structural findings related to the ageing literature were discussed suggesting that there is an extensive decline in brain integrity associated with poorer cognition. Taking into account the effect size of the atrophy in the ageing brain it is somehow surprising that older adults perform at levels as high as young adults especially after accounting for generalized slowing. One particularly intriguing enigma for neuroscientists is to understand how the ageing brain can manage in most cases to operate as well as that of a young adult, given the presence of age-related atrophy and loss of homeostasis on a molecular level (reviewed in the following section) (Park & Reuter-Lorenz, 2009). Interestingly, findings from neuroimaging studies show some potential for unravelling the enigma. It has been shown that the brain has the ability to change and adapt, referred to as the neuroplasticity of the ageing brain (Goh & Park, 2009). For example there can be recruitment of additional brain regions (apart from those specialized in a specific cognitive function) to compensate for reduced function with age. There is a vast number of studies showing additional recruitment of neural areas in older adults relative to young adults, however, with different patterns of activation. A number of theories have come up to generalize the patterns of activation.

One model is the Hemispheric Asymmetry Reduction in OLDer adults (HAROLD) model. This suggests that there is an age-related tendency for less lateralized brain activation during cognitive tasks for which younger adults show a unilateral recruitment (Cabeza, 2002). The HAROLD model has been mainly observed in the prefrontal cortex (PFC) during a variety of cognitive tasks involving visual attention

(Cabeza et al., 2004), working memory (Park et al., 2003; Cabeza et al., 2004), implicit memory (Bergerbest et al., 2009) and episodic memory (Daselaar et al., 2003; Anderson et al., 2000) in young and older participants with matched cognitive performance. For example, according to the hemispheric encoding/retrieval asymmetry (HERA) model (Nyberg et al., 1996) the left prefrontal cortex is recruited in encoding in young adults; in contrast, the right tends to be more active during retrieval,. In older adults this laterality is reduced and yet performance is maintained at high levels (Cabeza et al., 1997; Salami et al., 2012). The shift of HERA to HAROLD with ageing is also supported by studies using transcranial magnetic stimulation (TMS) to momentarily disrupt cognitive performance. For example, Manenti et al. (Manenti et al., 2011) applied TMS over the left and right PFC during encoding and retrieval in young and older adults. The findings showed that memory performance in young adults was affected by TMS in the left PFC during encoding and in the right PFC during retrieval. On the other hand, the same stimulation had less effect in older adults. The authors suggested that the unstimulated hemisphere in older adults might support the processing of the inhibited hemisphere. The HAROLD model proposes that the age-associated decrease in lateralization may result from compensatory alterations or network reorganisation, both of which can be influenced by either cognitive strategy changes or alterations in neural architecture (Cabeza, 2002).

Another model suggesting the involvement of compensatory mechanisms with age emerges from studies reporting an age-related decrease in occipital activity coupled

with increased brain activations in frontal areas to match performance of young adults (Smith et al., 2001; Langenecker et al., 2004; Grady et al., 1999; Cabeza et al., 2004; Fischer et al., 2005; Gunning-Dixon et al., 2003; Nielson et al., 2002). This pattern of activity refers to the Posterior-Anterior Shift in Aging (PASA) model (Davis et al., 2008). The PASA model was observed in a variety of conditions where demands are placed on visual attention including: sustained attention task (Cabeza et al., 2004) and visual target detection (oddball) tasks (Madden et al., 2004c), visual perception (Levine et al., 2000), working memory (Grossman et al., 2002; Rypma & D'Esposito, 2000), and both episodic encoding (Dennis et al., 2007) and retrieval tasks (Daselaar et al., 2003; Cabeza et al., 2004). This consistency of findings provides evidence for a general tendency of frontal recruitment with reduced hemispheric lateralization reflecting a compensatory function with ageing to aid cognitive performance.

1.2.2.1. Compensation

In theory, *compensation* should imply that older adults should recruit brain regions in addition to those specifically implicated in the task in young adults, to match the behavioural performance of the young adults (Cabeza et al., 2002). Another possibility might be that the BOLD signal change in those regions recruited only by older adults is correlated to the behavioural performance - larger BOLD signal change reflects better performance (Grady et al., 2005; Dennis & Cabeza, 2008; Davis et al., 2008; Vallesi et al., 2011). For example, Vallesi (2011) had young and older participants carry

out go/no-go task and found that the conflict resolution-related activity in areas including the dorsal PFC and PPC, as part of the dorsal attention network, was higher in older relative to young participants. More importantly, the modulation of the dorsal attentional network correlated with efficient inhibition only in older adults but not in young adults. Taken all together, these studies indicate that older adults recruit additional brain areas (often the frontal areas) relative to young adults and the extent to which these areas are activated by older adults is positively correlated with task performance.

However, there is also evidence that over-recruitment of additional brain regions in older adults is not necessarily associated with better performance. In the study of Morcom et al. (2007) it was shown that, after matching performance across age groups, older participants showed widespread over-activation in task specific regions coupled with less efficient inhibition of task-irrelevant brain regions. In addition, others have observed that over-activation in the PFC in older adults is inversely correlated to performance in cognitive tasks including memory encoding, retrieval and a number of visual tasks (de Chastelaine et al., 2011; Persson et al., 2011; Garrett et al., 2011). More interestingly, Garrett et al. (2011; 2012) showed that these over-activations associated with low behavioural performance were linked to lower variability of BOLD signal change coupled with greater behavioural variability on cognitive tasks in older relative to young adults. Taken together, while in some cases over-recruitment might be considered as a compensatory mechanism, there are some cases where over-recruitment might be

taken as an indication of less effective modulation of neural populations to support cognitive function (Rypma & D'Esposito, 2000; Reuter-Lorenz & Lustig, 2005).

1.2.2.2. Dedifferentiation

To further explain age-related over-recruitment with less efficient modulation of task-specific functional networks (Logan et al., 2002; Rypma & D'Esposito, 2000), it has been proposed that these observations might reflect a dedifferentiation of the neural system (see for a review Grady, 2012). Dedifferentiation in ageing relates to the loss of efficient modulation of functional specialization, coupled with an overrecruitment of non-selective brain regions in the cognitive task (Logan et al., 2002; Park et al., 2004a; Morcom et al., 2007; Park & Reuter-Lorenz, 2009; Garrett et al., 2011). According to this view, if older adults are showing widespread activation in specific tasks then there should be fewer distinct regions involved across a number of different tasks relative to young adults (Grady, 2012). For example, the retrieval of three different kinds of memory is associated with unique patterns of activity in specific brain regions in young adults: autobiographical memory in middle temporal lobe (MTL), episodic retrieval in dorsolateral PFC and parietal regions and semantic retrieval in the left temporal cortex (St-Laurent et al., 2011). In St-Laurent et al. (2011), older adults showed more widespread activation with an overlap of brain regions during episodic and autobiographical retrieval, but a similar pattern of activity in semantic memory when compared to young adults. This view is consistent with previous reports showing

increasing deficits in episodic and autobiographical, but not semantic memory throughout the lifespan (Grady, 2012). These studies suggest that while young adults are able to modulate task-specific mechanisms for a cognitive task, older participants show less efficient recruitment of task specific regions coupled with increased activations in task-irrelevant brain regions.

Interestingly, recent findings relating to reduced BOLD variability between cognitive states with ageing have been extended to the dedifferentiation hypothesis (Garrett et al., 2011; Garrett et al., 2012). Garrett et al. (2012) showed that in young adults and fast performing older adults there is increased variability in the neural response during a task condition relative to a fixation. In contrast, slow older adults show fewer changes in brain variability within and across experimental conditions. The authors proposed that the age-related decrease of task variability across and within conditions represents a more rigid and uniform neural system, which is switching less efficiently between different neural states, as well as showing a reduced ability to process efficiently varying and unexpected external stimuli (Garrett et al., 2012).

So far, I have covered two general observations in the research on ageing – the compensatory model, where overrecruitment coupled with better performance is treated as an indication of compensatory mechanisms, and the dedifferentiation model, where overactivations are coupled inversely with performance and are thought to be less efficient modulation of a particular network.

I would like also to note that overrecruitment in brain regions has not always been found in older relative to young adults. In some cases, older adults show weaker or even no brain activation recruitment, coupled with poor performance relative to young adults. This is particularly evident under conditions with high cognitive demands where cognitive capacity might be exceeded and the compensatory mechanism fails to deliver performance (Dennis & Cabeza, 2008). One theory that might explain these findings is the Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH)(Reuter-Lorenz & Cappell, 2008). This posits that, as the cognitive demands in a particular task increase, so will more brain areas be recruited; however, at high cognitive levels the compensatory mechanism is less effective resulting in less activation for older adults relative to young. An interesting study by Schneider-Garces et al. (Schneider-Garces et al., 2010) showed this clearly where young and older participants were required to carry out a Sternberg-like memory search task by varying the working memory (WM) load with the number (2 - 6) of letters to be encoded for a memory recall. When comparing two conditions at low WM load (e.g. 2 < 4 letters) young adults recruited the left PFC, while older adults activated the PFC bilaterally. Interestingly, at the comparison of conditions at high WM load (4 < 6 letters) young adults showed a bilateral PFC recruitment (similar to the pattern observed in older adults at low WM load), while older adults did not show any increase, suggesting a resource ceiling for older adults has been reached (Cappell et al., 2010; Rypma et al., 2007). The data also suggested that the differences in activation between age groups might be explained by overall different WM

demands for a specific condition, which could be accounted for if difficulty was adjusted subjectively (i.e. young participants to carry out the task with 6 letters, whereas the older only with 4 letters). Therefore, the CRUNCH account predicts that different patterns of activation reported in ageing studies might reflect overall WM demand available in a participant, rather than ageing per se and therefore, if possible, it becomes important to match task difficulty across age groups.

1.2.2.3. The STAC Theory – can it link all together?

Another model to explain both compensatory and dedifferentiation activity is the scaffolding theory of ageing and cognition (STAC), recently proposed by Park and Reuter-Lorenz (2009). STAC presents an integrative framework to accommodate age-related neurocognitive findings, where persistent overactivations of the prefrontal areas are taken as neural indications of the ability of the neuroplastic brain to engage in compensatory processing ('scaffolding')(Park & Reuter-Lorenz, 2009). The concept of scaffolding can be presented in view of the neural change of the brain in response to the acquisition of a novel skill (Petersen et al., 1998). The neural response during the initial stage of acquiring a new skill involves activation in a broad network of regions but as training progresses the neural mechanism "shapes up" into a more specific neural circuit. Petersen et al. (1998) also showed that after participants reached skilled performance the brain regions that provided initial scaffolding at the early stages of practice remained minimally active; regions that remain on "stand-by" for a recruitment under challenge to

aid performance (Petersen et al. 1998). Here, I will refer to these regions as *scaffolding areas* and the mechanism to activate these areas as the scaffolding process.

Park & Reuter-Lorenz (2009) propose in their STAC hypothesis that the scaffolding areas during initial stages of training might play an important role in cognitive ageing as an additional support in response to challenges posed by structural and functional deterioration. Thus, scaffolding processes are recruited in challenging situations by young and older adults. Young adults recruit scaffolding areas in highly challenging situations and during the acquisition of new skills, whereas older adults have an increasing need to activate scaffolding mechanisms to cope for basic cognitive operations that become more effortful as a result of neural deterioration.

Park & Reuter-Lorenz further suggest that scaffolding areas and scaffolding mechanisms provide a supplementary and a complementary role to achieve a cognitive goal. Furthermore, as scaffolding is developed later in life through the acquisition of new skills it is most likely that the brain structures involved are the most versatile and late to mature (Grieve et al., 2005), i.e., the prefrontal cortex. This proposal fits with most neuroimaging findings in ageing showing overrecruitment predominantly in the PFC (for a review see Grady, 2012), probably as an indication of scaffolding areas being active.

Another interesting aspect of the STAC hypothesis is that scaffolding can be regarded as a neurocognitive response to challenge, where the nature of the challenges can be external (novel, unexpected, or increased levels of task demand) or intrinsic (metabolic and structural changes within a neural circuitry). Intrinsic challenges can be

further subdivided to transient, e.g. in response to sleep deprivation (Drummond et al., 2004), or continuous, as a result of biological ageing (Bertoni-Freddari et al., 2006). A clear example is the study of Schneider-Garces (2010, the CRUNCH model), where it was shown that while young adults activated only the left PFC during low WM load conditions, older adults activated the PFC bilaterally at low WM load, similar to young adults under high WM load condition.

The STAC theory further predicts that (i) scaffolding networks are less efficient than task-specific cognitive networks; (ii) the ageing brain is less efficient at generating scaffolding than the young brain; (iii) the efficiency of scaffolding is determined by genetic factors, environment, illness, physical training and cognitive activity (Park & Reuter-Lorenz, 2009).

Furthermore, because the STAC model is so flexible, it could provide the backbone of a framework able to accommodate new measures from different modalities. One example is the obserservation of reduced variability of neural systems with ageing (described above, Garrett et al., 2011; Garrett et al., 2012). Variability within and across experimental conditions and brain areas was proposed to be an indication of invariability-based neural specificity which, according to STAC, would reflect recruitment of task-specific regions/networks, without the presence of scaffolding. Age-related decreases of task variability in the view of STAC model should reflect scaffolding regions.

Taken all together, STAC presents an integrative framework to accommodate age-related neurocognitive findings, where persistent overactivations of the prefrontal

areas are related to either compensation or dedifferentiation, which can be linked to the context of plasticity, challenge and the ability to adapt.

1.2.3. Neurotransmitter measures

Magnetic resonance spectroscopy (MRS) has been used for more than four decades in basic physical, chemical, and biochemical research, e.g. as an analysis technique or for determining the structure of complex molecules, but recently it has started drawing the attention of neuroscientists. MRS, like magnetic resonance imaging, is based on the principle of nuclear magnetic resonance, but the objective of spectroscopy is an analysis of a substance rather than imaging of an object. In-vivo MRS is focused on the measurement of metabolite concentration within an organ of interest. Proton MRS measured in 3T scanners can detect around a dozen metabolites including creatine (Cr), choline (Cho), N-acetyl-aspartate (NAA), lactate (Lac), myoinositol (MI), glutamate (Glu) / glutamine (Gln), lipids and gamma-amino butyric acid (GABA).

Although the main focus of this thesis examines the role of one particular metabolite (GABA), here is given a brief overview of age-related changes in metabolite concentrations as measured by standard magnetic resonant spectroscopy (MRS) methods. NAA is synthesized in the mitochondria of neural cell. MRS findings show evidence for an age-related decline in NAA concentrations, which are associated with a loss of neural cells, decreased neural metabolism, decreased microstructural integrity of white matter and a reduced number of dendritic structures (Birken & Oldendorf, 1989). There is a

consistency in the literature that absolute and relative concentrations of NAA decrease with aging (Haga et al., 2009). Pfefferbaum et al. (1999) reported a significant correlation between poorer cognitive performance during memory and language tests and lower NAA concentrations in the GM of older adults. Choline (Cho) concentration is directly associated to the degree of membrane proliferation and it is more useful in the detection of abnormalities such as cancer (Miller et al., 1996), rather than in ageing research. Creatine (Cr) is assumed to have constant concentrations within different brain areas and with age, which make it a candidate as a reference metabolite. The concentration of Lactate increases when oxidative metabolism fails, and has been reported to be a neuron energy substrate in stress conditions (Aubert et al., 2005) and therefore Lactate is not of primary interest in ageing research. Myoinositol is considered as a glial marker and has been reported to have slight concentration increases in cortical gray matter with age (Chang et al., 1996). Glutamate (Glu) is one of the major excitatory neurotransmitters released by neurons and there is evidence for an age-related decline in motor cortex (Kaiser et al., 2005). Glu has been suggested to play a major role in neurovascular coupling and the generation of the BOLD signal (Attwell & Iadecola, 2002), as well as in a 5:1 ratio with GABA across a number of regions and species (Lehmann et al., 2012). Therefore, the role of Glu should be investigated more thoroughly, since it could be important in understanding the link between the age-related changes in (i) BOLD signal, (ii) the inhibition/excitation relationship and (iii) cognition. Glutamine (Gln), a precursor

of glutamate, is mainly found in the glial cells and is observed to increase in white matter as a function of ageing (Martinez-Hernandez et al., 1977).

GABA is the main inhibitory neurotransmitter in the human brain and GABAergic interneurons play a major role in the modulation of cortical glutamatergic inhibition (Buzsaki et al., 2007; Jonas & Buzsaki, 2007). In addition, recent studies have shown that MRS measures of GABA concentration have been associated with cognitive function and BOLD signal change, where high BOLD signal change was inversely correlated with GABA (Muthukumaraswamy et al., 2009), whereas negative BOLD response was correlated with high GABA (Northoff et al., 2007).

With respect to the main inhibitory neurotransmitter, GABA, previous studies have demonstrated age-related decreases in resting GABA within the dorsolateral prefrontal cortex (dlPFC), the orbitofrontal cortex (OFC), the sensorimotor cortex (Grachev et al., 2001), the superior frontal gyrus (SFG) and the superior parietal lobule (SPL) (Gao et al., submitted). Gao et al. (submitted) measured GABA in the frontal (ACC) and the parietal cortices (middle PPC) in one-hundred healthy participants (age range 20 to 76 years of age, 20 participants per decade) and found significant decreases of GABA concentration with age in both frontal and parietal lobe. Non-age focused studies, with relatively limited age ranges, have looked at the relationship between GABA levels and age (e.g. in the frontal lobe (Goto et al., 2010; Aufhaus et al., 2012) and the occipital lobe (Goddard et al., 2001)), but did not find reliable differences between GABA levels and ageing. One possibility for the discrepancy might be that the

small age range included in these studies might have not been sensitive to track GABA changes associated with age. Taken together, these findings suggest that GABA concentrations in older adults may reduce across a number of regions. In support of the argument, Leventhal and colleagues (2003) demonstrated that decreased orientation sensitivity associated with increased age can be restored to that seen in young animals with GABAergic stimulation (e.g. GABA agonist), whereas administration of a GABAergic antagonist abolishes orientation sensitivity in young animals. The above findings suggest that the inhibitory transmitter GABA may play a role in cognitive ageing and its decrease in a number of brain regions as a function of age might be related to modulating age-related deficit in cognition.

1.2.4. Summary

Taken together, the neuroimaging studies consistently show a morphological and molecular decline in age. The decline of GM and white matter is not global, but rather region specific, suggesting that cognitive functions specific to these brain regions are sensitive to ageing, while others remain intact throughout the lifespan. However, a large portion of studies relate to age-related differences with measures that are particular to the publication, making it difficult to characterize the ageing brain. Future research in ageing might benefit from combining multimodal measures (fMRI + MRS) to provide a better understanding of the neural mechanisms for age-related cognitive decline. In addition, this might provide an opportunity to understand the commonality between ageing theories and models and to fuse them in a more general hypothesis.

The next section aims at converging the neuroimaging evidence and theories of cognitive ageing.

1.3. Convergence of Neuroimaging Evidence and Theories of Cognitive Ageing

In the following section I will cover two cognitive theories in ageing: the inhibitory deficit theory (IDT) and the capacity deficit theory (CDT) and attempt to link the theories to neuroimaging data.

Each subsection is composed of three parts: 1) an introduction to a cognitive theory of ageing with an example from the cognitive literature; 2) an outline of how the theory could be represented at a neural level, supported by an example; and 3) a discussion of how the theory could be represented on a neurochemical level.

1.3.1. Inhibition Deficit Hypothesis

One explanation for cognitive decline in ageing comes from the inhibition deficit theory (Hasher & Zacks, 1988; Lustig et al., 2007). This theory posits that the ability to suppress distracting information is less efficient as we get older and so irrelevant information may fall in the focus of working memory, resulting in increased interference in response to goal-driven information. Evidence for the inhibitory deficit theory emerges from studies using negative priming. Classic studies of negative priming have presented cases where an item's identity is inhibited (when another item presented at the same time has to be selected), slowing the item's subsequent identification (Tipper & Cranston, 1985).

Reduced negative priming in older adults might reflect less efficient cognitive inhibition[1] when stimuli are first encountered as distractors (Hasher et al., 1991). In addition, there is evidence for age-related changes in cognitive location-based inhibition, for example in preview search tasks. Preview search typically uses conjunction-like displays but presents distractors with one common set of properties prior to the second set of distractors plus the target (Watson & Humphreys, 1997). Provided there is a sufficient period between the two sets of distractors (of the order of 400ms or so), the first set of distractors can be efficiently ignored (Humphreys et al., 2004). There is substantial evidence that the lack of impact of the initial distractors is dependent, at least in part, on a process of active distractor suppression of its location (Watson & Humphreys, 2000; Humphreys et al., 2004; Allen et al., 2008) and features (Olivers & Humphreys, 2003). As in studies of negative priming it has been shown that older participants can show a selective reduction in the efficiency of preview search, particularly under conditions where distractor inhibition is challenged (e.g., with moving distractors). This is consistent with reduced distractor inhibition as participants age (Watson & Maylor, 2002).

According to Dennis & Cabeza (2008) the inhibitory mechanism might be represented as a coupling between two regions in a specific functional network implicated in cognitive tasks. The control region is an inhibitory control region, which will exert inhibition over the inhibited region in order that it remains "silent", so that the

[1] The term "inhibition" can be referred to either cognitive inhibition or neural inhibition (MacLeod, 2007). To reduce ambiguity between both concepts of inhibition, I refer to the full term ("cognitive inhibition", "neural inhibition") where necessary.

inhibited region does not process any information (distracting information). Thus, the pattern of BOLD signal change in brain regions implicated in an inhibitory mechanism might look like a positive BOLD response in the control region (e.g. prefrontal areas) coupled with a negative BOLD response in the inhibited region (e.g. sensory areas) (Dennis & Cabeza, 2008). Similarly, it has been argued that negative BOLD responses in the visual cortex are thought to be an indication of decreases in neuronal activity reflected by increased neuronal inhibition (Shmuel et al., 2006; Shmuel et al., 2002; Devor et al., 2007; Pasley et al., 2007; Mevorach et al., 2009b; Mevorach et al., 2010). For example, Mevorach et al. have reported reduced activation of left occipital cortex when distractors have to be ignored linked to increased activation of left PPC. They argued that the selection of the relevant information was mediated by the left PPC which suppresses distractor-related activity in early occipital cortex. Interestingly, suppressive TMS to the left PPC increases interference from distractors and this effect is greatest when TMS is applied prior to the onset of the stimulus (Mevorach et al., 2010). The above predicts that the pattern of activity representing an inhibitory deficit in older adults would be a reduced modulation of control regions (mainly frontal and parietal areas) coupled with an increased "uncontrolled" activity in early processing regions (generally sensory areas) reflecting an increased processing of irrelevant information (often referred to *disinhibition*). To further extend this to compensatory mechanisms, older adults might show more recruitment of control regions coupled with better cognitive performance as an indication of compensation for an inhibitory deficit in early processing areas.

One example of the inhibitory deficit theory in ageing from a neuroimaging point of view is the study of Milham et al (Milham et al., 2002) where young and older adults carried out a colour-word Stroop task in an fMRI environment. Relative to young adults, older adults demonstrated a reduced recruitment of dorsolateral PFC and parietal regions (control regions) as an indication of decreased inhibitory control, coupled with an increased activation in the temporal cortex (early processing region) as an indication of processing the word to be inhibited (*disinhibition* of the temporal cortex).

There is also evidence that older adults may compensate for reduced activity in inhibitory control by recruiting additional control regions relative to young adults. For example, young adults activate the right PFC in response to conflicting No-Go trials in a Go/No-Go task, whereas older adults have been shown to recruit both the right and the left PFC (Nielson et al., 2002). This is also consistent with the HAROLD model, where older adults show less lateralized patterns of activation in tasks where young adults show unilateral recruitment. The above examples support the idea that older adults may compensate for inhibitory deficits by recruiting additional control regions, while showing less ability to inhibit early processing regions.

From a neurochemical point of view, age-related reductions in GABA concentration (Gao et al, submitted) might play a specific role in the attentional processes related to cognitive inhibition of distracting information and they may play a part in any inhibitory deficit on cognitive level. As reported above, negative BOLD responses in visual cortex may represent a decrease of neural activity perhaps reflecting increased

neuronal inhibition. This pattern of response may be expected to be observed in regions implicated in early signal processing after successful inhibition posed by the control region. Evidence in support of this notion comes from the work of Northoff et al. (2007), where it was demonstrated that GABA levels in the ACC specifically modulated negative BOLD responses when emotional pictures were viewed, suggesting that GABA might play a role in attentional processes related to inhibition of early processing regions. Interestingly, Gao et al. (submitted) found that the GABA concentration in a similar region (ACC) in older adults is reduced compared to young adults. Taken together, age-associated decreases of resting GABA levels might be related to a decreased ability to modulate control regions indicated by underactivation in frontal areas and less efficient inhibition of early processing of signal indicated by overactivation in sensory areas. In other words there is increased *disinhibition* with ageing. In addition, GABA levels in individuals showing successful compensation (Cabeza & Dennis, 2013) should correlate positively with the modulation of inhibitory mechanisms as well as with better performance.

1.3.2. Capacity Deficit Theory

Older adults show deficits in maintaining and updating information in working memory (WM) for use in higher-order processing tasks (Reuter-Lorenz & Sylvester, 2005). In addition, it has been demonstrated that, according to the predictions of the capacity deficit theory, under conditions with reduced WM resources young adults show

similar cognitive deficits to older adults (Jennings & Jacoby, 1993; Anderson et al., 1998). Interestingly, the deficits in older adults are more pronounced under conditions including executive functions along with working memory, including memory updating, reordering, or inhibition, while being less noticeable under simple span tasks (Dobbs & Rule, 1989; Babcock & Salthouse, 1990). For example, Dobbs and Rule (1989) had participants ranging from 30 to 70 years of age carry out a number of working memory tasks. In the complex WM task (N-back task) older adults showed marked deficits relative to young adults, while in the easy WM condition (forward and backward digit span tasks) performance was matched across age groups. The authors suggested that ageing might have a consequence on the mechanisms related to processing information in WM, rather than mechanisms related to storing information. Theoretically, however, potential deficits in WM capacity should be detected in both easy and complex WM conditions. Related to this view, it has been hypothesized that executive processes might be recruited to support performance in older adults even when there are low cognitive demands and therefore WM-related deficits remain unnoticeable by behavioural measures with low cognitive demand (Craik & Byrd, 1982; Reuter-Lorenz & Cappell, 2008). If this is true then, on a neural level, there should be overrecruitment of brain regions in older adults relative to young adults even in conditions with low cognitive demand (Dennis & Cabeza, 2008). Neuroimaging findings support this view by showing an age-related overrecruitment in the contra-lateral dlPFC and the inferior frontal gyrus

(IFG) when performance levels are matched across age groups (Park et al., 2003; Cabeza et al., 2004; Nagel et al., 2009).

Another possibility that might reflect effects of WM capacity is the reduced ability to update and manipulate the content of WM with age (Hasher & Zacks, 1988; de Beni & Palladino, 2004; Babcock & Salthouse, 1990). In particular reduced WM capacity with age was previously associated with a poor ability to 'delete outdated' information irrelevant to current goals (Park et al., 2002; Hedden & Park, 2001) and to automate memory processes (Park & Reuter-Lorenz, 2009). A consequence of this is that higher demands are placed on cognitive resources to maintain performance, potentially by keeping task goals and targets as active memory representations (Park & Reuter-Lorenz, 2009). It follows that if older participants find it more difficult to automate a representation of a task template they may need to allocate cognitive resources for the maintenance of the task template, leaving less WM capacity for other task-related processing.

Further linkages between the neuroimaging findings and WM processing are suggested by results that, on a functional BOLD level, WM-related processes recruit multiple brain areas depending on the nature of the task: remember letters – left Broca's area, vlPFC, supplementary cortex areas (SMA) and parietal areas (Cabeza & Nyberg, 2000); spatial WM – right hemisphere; maintenance – dlPFC (D'Eposito et al.,1998;Smith & Jonides, 1999); item manipulation - dlPFC and frontopolar cortex (BA10) (Fletcher & Henson, 2001). The above findings suggest that there are possible

combinations of multiple regions activated during different WM tasks, however probably all of them are relying on the recruitment of executive processes in the frontal cortex. Taking this assumption into account, the capacity deficit theory predicts that there is weaker recruitment of the PFC in older adults relative to young adults, at least in those brain areas recruited by young adults. In order to complement the capacity deficit theory in its fit to the neuroimaging findings, Dennis & Cabeza (2008) further suggested that overrecruitment in brain areas other than those recruited by young adults (e.g. the contralateral hemisphere) should be observed in older adults to compensate for age-related deficits in WM capacity.

Evidence from neuroimaging research in support of the extended capacity deficit theory comes from studies where the memory load was varied parametrically in young and older adults (Cappell et al., 2010; Schneider-Garces et al., 2010). At lower memory load, older adults recruited the right PFC compared to young adults as an indication of compensatory recruitment to support WM deficits. Interestingly, at higher WM load, young adults recruited similarly the right PFC, which is consistent with early cognitive observations that young adults under reduced WM resources perform similarly to old adults (Anderson et al., 1998; Jennings & Jacoby, 1993). However, when the WM load increased there was marked underactivation associated to poor cognitive performance in older compared to younger adults, which was considered as an indication that the limits of the capacity were reached (Cappell et al., 2010; Rypma et al., 2007). Taken together, the extended capacity deficit theory suggest that deficits in WM capacity might be

compensated for by overrecruitment in frontal cortices in older adults, though the compensation is resource dependent and resource limited (Craik & Byrd, 1982; Reuter-Lorenz & Cappell, 2008; Reuter-Lorenz & Park, 2010).

Based on the above assumption that cognitive performance relies on the ability to recruit task-specific circuits, while efficiently inhibiting other non-selective networks, it can be hypothesized that the inhibitory neurotransmitter GABA plays an important role in the modulation of neural networks/circuits – particularly in the inhibition of the non-selective networks (on the role of GABA in cortical networks see Jonas & Buzsaki, 2007; Buzsaki et al., 2007). It follows that age-related decline of GABA might result in less efficient inhibition of the non-selective networks. This proposal is consistent with recent computational models suggesting that increases in GABA model more efficiently neuronal inhibition of ongoing-spontaneous activity (as noise activity), while increasing stimulus-related activity (as signal activity) of neurons and their signal-to-noise ratio activity (Fujiwara et al., 2011). The authors suggested that age-related reduction in GABA concentration (Gao et al., submitted) might increase ongoing-spontaneous neuronal activity and be related to the inhibitory deficits with ageing. Taken together, GABAergic inhibition (i) decreases with ageing and (ii) is essential for the modulation of particular regions or even whole networks/circuits as part of efficient signal processing. Therefore the study of GABA might play an important role in understanding the underlying mechanisms driving the alterations in neurocognitive functioning with age.

1.4. Models to Test Theories of Cognitive Ageing

Below are presented two models that put into test the extended deficit theories in ageing initially expanded by Dennis & Cabeza (2008). I have further extended these theories with respect to accommodating findings on the inhibitory neurotransmitter in the central nervous system (GABA) and the role of GABA in the neurocognitive processes of ageing. Next, each model is presented in turn together with the predictions about the results derived from behavioural, functional and molecular measures based on the assumptions of each theory.

1.4.1. Saliency model to test Inhibition Deficit Theory

The *inhibitory deficit theory* posits that older adults are generally less able to inhibit unwanted information, allowing irrelevant stimuli to gain access to attention and working memory resulting in worsening attentional selection in older adults (Hasher & Zacks, 1988, Lustig et al., 2007). Deficits in filtering out distractor information have been observed across a range of conditions, with different types of stimuli (e.g., in reading (Carlson et al., 1995), language comprehension and production (Burke & Mackay, 1997; Burke, 1997; Tun et al., 2002), visual memory (Gazzaley et al., 2005) and spatial visual

selection (Watson & Maylor, 2002; Schlaghecken et al., 2012). However, there is no evidence examining whether age-related deficits in inhibition are observed in the domain of non-spatial visual selection.

Recently, Mevorach and colleagues showed in a series of studies that by manipulating the relationship between the features of elements in hierarchical letters, the *saliency*[2] of global and local level can be varied (Mevorach et al., 2006b; Mevorach et al., 2005; Mevorach et al., 2006a; Mevorach et al., 2009b). There were two sets of stimuli (Figure 3.1). One of the sets used global salience stimuli, which would make the shape of the large letter (global level) from a combination of blurred local elements, where processing of the global level was easy and in bottom-up manner (*target salient condition*), whereas processing of the local level in a set of global salience stimuli was more difficult as it posed demands on inhibition of the global level in top-down manner (*distractor salient condition*) (Figure 3.1). In contrast, the set with local salience stimuli would consist of small elements with high contrast, setting the focus of attention to local elements, where processing of the local level is easy and in a bottom-up manner (*target salient condition*), whereas processing of the global level is more difficult as it requires top-down suppression of the high-saliency information (the *distractor salient condition*) (Figure 3.1). On a neural level, the right posterior parietal cortex (PPC) has been linked to the guidance of attention towards the more salient of the levels when the target is at that

[2] Saliency refers to the state of an item based on the configuration of its features, which make the item to stand out from its neighbouring items. High saliency is processed on a bottom-up basis e.g. flickering headlight of a bicycle in the night. Low saliency requires endogenous (top-down) control to meet behavioural goals, e.g. identify the bicycler while trying to ignore the flickering headlight.

level (Hodsoll et al., 2009; Mevorach et al., 2009b), while the left PCC is involved when the target is at the less salient level and the distractor at the higher level of salience (Mevorach et al., 2009b). This PPC system has been linked also to a down regulation of the early visual regions which would otherwise respond differentially to the salient stimulus (in this case the distractor level). For example, increased activity in the left PPC is associated with decreased activity in left occipital cortex, while the application of transcranial magnetic stimulation (TMS) to the left PPC (to suppress activity there) leads to increased activity in left occipital cortex under conditions of high distractor salience (Mevorach et al., 2010). To date, work addressing the decreased ability to inhibit salient distractors, as people age, has been conducted under conditions of spatial selection and we know little about how ageing affects the ability to suppress irrelevant distractors when other forms of selection are demanded, such as selecting the level of form and/or selecting stimuli according to their relative salience.

The paradigm introduced by Mevorach et al. (2006b) and the findings related to the identification of specific neural mechanisms involved in processing high and low saliency targets makes it a solid foundation to test the inhibitory deficit theory on behavioural, functional and molecular levels. Based on findings in the literature related to less efficient ability to suppress distracting information as we age, it is possible that the ability to select low saliency targets through the non-spatial suppression of high saliency distractors (e.g. through top-down inhibition via the left PPC; Mevorach et al., 2010) may reduce in older participants.

1.4.1.1. Behavioural predictions based on the saliency model

Behavioural data in support of the proposed model should provide evidence for reduced performance (slower response times and poorer accuracy levels) in processing low-saliency (distractor salient) stimuli irrespective of the processing level (global or local) at which attentional selection was required for older adults relative to young adults. This hypothesis was tested in **Chapter 3**.

1.4.1.2. Functional predictions based on the saliency model

Based on fMRI and TMS findings in young adults (Mevorach et al., 2006b; Mevorach et al., 2005; Mevorach et al., 2006a; Mevorach et al., 2009b; Mevorach et al., 2009a) I predict that age-related deficits in suppressing high-saliency distracting information could emerge from less efficient modulation of the neural circuit involved in suppressing such distractors. In particular, we should observe a reduced activation in the left PPC as an indication of less efficient modulation of the left PPC coupled with an "uncontrolled" increase of activation in the early visual cortex as an indication of processing high salience distracting information. We may further speculate that the age-related decline in the suppression of salient information may be due to age-related

decreases in the effectiveness of connection between occipital and parietal cortices (Head et al., 2004; Hasher & Zacks, 1988). This hypothesis was not tested in the current Thesis.

1.4.1.3. Neurochemical predictions based on the saliency model

Animal models demonstrate that decreased orientation sensitivity associated with increased age can be restored to that seen in young animals with GABAergic stimulation (e.g. GABA agonist or electrophoretic application of GABA), whereas administration of GABAergic antagonist abolishes orientation sensitivity in young animals (Leventhal et al., 2003). Similarly, age-related depletion of GABAergic inhibition in early visual cortex associated with decreased orientation sensitivity is accompanied with increased spontaneous activity (Hua et al., 2006; Wang et al., 2006), also referred to as *homeostatic disinhibition* (Gleichmann et al., 2011). This suggests that the modulation of early visual areas to process or inhibit a bottom-up signal might be influenced by resting GABA concentrations. Most relevant to the current thesis I propose that, following Mevorach et al. (2010), during the presentation of high salient distractors, well characterised age-related decrease of GABA in visual areas might reduce the efficiency of the left IPS to control EVC, resulting in increased activity in EVC related to processing high salience distracting information.

1.4.2. WM-biased Visual Selection model to test Capacity Deficit Theory

Work in this thesis aims to test the *capacity deficit theory* using a combined memory and search paradigm developed by Soto et al (2006), in which the interaction between working memory (WM) and selective attention is measured by manipulating the relevance of WM content to the search task. In this paradigm participants are required to perform a dual task: 1) either hold a cue in WM (WM condition) or match two cues merely (the 'mere repetition', MR condition) (**cue event**); while simultaneously 2) performing a search for a cue-irrelevant target (**search event**). The search array may display the WM item, though it is never the search target. The effects of WM on search can be measured by manipulating the relations between the cue and the search target in the search display – referred to as the *validity effect,* where response times (RTs) to the search target are faster if the cue shares the location of the memory item (valid trials) compared with when the cue falls at the location of the search target (i.e. on invalid trials). In contrast to the effects of an item coded in WM, there is reduced memory guidance if the initial cue has to be identified but representation in WM is not required (MR, condition) (Soto et al., 2007; Soto et al., 2012a).

1.4.2.1. Behavioural predictions based on the interaction model

According to Lavie's load theory (1995) the effect of carrying a greater cognitive load might decrease the selectivity in information processing by increasing effects of irrelevant distractors. Here it could be argued that an age-related decrease in WM

capacity might result in a greater validity effect from the contents of WM due to there being fewer cognitive resources to suppress distracting information; this would allow the represented irrelevant WM distractor memory to be selected. In RT data, this would be indicated by a larger validity effect (invalid - valid) in older adults relative to young adults.

In contrast, there is some evidence suggesting that guidance from WM remains robust even under increased load conditions provided the item to be remembered is kept "at the forefront of WM" (Olivers et al., 2011; Dombrowe et al., 2010). This is in line with Cowan's model of WM suggesting that only one item can be actively represented in the forefront of WM (Cowan, 2001; Cowan, 2005; Oberauer, 2002). Older adults however, show a reduced ability to update and manipulate the contents of working memory (Hasher & Zacks, 1988; de Beni & Palladino, 2004; Babcock & Salthouse, 1990), as well as having difficulties in automating memory processes (Park & Reuter-Lorenz, 2009). This suggests that, if older adults find it difficult to automate the task template in the proposed search paradigm, then they may need to keep the task template at the forefront of the WM, while "pushing" the WM item at the background of the WM. If this is the case, then we should observe weaker effects of WM on visual selection in older adults relative to young adults. The predictions would be supported by detecting a reliably weaker validity effect (smaller invalid-valid difference) in older relative to young adults. The hypothesis is tested in **Chapter 5**.

1.4.2.2. Functional predictions based on the WM-biased Visual Selection model

Evidence on the neural underpinnings of WM-based guidance on attention shows that there can be an increased signal for valid compared to invalid trials. This is reported to affect a fronto-thalamic-occipital network including regions in the prefrontal cortex (Soto et al., 2007), inferior gyrus and anterior insula (Soto et al., 2012b), superior temporal cortex, thalamus (Soto et al., 2007, Soto et al., 2011; Rotshtein et al., 2011) and lateral occipital (Grecucci et al., 2009). Most relevant here, Soto et al. (2012) had young adults perform the dual-task paradigm under low WM load (remember one item) and high WM load (remember three items). Young participants showed increased bilateral PFC activation (including the right anterior insula (AI) and the inferior frontal gyrus (IFG)) when there was a high WM load (remember three items) relative to a low WM load (remember one item). In addition, a functional connectivity analysis revealed a strong coupling between the AI/IFG and the early visual cortex (EVC) related to the effects of cue validity under low but not high WM load conditions.

As noted above, according to predictions of the capacity deficit theory, young adults under conditions with reduced WM resources (high WM load) should show similar cognitive deficits to older adults (Jennings & Jacoby, 1993; Anderson et al., 1998) and similar functional patterns of activation (Schneider-Garces et al., 2010). Therefore it can be predicted that the neural mechanism involved in the dual-task paradigm proposed by Soto et al. might be similar for young adults under high WM load (remember 3 items)

and older adults with low WM load (remember one item). Thus, we might expect to observe on a functional BOLD level, that older adults recruit the right AI/IFG during encoding of WM item as a compensatory effect of reduced WM capacity, while the modulation of attention from irrelevant information held in the WM weakens. The hypothesis is tested in **Chapter 6**.

1.4.2.3. Neurochemical predictions based on the WM-biased Visual Selection model

Based on the functional predictions and evidence that resting GABA concentrations are inversely related to BOLD signal change, I expect to detect reduced GABA concentrations within the right AI/IFG. The proposal is also in line with previous studies showing age-related decrease in resting GABA within the dorsolateral prefrontal cortex (dlPFC), the orbitofrontal cortex (OFC), the sensorimotor cortex (Grachev et al., 2001), the superior frontal gyrus (SFG) and the superior parietal lobule (SPL) (Gao et al., submitted). The hypothesis is put into test in **Chapter 7**.

1.5. Aims and Thesis Overview

Aging has its effects on molecules, cells, vasculature, signalling pathways, neurotransmitters, gross morphology and cognition. The effects of aging cause a disturbance of homeostasis on a micromolecular level, leading to alterations of system-

level function, which causes an impact on cognition. To date most studies of cognitive ageing look only at one or a maximum of two levels of analysis, e.g. using two neuroimaging techniques (e.g. conventional MRI and DTI) or a single neuroimaging technique with behavioural data (e.g. fMRI and behavioural data). In addition, current theories of ageing are discipline specific (cognitive, neural). There is a pressing need to combine multimodal measures and to develop an integrative theory for different measures in order to understand better the complex interaction between biological and cognitive factors of ageing.

The major goal of the present work is to combine recently developed MRI techniques together with behavioural data to investigate the cognitive neuroscience of aging. More specifically, the thesis aims at investigating two existing cognitive frameworks on behavioural, functional and molecular levels and it attempts to explain the findings within the extended deficit theories of cognitive ageing (proposed above).

The thesis is composed of five empirical chapters, the content of which is presented below.

Chapter 3. The effects of ageing on selection by visual saliency (Behavioural study)

In **Chapter 3**, I report a first analysis of non-spatial selection by saliency in older participants. To do this, I used the paradigm introduced by Mevorach et al. (2006b), where local and global letters have to be selected under conditions of varying saliency using a blocked design – with either the target level being highly salient and the distractor

having low saliency (when there should be bottom-up guidance of attention to targets), or the distractor level having high saliency and the target low (when there are greater demands on distractor inhibition to select the low saliency target; see Figure 3.1).

I assessed whether the effects of ageing are best characterized in terms of a greater local bias (Muller-Oehring et al., 2007) or a greater sensitivity to stimulus saliency in a directed attentional task on behavioural level. I also assessed whether any effects of saliency might be particularly pronounced when distractors are salient and need to be suppressed to generate efficient target selection (cf. Mevorach et al., 2010).

Chapter 4. Dissociating effects of stimulus identity and load on working memory attentional guidance: Lengthening encoding time eliminates the effect of load but not identity (Behavioural study)

Chapter 4 focuses WM-based guidance of attention. It assessed whether effects of WM-based guidance emerge from secondary mechanisms such as (i) encoding of information in the WM or (ii) overall cognitive load during WM conditions relative to control conditions where no memory is required. To assess these possibilities I tested young adults on an adopted version of the memory-search paradigm used in Soto et al. (2007). Here, the effect of the WM cue on selection of the cue-irrelevant target during search was evaluated while varying the interstimulus interval (ISI) between the cue and the search event from 250ms to 3.0 seconds. Significant validity effects (RTs in invalid trials significantly slower than valid trials) at the longest ISI (3 seconds) might be an

indication that attention is driven by contents stored in WM, rather than information being consolidated into WM (Olivers et al., 2009).

Chapter 5. Attentional guidance from WM: Age dissociates effects of overall load and attentional guidance (Behavioural Study)

In **Chapter 5**, ageing is taken as a model of decreased WM capacity (relative to young adults) to test how the guidance effect is affected as overall load increases. I evaluated the effects of aging on (i) the general effects of memory load on search, found when memory items do not re-appear in the search displays, and (ii) the guidance of attention by WM specific cueing effects. Does ageing influence these effects in the same or in opposite ways?

Chapter 6. Reduced memory guidance of selection in ageing: Increased responses in anterior insula and reduced frontal-occipital coupling (fMRI study)

In **Chapter 6**, I report data on the effects of neurocognitive ageing on attentional guidance from contents stored in WM in young and older adults. The main focus is to identify the age-related alterations in the neural mechanism selective to the effects of cue validity on search.

Chapter 7. Effects of ageing on the link between resting GABA and BOLD-signal in the right anterior insula / inferior frontal gyrus (MRS study)

In **Chapter 7**, the experiment measured resting GABA concentrations of brain regions showing age-related alterations in WM-based guidance of attention. The regions of interest were selected *a posteriori* based on findings in **Chapter 6.** I rescanned most

of the participants in **Chapter 6** using *in-vivo* MRS measurement sensitive to GABA concentrations and correlated resting GABA to neural correlates within the same brain region.

Taken together the results from these experiments reveal conditions in which there is both increased and decreased control of visual selection in older adults, linked to changes and compensatory processes at neural and molecular levels. The experiments advance our understanding of age-related change in attention.

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CHAPTER 2. Methods

The following chapter will provide an introduction to the relevant biophysical concepts of MRI and to the two applications of MRI in this thesis - functional magnetic resonance imaging (fMRI) and magnetic resonance spectroscopy (MRS). Both fMRI and MRS will be described in four sections: (i) beginning with the biophysical origins of the signal and principles for signal measurement; (ii) methods for data processing and the assumptions made to interpret the results; (iii) the applications of the technique to research on the ageing brain; and (iv) the limitations of the technique for research on ageing.

2.1. Functional Magnetic Resonance Imaging

2.1.1. Introduction to fMRI: the origin of a signal

Functional MRI allows the visualization of the brain in action by measuring increases in blood supply and oxygenation to active brain areas in response to stimulation. The local increases in blood supply reflect the increase in neuronal energy consumption as cognitive demand increases. fMRI provides in a standard whole-brain measurement a temporal resolution at about 2 seconds and a spatial resolution at about 2-

3 mm^3, which makes it a preferred choice by neuroscientists to studying the effects of ageing on neurocognitive processing.

2.1.1.1. The biophysics of fMRI

The human body is composed of ~70% water and the MRI principles make use of the hydrogen atoms in the water molecule. The hydrogen atom consists of a proton and an electron, both of which have an intrinsic quantum mechanical property called *spin*. The strong magnetic field of the scanner ($\boldsymbol{B_0}$, in our case 3 Tesla, which is 30000 times stronger than the earth's magnetic field) aligns the spins of a portion of available hydrogen protons either parallel to $\boldsymbol{B_0}$, or anti-parallel to $\boldsymbol{B_0}$. The total difference between the number of protons in each alignment increases with greater magnetic field strength, which is referred to as a net magnetization ($\boldsymbol{M_0}$) in the tissue. The frequency of the protons' spin at a given $\boldsymbol{B_0}$ creates an intrinsic property known as the Larmor frequency which can be exploited by MRI techniques to create a signal measurement for a specific tissue. When a radio frequency (RF) pulse is applied at this Larmor frequency, protons absorb the energy of the RF pulse and are consequently excited into a higher energy level. Upon termination of the RF pulse, the excited protons return to a state of thermal equilibrium by emitting energy in the form of a weak RF signal. The recovery of the MR signal has a number of properties that are specific to the imaged tissue. For instance, the recovery of longitudinal magnetization (giving rise to tissue specific time-constant T1) is widely exploited to create high-resolution images of body and brain structure. The

recovery of T2* magnetisation is particularly affected by the homogeneity of the local magnetic field and so is sensitive to the paramagnetic form of the haemoglobin, deoxyhaemoglobin, which creates small inhomogeneities in the magnetic field. Thus, the excess of oxygenated blood mediated by neuronal activity in response to increased energy demands reduces the concentration of the deoxyhaemoglobin, resulting in an increase of MRI signal. This MR signal change forms the basis of the fMRI specific acquisition, blood oxygenated level-dependent, BOLD imaging.

2.1.1.2. Physiological basis of the haemodynamic response

The intensity of the BOLD signal reflects the oxy-/deoxyhaemoglobin ratio. Neuronal changes have an effect on the ratio by modulating a number of factors including the cerebral blood flow (CBF), cerebral flow volume (CBV) and the cerebral metabolic rate of blood oxygen consumption ($CMRO_2$) (Logothetis, 2008; D'Esposito et al., 2003). Therefore the BOLD signal reflects a complex summation of multiple vascular components, resulting in a delayed temporal profile relative to neural activity.

The BOLD response follows the CBF response with about a one second delay, by the so called “initial dip” (Hu & Yacoub, 2012). Generally the temporal profile of the BOLD response depends on the duration of the stimuli. However, following a brief stimulus impulse increases in BOLD signal are observed with the response peaking at approximately 5 seconds post stimulation onset followed by an undershoot that can last as long as 30 seconds, until the signal returns to baseline level. Thus, the design of early fMRI experiments allowed long periods between different conditions to allow the

response to return back to baseline. However, as the understanding of the HRF has increased, more complex designs with shorter inter-stimulus interval (ISI) have been approached (see below 1.2.2 Post-Processing).

2.1.1.3. Neurovascular coupling

Neurovascular coupling refers to the mechanism representing the influence of neural activity on the haemodynamic properties of the vascular bed. The signalling mechanisms of neurovascular coupling represent a complex chain of events that has been an area of intense research for many years (for excellen reviews Attwell et al., 2010; Petzold & Murthy, 2011; Kim & Ogawa, 2012). There is a consensus that the increase of blood flow in response to neuronal stimulation originates because of the increased energy demands of neuronal signalling. As the neurons start firing, glutamate is released into the synaptic space activating glutamate receptors on astrocytes. In response, the concentration of calcium ions (Ca^{2+}) increases, causing the release of vasodilatory molecules such as nitric oxide to trigger increases in CBF. This results in an increased glucose and oxygen delivery to the area to compensate for increased energy needs of the neurons. It is this influx of CBF, rich in oxyhaemoglobin that creates the BOLD response to stimulation.

2.1.2. Data Analysis

There are a number of software packages available for processing fMRI data. Each software has different processing steps, although their processing pipelines follow

somewhat similar ideas. In the current thesis SPM8 was used for the pre-processing and statistical analysis of the fMRI data. The choice of software was based on personal preference as it is MATLAB based and all other experiments (behavioural and spectroscopy) were also designed, presented and analyzed within the same environment. In this section I will briefly describe the analysis stages that are required to create a statistical map of BOLD activation to an external stimulus.

2.1.2.1. Pre-processing

The preprocessing of the fMRI data is essential as there is the need to reduce variability in the data that is not related to the experimental task, such as subject movements throughout the session, or factors such as physiological changes or scanner drifts over time.

2.1.2.1.1. Unwarping and spatial realignment

fMRI experiments repeatedly acquire 3D images of the brain (referred to as volume), which enables a researcher to discern changes in brain activity in response to different stimuli presented thorough the whole brain acquisition. This technique, however, presents some technical difficulties of which subject movement is the first one to be taken care of. SPM8 uses spatial realignment and unwarping tools to control for possible movements throughout an acquisition. Spatial alignment accounts for movements that occur between consecutive image volumes. The assumptions in spatial alignment are that the size and the shape of the head between volumes does not change and therefore a rigid-body transformation can be applied to align all volumes to a

reference volume (usually the first volume). The alignment is applied using translation and rotation in the three possible orientations to align each volume to match the reference image.

While spatial realignment corrects for movement between volumes, the unwarping procedure corrects for movements within volume acquisition. Movement within the acquisition of a volume results in distortion of the image. To correct for this, knowledge about the head shape and the orientation of the movement is derived based on information from the spatial realignment procedure. Once a volume is unwarped, the spatial realignment procedure is applied again, which results in an iterative procedure between unwarping and spatial realignment until a maximum likelihood solution is reached.

2.1.2.1.2. Temporal realignment

Each volume (a single 3D image of the brain) is a sequence of two-dimensional slices acquired in a prespecifed order (e.g. ascending, from bottom (cerebellum) to the top of the brain) within a couple of seconds. A drawback of this approach is that the haemodynamic response of the bottom slice will be measured at a different time to that of the haemodynamic response of the top slice. A solution to this concern is to implement a temporal interpolation that shifts all slices to the latency of the middle slice (e.g. the time course of the first slice's HRF will be shifted ('lagged') by 1 second to the right if the volume was acquired within 2 seconds).

2.1.2.1.3. Normalisation

The idea of the spatial normalization step is to warp images across sessions and subjects so that functionally homologous regions from different subjects are aligned to facilitate comparison of regional brain activations between individuals. This is achieved by spatially aligning and warping the functional Echo-planar images (EPI) of each subject to a reference anatomical image e.g. 152 Montreal Neurological Institute average image created from the MRI data of 152 healthy subjects (mean age 25 years), using multi-parameter linear transformation, translation and then low degrees-of-freedom of non-linear registration (Ashburner et al., 1997) (Figure 2.1). After all EPI images spatially normalized to the MNI template they were visually inspected to ensure correct registration.

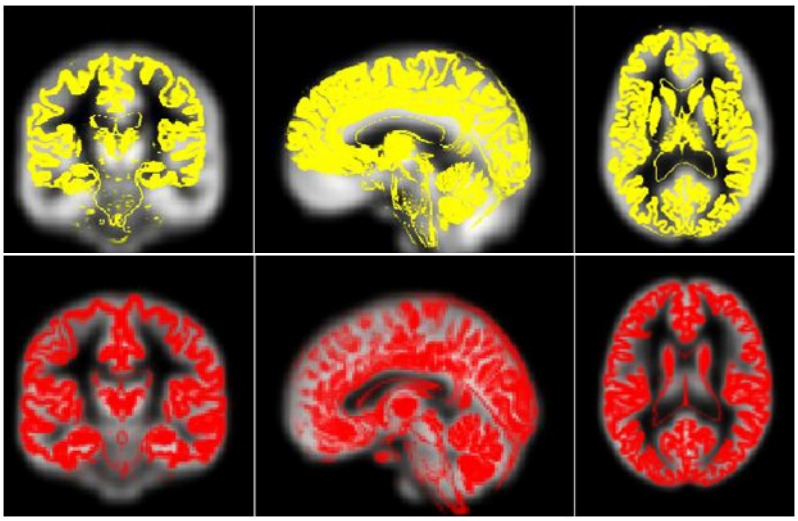

Figure 2.1. In yellow and red are presented non-normalized and normalized images of one participant, respectively, overlaid on the template image (background image).

2.1.2.1.4. Smoothing

Spatial smoothing increases the signal-to-noise ratio of the data and improves reproducibility across different experiments. Here, normalized data were smoothed by applying a 6mm Gaussian filter to each voxel. Furthermore, this prepared the processed data for statistical analysis as the data was already distributed closely to the Gaussian field model.

2.1.2.2. Statistical inference

In the final step, univariate analysis of the time-course of individual voxels was performed using the General Linear Model (GLM). Statistical inference concerning whether an individual voxel was activated in response to the stimulus was calculated by correlating the voxel's BOLD signal time course with the temporal sequence of events in the stimulus paradigm. One important point that has not been covered yet is that the BOLD signal is a relative measure than a quantitative measure. Therefore inferences about the signal require comparison between different cognitive conditions, e.g. contrasting between a stimulus condition and either a resting baseline or another experimental condition. The assumption of the GLM is that the time course of a voxel responding to an event (a stimulus), can be explained by a linear combination of hypothetical time-series based on (i) the experimental effect, (ii) confounding effects, and (iii) residual noise. For this purpose, a design matrix is created which specifies the

experimental effects (the times when a stimulus condition is active) and the potentially confounding effects (such as movement parameters, heart rate, respiration etc.). Because of the ~5s temporal delay between a neuronal event and the haemodynamic response, modelling of the BOLD response by the design matrix is optimized by making an assumption about the shape and timing of the haemodynamic response (Friston et al., 1994). To represent a typical BOLD response to exogenous stimulation, the so called canonical haemodynamic response function (HRF) is used (Kiebel & Holmes, 2007). The prediction of the BOLD response, an index of the underlying neuronal activity, to known experimental effects can be attained by convolving the experimental effects with an HRF. However, assumptions about the HRF shape need to be treated with caution, as designs that are optimized for one HRF may not account for any alteration of the HRF across conditions, brain regions or subjects (Liu et al., 2001). Furthermore, recent views suggest that analyses assuming the shape of HRF might be beneficial in blocked-design experiments, whereas model-free analysis (no assumptions of the HRF shape are made) might be more appropriate in event-related designs (Liu, 2012). The output from the GLM analysis is a statistical map containing clusters of "activated" voxels whose time course shows a significant correlation with the hypothetical time course of underlying neuronal activity that is modelled in the design matrix. T-statistics representing how well the GLM model explains the BOLD time course in each voxel are calculated by comparing the coefficient of the linear model fit with an estimate of the residual noise of the fit. Depending on the experimental paradigm and the exact research question a

statistical threshold is set upon these images to identify significant areas of BOLD response. Commonly, voxels are thresholded to correct for multiple comparisons by adjusting the threshold to the number of voxels in the data (e.g. Bonferroni correction).

2.1.3. fMRI in the research of Ageing

It is important to note that while the BOLD signal is known to be strongly coupled with the synaptic activity indicated by local field potentials (Logothetis et al., 2001) it does not directly reflect neuronal firing but is the product of changes in CBF, CBV and $CMRO_2$ (Leontiev & Buxton, 2007). Thus, vascular changes in the ultrastructure integrity that are known to occur with ageing, such as stenonsis, tortuosity or elasticity, are likely to have an influence on the BOLD signal and HRF (D'Esposito et al., 2003). For example, age-related decreases in vascular reactivity have been suggested to be a cause of less compliant vasculature, which is further associated with a decrease in CBF (Ito et al., 2002). Therefore it is possible for BOLD responses to be altered in elderly subjects through vascular mechanisms that are independent of neuronal activity. Consequently, changes in the BOLD response to an experimental task that are observed between young adults and aging adults should always be interpreted with caution. One method that could examine whether the observed age-related differences in brain activation are related to cognitive/neuronal processes or cerebrovascular changes is to implement a scaling to control for purely haemodynamic factors. Here, an additional BOLD measurement is made using a condition that has minimal demand on neural

processing. i.e. to normalise fMRI data by a measurement of the signal change induced by hypercapnia using simple breath-hold techniques (BH) (Thomason et al., 2005). Another technique that could be used as a potential haemodynamic scaling factor is the resting state fluctuation amplitude (RSFA), as resting state fMRI signals are hypothesized to contain vascular reactivity information (Kannurpatti & Biswal, 2008).

An additional consideration surrounds whether the shape or the timing of the HRF is affected by ageing. For example, Huettel et al. (2001) examined the influence of checkerboard stimulation on the hemodynamic response in young and older participants and found that although the HRF had a similar onset in both groups, the peak reached its maximum in young adults later than in older adults. In contrast, another study used a simple-sequential-grasping response and showed that the HRF in the motor cortices peaked more slowly in older adults compared to young adults (Taoka et al., 1998). Another two studies by D'Eposito et al. (D'Esposito et al., 1999a; D'Esposito et al., 1999b) also suggest that the timing of the HRF is affected by age. In addition, there is evidence for more variability in the HRF timing within a specific condition of older adults relative young adults (Kannurpatti et al., 2010). The above findings suggest that the HRF might be differentially affected by age in different brain areas during different cognitive tasks.

The previous section discussed the assumptions underlying the canonical HRF which facilitate analysis in most cases, however, if the HRF of a studied population differs from the canonical function this will affect the accuracy of response detection

using the GLM (Liu et al., 2001). Therefore, drawing inferences based on analyses, where assumptions about the HRF were made to model the ageing data, need to be approached with caution. An alternative approach would be to make no assumptions about the shape of the HRF. This has been shown to be the preferred choice also for event-related designs (Liu, 2012). Therefore, in the analysis of the fMRI data in this thesis (Chapter 6), I have used model-free group analysis with the finite impulse response (FIR) approach as implemented previously in (Padmala & Pessoa, 2011). This application is described in detail in Chapter 6.

An additional method to reduce the influence of the above concerns would be to implement stringent screening criteria for selection of participants in neuroimaging studies. Potential exclusion criteria include evidence of dementia or depression, history of psychiatric or neurological disease, cardiovascular-related diseases or use of medications that may affect the cardiovascular or nervous system. However, this could present a potential concern as the selection of participants to meet these criteria becomes narrowed to representatives of the extremely healthy population and may not be any more regarded as "normally" aged population.

2.1.4. Limitations of BOLD fMRI to study neurocognitive ageing

- BOLD signal is an indirect measure of neural activity
- BOLD signal is a product of CBF and $CMRO_2$, which might be affected differentially by age, so that significant changes of activation between

groups might only result from changes in CBF/$CMRO_2$ due to age-related changes in vasculature

- The most commonly used approach for analyzing fMRI data by assuming the HRF shape might not be optimal
- Relatively low temporal resolution making it difficult to separate the neural mechanisms involved in the processing of two different events close in time (< 4 seconds)
- Normalisation of fMRI data using age-biased templates
- Stringent inclusion criteria of the cohort might be a representation of the "graciously" aged population, rather than normally aged population

2.2. MR-Spectroscopy

Magnetic resonance spectroscopy (MRS) has been used for more than four decades in basic physical, chemical, and biochemical research, e.g. as an analysis technique or for determining the structure of complex molecules. MRS, like magnetic resonance imaging (MRI), is based on the principle of nuclear magnetic resonance, but the objective of spectroscopy is the investigation and identification of specific chemicals in the brain, as opposed to differentiation between larger scale tissue types in MRI.

2.2.1. Introduction to MRS

2.2.1.1. The biophysics of MRS

Section (2.1.1.1) described how the fMRI signal was mainly derived by applying a weak RF pulse with a specific frequency (Larmor Frequency) to the hydrogen atoms in the water (H_2O) molecules contained in biological tissue. In contrast, MRS measures signal not only from hydrogens' spins in water molecules, but also other hydrogen-based molecules such as choline (Cho), creatine (Cr), or N-Acetylaspartate Acid (NAA). When the water molecules in brain tissue are introduced to a 3T magnetic field, their hydrogen nuclei will precess at a frequency of ~127MHz (Larmor frequency for water at 3T) and as a result signal will be detected at a frequency of ~127MHz, represented as the most significant peak in Figure 2.2. Theoretically, in this example the water should be represented with a single spectral line at the position of the resonance frequency. In practice, due to imperfections of the magnetic field in the region of interest, participant motion and other factors, the spectral line broadens to become a less distinct peak, where the full width at half maximum (FWHM in Hz) represents the goodness of the measurement (low Hz, thin spectrum, good measurement). Another characteristic of the spectrum is the area under the peak, which is used as a measure of the concentration in arbitrary units of a particular chemical compound. The measurement, however, can be quantified by comparison with a reference signal (i.e. providing an additional measurement of a non-suppressed water signal, see below).

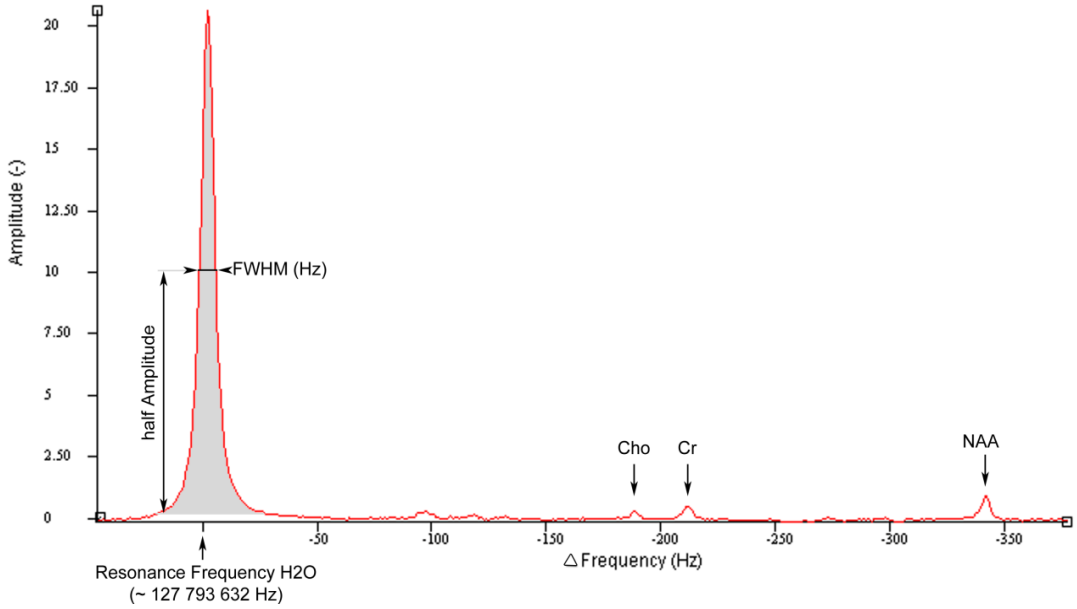

Figure 2.2. Example of water-suppressed spectrum of the human brain at 3T. The high peak on the left represents remaining signal from water with the following characteristics: amplitude, Full-Width at Half Maximum (FWHM) in Hz, and area under the peak (gray colour). On the right hand-side of the spectrum, a number of peaks of brain metabolites (e.g. Choline (Cho), Creatine (Cr) or N-Acetylaaspartate) at a reference frequency at a distance away from the reference frequency of the water in Hz.

The resonance frequency in a ^{1}H-MRS measurement of a particular chemical compound depends on the chemical environment of the hydrogen atoms e.g. the spins of water hydrogen will precess at a different frequency to that of the hydrogen spins of other ^{1}H-based compounds; NAA spins precess at resonant frequency that is ~350Hz smaller than the resonant frequency of water spins in the human brain at 3T (Figure 2.2). To represent these differences in a universal unit (same representation for measurements at different magnetic field strengths) the relative difference in resonant frequency is

compared to a standard signal (in ^{1}H – NMR this is tetramethylsilane (TMS)), which is referred to chemical shift with the unit parts per million (ppm) (Figure 2.3).

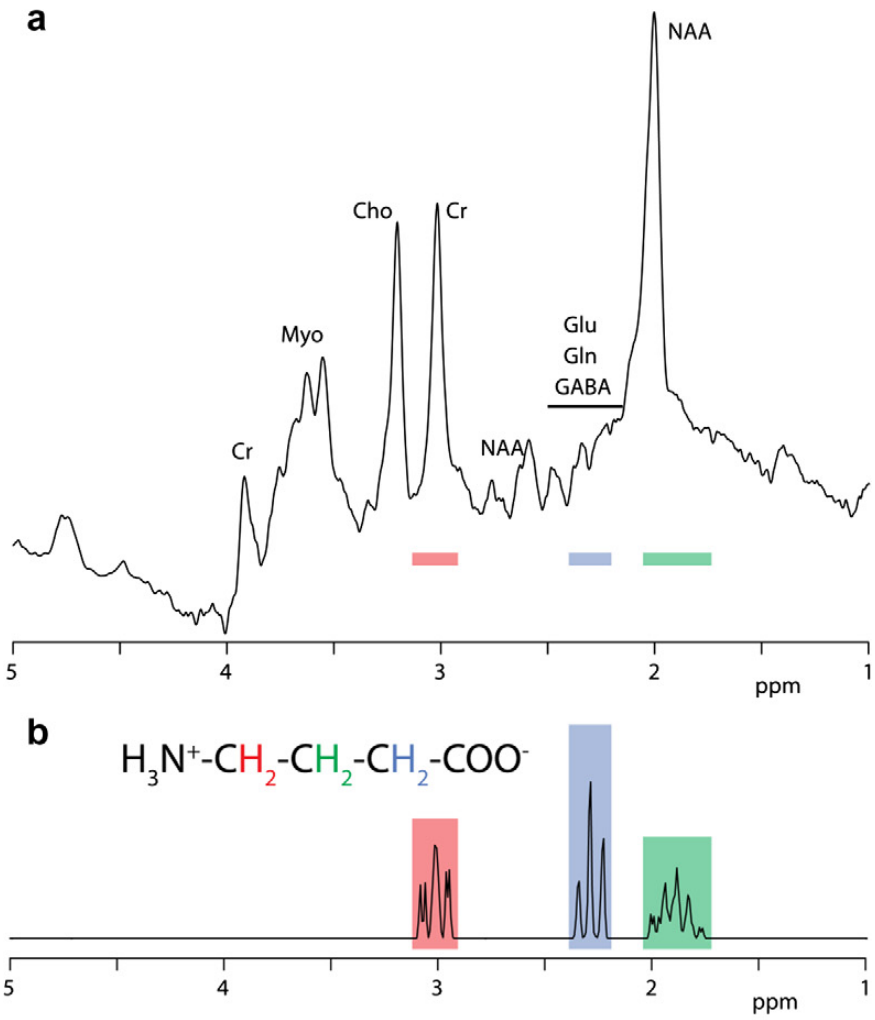

Figure 2.3. MR spectra of GABA. (a) ^{1}H MR spectrum showing a number of brain metabolites (NAA – N-Acetylaspartate, Glu – Glutamate; Gln – Glutamine; Cr – Creatine; Cho –Choline; Myo – Myoinositol; GABA – γ-Aminobutyric acid); (b) Chemical formula of GABA and simulated MR spectrum of GABA at 3T, showing corresponding ppm for the spins of each CH_2 group in the GABA molecule. (Adopted from Puts et al., 2011 with permission)

2.2.1.2. GABA measurement with MEGA-PRESS at 3T

As outlined in the introduction, γ-Aminobutyric Acid (GABA) is the main inhibitory neurotransmitter in the brain and its of great interest to neuroscientists to investigate whether GABA is playing a role in neurocognitive ageing. GABA contains three methylene groups (-CH_2) (Govindaraju et al., 2000) and offers the possibility to be measured with ^{1}H-MRS. However, the *in-vivo* detection and quantification of GABA using MRS is hindered because resonances of GABA overlap with other metabolites such as Glu, NAA and total creatine (tCr) and has not been possible until the recent development of GABA-dedicated methods for 3T scanners.

MEscher-GArwood Point RESolved Spectroscopy (MEGA-PRESS) (Mescher et al., 1998) is one of the MRS methods developed to measure GABA *in-vivo* and its application has recently quantitatively associated GABA with behaviour (Sumner et al., 2010), BOLD signal (Muthukumaraswamy et al., 2012) and age (Gao et al., submitted).

MEGA-PRESS is based on an implementation within a PRESS sequence, where the acquisition of two interleaved datasets carries information about the different states of the GABA spin system. In more detail, MEGA-PRESS acquisition starts with an RF excitation pulse with a broad resonant frequency range to excite hydrogen spins of water and other metabolites, including GABA. A short time after the initial excitation, a second refocusing RF pulse at a specific resonant frequency is applied, so that hydrogen spins at that frequency are refocused and not present in the measured signal. On an odd number of acquisitions the refocusing pulse is set at the resonant frequency at 1.9ppm (*Edited ON*),

which refocuses (i) hydrogen spins at 1.9ppm (e.g. GABA's CH_2 group, NAA) and (ii) the spins of hydrogens coupled to hydrogens at 1.9ppm, e.g. GABA's second CH_2 group at 3.00ppm is coupled with GABA's third CH_2 group at 1.9ppm (Govindaraju et al., 2000) (see Figure 2.4 blue spectrum). On an even number of trials the refocusing RF pulse (*Edit OFF*) is set at another frequency (e.g. 7.46ppm) where the spins of brain metabolites are unaffected (non-refocused). Subtraction of the refocused spectrum (*Edit ON*) from the non-refocused spectrum (*Edit OFF*) removes peaks shared in both spectra and retains those peaks that are affected by the refocusing pulse (Figure 2.4, red spectrum – blue spectrum = black spectrum). Thus, the edited spectrum (Figure 2.4, black colour) should contain peaks of spins close to 1.9ppm (GABA, NAA) and spins coupled to those close to 1.9ppm (GABA at 3ppm) coupled to spins at 1.9ppm; Macromolecules (MM) at 3ppm coupled to spins at 1.7ppm; Glutamate/Glutamine/Glutathione (Glx) at 3.75ppm couple to Glx spins at 2.1ppm; Homocarnosine at 3.00 ppm coupled to spins at 4.47ppm). More detailed information about the MEGA-PRESS sequence and other GABA-dedicated sequences can be found in the following literature (Mescher et al., 1998; Edden & Barker, 2007; Near et al., 2011; Evans et al., 2010).

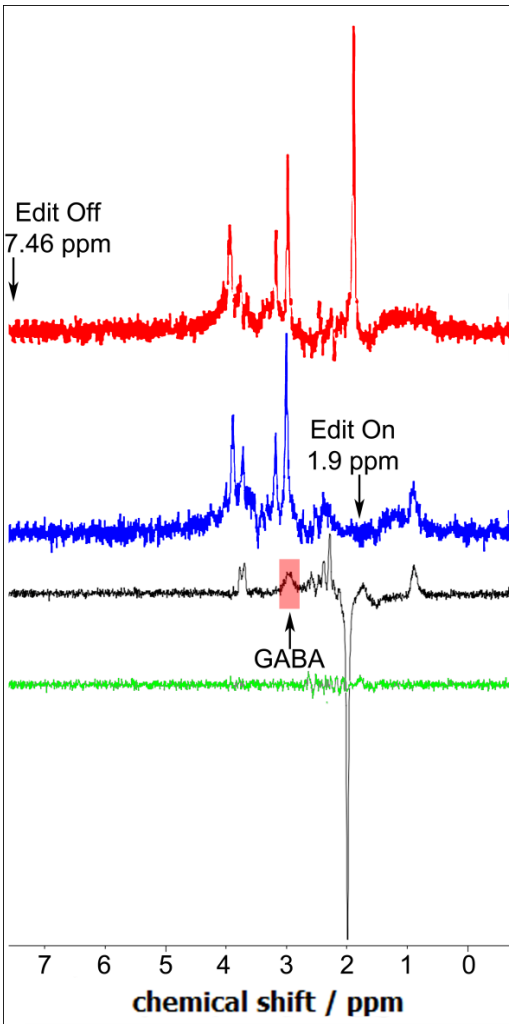

Figure 2.4. Four water-suppressed spectra representing *in-vivo* human brain MEGA-PRESS measurement using a 3T scanner: (i) red colour – the spectrum is acquired with non-refocused pulse (*Edited OFF*) and includes peaks of brain metabolites; (ii) blue colour – the spectrum is acquired with refocused pulse (*Edited ON)*) where spins affected by the pulse are not present in the spectrum. Spin signals from other ^{1}H-based groups in "bond" to ^{1}H-based groups at 1.9ppm were refocused as well; (iii) difference spectrum between *Edited ON* and *Edited OFF* with resulting GABA+ peak at 3ppm, and (iv) residual from subtraction (green colour)

2.2.1.3. Setting up the MEGA-PRESS sequence at BUIC

Although the MEGA-PRESS sequence is available at the imaging centre where the work was based (BUIC) and can be provided for use upon request, the sequence requires additional setup. In order to complete the experiments in this thesis, I set up the sequence following the advice of one of the collaborators, Dr. Richard Edden. The most current recommendations on setting up MEGA-PRESS sequence could be found on Richard Edden's blog (http://gabamrs.blogspot.co.uk/) .

Steps to setup a basic PRESS sequence at BUIC scanner (Philips Achieva 3T):

1. Start with a short-TE PRESS sequence
2. In Initial tab, set the TE to 68 ms.
3. In Geometry, set the voxel size to 3x3x3 cm^3.
4. In Contrast:
    a. Change Gradient mode to Maximum and PNS mode to high.
    b. Select MEGA basic in BASING pulse options.
    c. Pulse duration 14ms, freq1 7.46 ppm, freq2 1.9 ppm. [OFF first]
5. In Motion, set phase cycles and averages to 8, startup acquisitions 0 and turn off frequency correction.
6. In Dyn/ang, Dynamic study individual, 40 dynamic scans.
7. In Postproc, turn off spectral correction.
8. Set up a separate non-water-suppressed PRESS scan for quantification relative to water.

2.2.2. Data Analysis

2.2.2.1. Pre-processing

One MEGA-PRESS measurement in the MRS experiment consisted of 40 acquisitions, of which half were *Edited ON* and the other half *Edited OFF*. Both type of measurements followed the same steps of pre-processing, using MATLAB-based

software, Gannet (available at gamamrs.blogspot.com), with a fully automatised processing pipeline of the MRS data, although other alternatives could be used (e.g. Tarquin, LCModel, jMRUI). Data were fourier-transformed from the time-domain to the frequency-domain prior to frequency-correction, phase-correction, apodization and fitting were applied. The Creatine and water peaks were used for correction of potential frequency drifts, which could arise from scanner instability or subject motion. Next the edited spectrum was calculated by subtracting refocused and non-refocused spectra followed by quantification of the GABA signal with a water non-suppressed measurement as an internal reference.

2.2.2.2. Post-Processing – correction for partial tissue volume effects in the voxel

One essential step regarding quantification of the GABA concentration was to account for partial volume effects from different tissue types (grey matter, white matter and CSF) contained in the voxel. The obvious approach to account for partial volume effects would be to adjust GABA concentrations to each tissue type. However, there are two concerns related to this approach: there is no consensus about the GABA concentration in grey or white matter, although there is some evidence that GM concentration is double that of white matter (Jensen, deB Frederick, & Renshaw, 2005a; Petroff, Ogino, & Alger, 1988; Petroff, Spencer, Alger, & Prichard, 1989). The other concern is related to the "excitability" of GABA in different tissues. It was suggested that

GABA T2-relaxation times may differ slightly across grey and white matter (Edden & Barker, 2007) and therefore the excitation of GABA might not be uniform across large voxel sizes that include different tissues. Therefore, it might be difficult to adjust GABA concentration to tissue type while controlling for the heterogeneous excitability of GABA spins within grey matter and white matter. The current practice to control for the partial volume effects of different tissue types in the voxel is to include the ratio between grey matter and white matter as an additional regressor in the statistical analysis (Sumner et al., 2010; Aufhaus et al., 2012; Boy et al., 2010; Puts et al., 2011).

As the MRS output does not carry any spatial information it is not that straightforward to determine partial tissue volume effects within the voxel. There is no software available that implements this procedure. For the tissue segmentation of the voxel in the current thesis the following semi-automatic approach was developed.

2.2.2.2.1. Extraction of VOI coordinate information from the dicom file

Before an MRI session starts, the participant's head is positioned in the isocenter of the magnet bore. The isocenter can be regarded as the centre of a coordinate system for all measurements within the session. In this case, images can be related to each other using a set of parameters describing the images position relative to the isocentre. These parameters can be found in the dicom header of the image file and can be used to align the spectroscopy volume of interest (VOI) with another MRI image acquired within the same session.

Three of the parameters contain information about the distance of the centre of the voxel from the isocentre. Another six parameters contain the direction cosine, which provides information about the angulation of the VOI. These direction cosines can be used for the calculation of the transform matrix. Matlab code was developed to extract the information about the image position and calculate the transform matrix for each VOI.

2.2.2.2.2. Generation of artificial mask representing VOI

A shell script was developed to generate an artificial mask with the dimensions, localisation and angulations (parameters from step 1) of each VOI using *volsynth*, part of the Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu).

2.2.2.2.3. Coregistration of the VOI mask to the T1-image

In the MRS session three fast T2-images were used for the localisation of the VOI in the region of interest and to generate masks in order for the VOI to be co-registered to the T2-images. However, the T2-images did not share the spatial resolution of the T1-images (acquired in the fMRI session), suggesting that for a more efficient tissue segmentation it would be useful to coregister T2-images, together with theVOI mask, to the high resolution T1-images. An additional Matlab script was generated to coregister T2- to T1-images using the SPM coregistration tool.

2.2.2.2.4. Segmentation of the VOI

A further Matlab code, segmented the T1-image into three separate images containing only one tissue type: grey matter, white matter and CSF. These were used for the estimation of tissue type as a percentage of the total volume of the VOI mask.

2.2.3. MRS in the research of Ageing

As outlined in the introduction, GABA is the major inhibitory neurotransmitter and has been shown to be associated with cognitive functions. This suggests that GABA may play an important role in neurocognitive ageing. Recent developments in MRS provide quantitative measurement of GABA and suggest that GABA concentration may decline with age. However, inferences about age-related decline based on MRS measures need be approached with caution. In particular, there are two concerns that need addressing.

One factor is that a particular property of the GABA spins related to their excitability (T2- relaxation times) might be affected by ageing. Change in T2-relaxation times might affect quantification of the MRS measurement, so that age-related reduction in metabolite signal intensity does not reflect a decline in concentration, but rather a change of T2-relaxation times. For example, a recent study by Kirov et al (Kirov et al., 2008) demonstrated an age-related shortening of the T2-relaxation times of NAA, Cr and Cho, which might partially account for observed age-related decrease of concentrations in these metabolites. These findings suggest that potential age-related changes in the GABA T2-relaxation times might account for some of the findings in the literature. Additional

research in this area requires attention to rule out or adjust for possible age-related changes of GABA's T2-relxation times.

Another factor that was discussed in the previous section is the inclusion of different tissue types in the imaged voxel. GABA might have different T2-relaxation times and concentrations across different tissues, therefore introducing confounds in concentration measurements. One way to reduce the effect of this factor is to opt for a higher spatial resolution (smaller voxel or multi-voxel spectroscopy) as the technology develops so that the effects of different tissue types can be separated into different voxels.

2.2.4. Limitations of MRS to study neurocognitive ageing

The acquisition method for detection of GABA in ageing using MEGA-PRESS has several limitations.

- MMs account for 45% of the GABA+ signal. This suggests that an age-related reduction in MM might significantly contribute to measured GABA decreases in older adults. However, this is unlikely to explain the observed findings related to GABA+ in ageing studies, as the MM signal at 3.0ppm has been shown to increase with age (Hofmann et al., 2001; Mader et al., 2002; Aufhaus et al., 2012). Further studies with newly developed techniques accounting for MM suppression should confirm reduction of GABA with age.
- The signal of GABA detected by MRS at 3T is relatively low, requiring the use of large VOIs (3 x 3 x 3 cm). An approach allowing the measurement of smaller VOIs might be of more use for small region-specific analysis.

- Change of GABA excitability (T2- relaxation times) with ageing requires better quantification and control methods.
- Accounting for partial tissue volume in VOI requires more work and better methods.

CHAPTER 3.
The Effects of Ageing on Selection by Visual Saliency

3.1. Abstract

We examined the ability of older adults to select local and global stimuli varying in perceptual saliency – a task requiring non-spatial visual selection. Participants were asked to identify in separate blocks a target at either the global or local level of a hierarchical stimulus, while the saliency of each level was varied (across different conditions either the local or the global form was the more easy to identify). Older adults were less efficient than young adults in ignoring distractors that were higher in saliency than targets, and this occurred across both levels of form. The increased effects of distractor saliency on older adults occurred even when the effects were scaled by overall differences in task performance between young and older participants. The data provide evidence for an age-related decline in non-spatial attentional selection of low-saliency stimuli, not determined by the (global or local) level at which selection was required. We discuss the implications of these results for understanding both the interaction between saliency and hierarchical processing and the effects of aging on non-spatial visual attention.

3.2. Introduction

In order to survive in complex, dynamic environments we need efficient mechanisms of attention to select information relevant to our behavioural goals. Current theories of visual attention hold that selection is determined by the interaction between bottom-up and top-down signals. Bottom-up signals act to draw attention to salient items that differ from their local surroundings (Theeuwes, 1992; Theeuwes, 2005). Top-down forms of selection become involved when participants have particular expectations about the target they are required to select (e.g., knowing its location or one of its features) (Wolfe et al., 2003) and/or when the target is less salient than particular distractors – when the bottom-up attraction of attention to the salient distractors must be overcome. In addition, top-down selection itself can be fractionated into excitatory processes, that guide attention to targets, and inhibitory processes, which can filter out irrelevant distractors (see Braithwaite et al., 2005). The inter-play between these selection processes can change as we age. For example, there is evidence that the role of top-down expectations for targets, and of excitatory guidance, may be stronger in older than younger adults (Madden et al., 1999). On the other hand, the ability to suppress irrelevant distractors may decrease – as argued by the inhibition deficit theory of cognitive ageing (Hasher & Zacks, 1988; Lustig et al., 2007). According to this account, cognitive ageing is associated with a selective decrease in the ability to inhibit irrelevant stimuli and responses, worsening attentional selection in older adults. Though the loss of inhibitory control may be compensated for by increased top-down excitatory guidance, problems

will emerge under conditions in which distractors strongly compete for selection with targets (e.g., under conditions in which distractors have the higher saliency).

3.2.1. Inhibitory Deficits in Visual Selection

Evidence for the inhibitory deficit theory emerges from studies using negative priming. Negative priming tasks measure the unfavourable influence of a prior exposure to a distractor stimulus on the response to the same stimulus when a target. Classic studies of negative priming have contrasted cases where an item's identity is inhibited, slowing its subsequent identification (Tipper & Cranston, 1985). Reduced negative priming in older adults might reflect less efficient inhibition when the stimuli are first encountered as distractors (Hasher et al., 1991). In addition, there is evidence for age-related changes in location-based inhibition, for example in preview search tasks. Preview search typically uses conjunction-like displays but presents distractors with one common set of properties prior to the second set of distractors plus the target (Watson & Humphreys, 1997). Provided there is a sufficient period between the two sets of distractors (of the order of 400ms or so), the first set of distractors can be efficiently ignored (Humphreys et al., 2004). There is substantial evidence that the lack of impact of the initial distractors is dependent, at least in part, on a process of active distractor suppression of its location (Allen et al., 2008; Watson & Humphreys, 2000; Humphreys et al., 2004) and features (Olivers & Humphreys, 2003). As in studies of negative priming it has been shown that older participants can show a selective reduction in the efficiency of preview search, particularly under conditions where distractor inhibition is challenged

(e.g., with moving distractors). This is consistent with reduced distractor inhibition as participants age (Watson & Maylor, 2002).

However there are many instances in everyday life where selection is neither feature nor space-based, but rather dependent on the ability to select the appropriate level of a form. For example, when trying to make a judgement about someone's identity we may want to select the whole face to take advantage of configural relations between features, but in doing this we may not want to attend to the local features themselves. On the other hand, when we make a judgement about part of a face (is the person smiling?), we may want to focus attention on the local part without processing the whole. For such cases, we need to be able to flexibly select with the local or the global level of a form, an ability that likely depends on different underlying mechanisms to those studied through feature or space-based selection. For example, while there is much evidence for spatial selection being dependent on a largely bilateral fronto-parietal network (Corbetta & Shulman, 2002), the selection of local and global forms is associated to lateralized brain recruitment, with the left hemisphere being selectively linked to local processing and the right hemisphere to global processing (Lux et al., 2004). In addition, other regions may be recruited irrespective of whether the local or global level of form needs to be selected, as a function of whether the target level (local or global) is high or low in salience. For example, the right posterior parietal cortex (PPC) has been linked to the guidance of attention towards the more salient of the levels when the target is at that level (Hodsoll et al., 2009), while the left PCC is involved when the target is at the less salient level and

the distractor at the higher level of salience (Mevorach et al., 2009b). This PPC system has been linked also to a down regulation of the early visual regions which would otherwise respond differentially to the salient stimulus (in this case the distractor level). For example, increased activity in the left PPC is associated with decreased activity in left occipital cortex, while the application of transcranial magnetic stimulation (TMS) to the left PPC (to suppress activity there) leads to increased activity in left occipital cortex under conditions of high distractor salience (Mevorach et al., 2010). To date, work addressing the decreased ability to inhibit salient distractors, as people age, has been conducted under conditions of spatial selection and we know little about how ageing affects the ability to suppress irrelevant distractors when other forms of selection are demanded, such as selecting the level of form and/or selecting stimuli according to their relative salience. Here we set out to address this issue by evaluating how cognitive ageing might alter an individual's ability to select a target at the low saliency level of a form, compared to when the target is highly salient.

3.2.2. Global and Local Processing in Ageing

There are several previous studies of the effects of cognitive ageing on the ability to select local and global levels of form. However the results are very mixed. Roux and Ceccaldi (2001), for example, used stimuli that showed an overall global processing advantage and reported that older participants had stronger global interference (when responding to local targets) than younger observers. In direct contrast, Muller-Oehring et al. (2007), employing stimuli with an overall local advantage, found greater local-on-

global interference in older participants. Others have reported null effects of ageing on local and global interference (Bruyer et al., 2003). These contradictory results may be understood if cognitive ageing affects the ability to select stimuli varying in saliency rather than the ability to select local and global forms per se. For example, in studies showing greater interference effects in older participants, the interfering distractors were typically more salient than the target (global distractors in Roux & Ceccaldi, 2001; local distractors in Muller-Oehring et al., 2007), while experiments showing no differential interference effects have tended to have local and global forms more balanced for saliency (e.g., judged by overall RTs; Bruyer et al., 2003). The conflicting results may be accounted for by differential selection of stimuli varying in saliency. We investigated this for the first time using stimuli modelled on investigations of selection by saliency by Mevorach and colleagues.

3.2.3. Saliency Processing in Global/Local Level

In contrast to prior studies in this field, Mevorach orthogonally varied whether the target was at the local or global level of the forms and whether it had high or low saliency (in relation to the distractor level of form) (Mevorach et al., 2006b; Mevorach et al., 2005; Mevorach et al., 2006a; Mevorach et al., 2009a; Mevorach et al., 2009b). The saliency of the forms was varied by either presenting high contrast local forms in alternating colours (high local saliency, low global saliency) or by blurring the hierarchical letter and presenting the local forms in uniform colour (high global saliency, low local saliency; See Figure 3.1). Performance was analysed by pooling the data across

conditions where the target was at the local level and when it was at the global level and contrasting the results when the target had high salience (distractor low salience) and when the target had low salience (and the distractor high salience). When the target level was high in salience, the demands on inhibition of the distractor level were low as selection could have been driven by bottom-up cues; however when the target was low in salience and the distractors had high salience, then the demands on distractor inhibition were high in order to overcome bottom-up cues which would bias selection in favour of the distractor. Consistent with the argument for the inhibition of high saliency distractors, Mevorach reported reduced activation of left occipital cortex when distractors had high salience, linked to increased activation of left PPC. They argued that the selection of the low saliency target was mediated by the left PPC suppressing distractor-related activity in early occipital cortex. Interestingly, suppressive TMS to the left PPC increases interference from salient distractors and this effect is greatest when TMS is applied prior to the onset of the stimulus. In this case, TMS appears to block the top-down setting up of perceptual suppression.

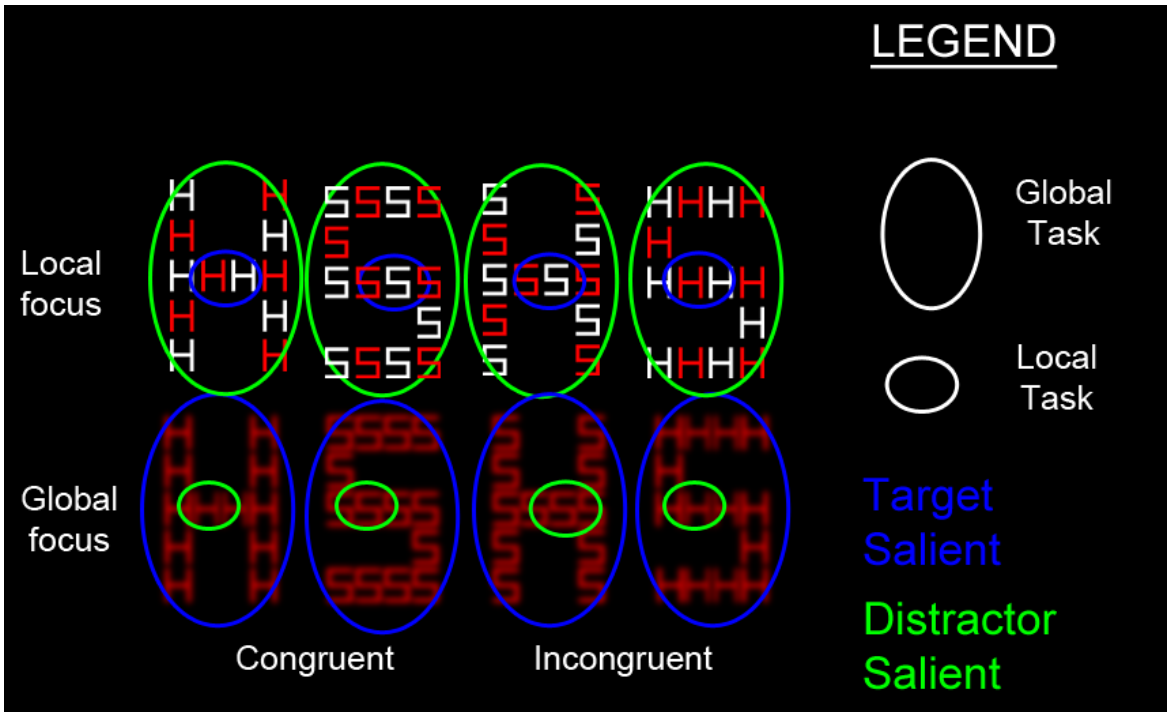

Figure 3.1. All compound letters with either high local saliency (top row) or high global saliency (bottom pair), used in the experiment. Target saliency was varied orthogonally with the task (Local task [small circles] / Global task [Large ellipses]). The color of the ellipse denotes the saliency level in particular condition - target salient in blue (e.g. in a block with global task using the set of stimuli with global focus, bottom row) and distractor salient in green (e.g. in a block of local task using the set of stimuli with global focus, bottom row).

3.2.4. Ageing and Saliency Processing

In older people the ability to inhibit irrelevant distracting information can be disrupted. Age-related deficits in inhibition have been observed in a number of cognitive domains including reading (e.g. language comprehension and production in the presence of background speech or noise Morrone et al., 2010; Burke, 1997; Tun et al., 2002), motor and somatosensory processing (Bolton & Staines, 2012; Anguera & Gazzaley,

2012), visual memory (Gazzaley et al., 2005) and visual spatial selection (Watson & Maylor, 2002).

Evidence of poor distractor inhibition specifically affecting visual selection comes from studies using preview search (described above). In older participants, the ability to ignore distractors in preview search can be disrupted, particularly when search becomes more taxing (e.g., with moving distractors Watson & Maylor, 2002; Allen & Payne, 2011).

Imaging studies support the view of less efficient inhibitory control in ageing (Gazzaley et al., 2005). Gazzaley et al. had participants observe two types of stimuli (scenes and faces) and required them to remember one stimulus type while ignoring the other (i.e. remember scenes and ignore faces, or remember faces and ignore scenes). The difference in brain activity when scenes were successfully remembered scenes, compared to when the scenes were passively viewed, was similar for both young and older adults. On the other hand, younger participants showed a negative response to ignored scenes compared to baseline, but there was no difference between these conditions for older adults, suggesting decreased distractor inhibition. Interestingly, the level of deactivation for ignored stimuli during encoding was the only neural measure that predicted brain responses in the memory test phase. These data suggest that distractor suppression may be disrupted in older subjects, particularly under conditions where distractor suppression is more difficult to impose (e.g., with moving distractors, If this is the case, then the ability to select low saliency targets through the non-spatial suppression of high saliency

distractors (e.g. through top-down inhibition via the left PPC; Mevorach et al., 2010) may reduce with age. To our knowledge this is the first study examining age-related deficits in the inhibition of non-spatial visual information, and whether stimulus saliency is critical to age-related differences in the selection of local and global forms.

3.2.5. The Present Study

In the present study, we report a first analysis of non-spatial selection by saliency in older participants. To do this, we used the paradigm introduced by Mevorach et al. (2006b), where local and global letters have to be selected under conditions of varying saliency using a blocked design – with either the target level being highly salient and the distractor having low saliency (when there should be bottom-up guidance of attention to targets), or the distractor level having high saliency and the target low (when there are greater demands on distractor inhibition to select the low saliency target; see Figure 3.1).

We assessed whether the effects of ageing are best characterized in terms of a greater local bias or a greater sensitivity to stimulus saliency in a directed attentional task. We also assessed whether any effects of saliency might be particularly pronounced when distractors are salient and need to be suppressed to generate efficient target selection (cf. Mevorach et al., 2010).

3.3. Methods

3.3.1. Participants

The participants were twenty-four young (eleven males; group mean age, 24 years; age range 19 to 29) and nineteen older (ten males; group mean age, 74 years; age range, 65 to 84) healthy volunteers who took part in the study for exchange of course credit or cash (£6 per hour). The participants were recruited by advertisements in local communities, word-of-mouth information and advertisements on an online experiment management system (Research Participation Scheme, University of Birmingham). All the subjects had normal or corrected-to-normal vision and were healthy with no history of psychiatric or neurological disease.

3.3.2. Stimuli

Two sets of compound-letter stimuli were created to have either high global saliency or high local saliency. The stimuli comprised the letters "H" and "S" and their combinations created figures in the shape of large orthogonal "H" and "S" letters (see Figure 3.1).

In the set of stimuli where local information was manipulated to pop out over the global information the compound letters were made of red and white local letters (Figure 3.1, top row). The size of the local letters was 1.34° × 1.76° of visual angle (in width and height, respectively) with a distance between the letters of 0.46°. The total width and height of the global letters was 6.7° × 10.81° of visual angle, respectively.

The local elements in the set of compound letter weighted for global processing consisted of red blurred letters (Figure 3.1, bottom row). The width and the height of the

local letters was 1.34° × 1.76° of visual angle respectively, with an inter-letter distance of 0.15°, resulting in a global letter subtending 5.83° × 9.22° of visual angle (in width and height, respectively). These images were additionally blurred in MATLAB using a Gaussian lowpass filter (FWHM of 1.56 mm).

To reduce strategic focusing on a local area of the screen there were three possible positions for presentation of the stimuli – the centre or 13.16° to the left or right of the centre of the screen.

3.3.3. Procedure

In a directed attentional task, participants undertook different trial blocks in which they were asked to concentrate only on the global or the local letters across a block of trials while ignoring the information at the other level. The task was to identify the letter (H or S) on the designated target level by pressing pre-specified buttons on a serial mouse (e.g., "Is the letter on the global level H or S?"). The experiment had four types of blocks formed from the orthogonal combination of task and saliency (see Figure 3.1). There were two target-salient block types: (i) identify the global letters in stimuli where global information pops out over local information (Figure 3.1, bottom row); and (ii) identify small letters in stimuli where local information pops out over the global shape (Figure 3.1, top row) with high local saliency and low global saliency. Likewise there were two distractor-salient block types: (i) identify the global letters in a stimulus with set focus on the local level (Figure 3.1, top row); and (ii) identify the local letters in stimuli weighted to the global level (Figure 3.1, bottom row). Blocking the task – saliency combinations

allowed participants to adopt a top-down set to the designated target level[3]. This top-down set is more critical for efficient task performance when the target level has low saliency and the distractor high, relative to when the opposite occurs (i.e. target salient, e.g. with stimuli weighted to the global level, Figure 3.1 bottom row, are identified on the global level). Each block had 48 trials. On half of the trials the same letters appeared on the global and local levels (congruent trials), whereas on the other half there were different letters on the two levels (incongruent trials). The first two blocks and the last two blocks of the experiment were both either globally salient displays or locally salient displays. Each pair of these blocks consisted of a global and a local identification task. The order of the blocks was randomized across participants. Each experimental trial started with a white fixation point presented for 2000ms followed by a 150ms presentation of a compound letter on a black background. The trial ended after the participant identified the letter (H or S) on the target level (global or local) and gave a speeded response by pressing one of the two mouse buttons (one for each letter). The inter-stimulus interval was variable (1 - 4 seconds from the response of the subject in one trial to the onset of the stimulus in the next trial) to avoid possible predictions of stimulus onset. The viewing distance was controlled with a chinrest at 65cm from the monitor. Psychophysics Toolbox for Matlab (Kleiner et al., 2007; Brainard, 1997; Pelli, 1997) was used for the presentation of the paradigm and the collection of the responses.

[3] Example scenario of block order: 1st Block - Target salient condition, global task using the set of stimuli with global focus (Figure 3.1, bottom row); 2nd Block - Distractor salient condition, local task using the set of stimuli with global focus (Figure 3.1, bottom row); 3rd Block - Distractor salient condition, global task using the set of stimuli with local focus (Figure 3.1, top row); 4th Block – Target salient condition, local task using the set of stimuli with local focus (Figure 3.1, top row).

Response times and performance accuracy were recorded. Incorrect responses were excluded from the analysis. RTs were screened for outliers after an exGaussian function was fit to each cell data to reject any RTs over 3.5 standard deviations away from the mean.

3.4. Results

Exploratory analysis indicated that there were lower accuracy rates for older compared to young adults, which were driven mainly by errors in distractor-salient conditions. In order to account for speed/accuracy tradeoffs in the analysis the data were analyzed by combining RT/proportion correct (see Townsend & Ashby, 1983), as well as analysing Z-transformations. The Z transformations aim to dissociate group differences from effects of generalized slowing/decreases in processing efficiency by examining effects of task conditions normalised by the average performance for each participant participant (Faust et al., 1999)[4].

3.4.1. Left vs Right Visual Field

An initial ANOVA on data from the left and right field locations (excluding centrally presented stimuli) assessed whether there were differential effects of visual field on performance. There was a reliable main effect of field, with Z-transformed efficiency

[4] To the best of our knowledge this is the first time that analyses based on Z transformation have been conducted on measures of performance efficiency rather than reaction time. However, since efficiency is likely to have the same distribution as the reaction time data from which it is derived then it should be applicable here. In addition, to have analyzed the RT data alone would have been to miss the critical trade-off in accuracy in older participants.

values lower to targets in the left field [left visual field: 842ms, right visual field: 858ms, F (1, 41) = 147.17, $p < 0.001$]. However, there were no other significant interactions with age and the data were subsequently pooled across field.

3.4.2. Main Analysis

Performance was assessed in mixed design ANOVAs over the mean efficiency data, and Z-transformed values of efficiency (Figure 3.2). The within-subject factors were task (select Global or Local targets), saliency (Target-salient (e.g. global task with global saliency) vs. Distractor-salient (e.g. global task with local saliency)), congruency (Congruent vs. Incongruent) and visual field (Central vs. Peripheral). The between-subject factor was age group (Young adults vs. Older adults). F- and p-values for significant main effects and interactions are displayed in Table 3.1.

Table 3.1. Significance levels (*F*- and *p*-values) for the main effect and interactions involving the factors task, saliency, congruency, visual field (VF) and age.

		Efficiency (RT/Accuracy)		Z-score	
	Interaction	F-Value	p-Value	F-Value	p -Value
Main Effects	Task	20.82	<0.001	29.81	<0.001
	Saliency	54.76	<0.001	188.16	<0.001
	Congruency	28.71	<0.001	166.09	<0.001
	VF	43.07	<0.001	147.17	<0.001
	Age	22.76	<0.001	-	-
General Interactions	Task x Saliency	17.93	<0.001	-	-
	Saliency x Congruency	13.82	<0.001	38.82	<0.001
	Task x VF	32.83	<0.001	140.98	<0.001
	Saliency x VF	15.81	<0.001	43.58	<0.001
	Task x Saliency x VF	9.83	0.003	18.69	<0.001
	Congruency x VF	9.17	0.004	-	-
	Task x Congruency x VF	12.45	0.001	13.06	0.001
	Saliency x Congruency x VF	7.83	0.008	-	-
	Task x Saliency x Congruency x VF	4.36	0.043	-	-
Interactions with Age	Task x Age	-	-	5.35	0.026
	Saliency x Age	21.42	<0.001	8.72	0.005
	Congruency x Age	11.55	0.002	-	-

VF x Age	11.12	0.002	-	-
Task x Saliency x Age	8.27	0.006	-	-
Saliency x Congruency x Age	7.68	0.008	4.45	0.041
Task x VF x Age	9.99	0.003	-	-
Saliency x VF x Age	7.86	0.008	-	-
Task x Saliency x VF x Age	5.54	0.023	-	-
Congruency x VF x Age	6.14	0.017	-	-
Task x Congruency x VF x Age	5.27	0.027	-	-

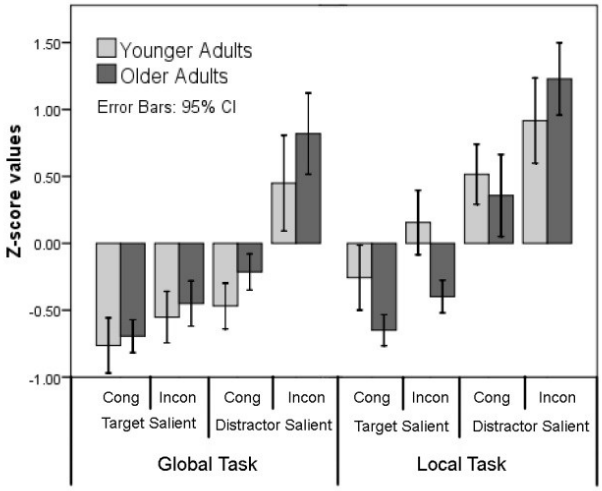

Figure 3.2. Mean Z-score values (± 95% confidence interval) as a function of congruency, saliency and level of identification for young and older adults. Values indicate the difficulty (-1 being easiest, +1 being most difficult) of a condition in relation to the averaged efficiency across all conditions (baseline).

The three way interaction between saliency, congruency and age suggests that there may be a differential effect of congruency for older adults. Performance on trials with low saliency targets (e.g. select the local element in a stimulus with set focus on the global level), in particular, may be difficult for older adults if they lose the ability to suppress high saliency distractors – a result mimicking the effects of TMS on left PPC (Mevorach et al., 2010). To quantify this, the cost of congruency was calculated individually for each target saliency condition from the difference in the Z-score values

between incongruent and congruent trials (incongruent - congruent), see Figure 3.3. A two-way ANOVA was conducted with a within-subject factor of salience (Target-salient vs. Distractor-salient) and a between-subject factor of age. This revealed significant main effects of salience and ageing [F (1, 41) = 38.82, $p < 0.001$, F (1, 41) = 8.72, $p = 0.005$]. The interaction between salience and ageing was also significant [F (1, 41) = 4.45, $p = 0.041$] (Figure 3.3). Further t-tests revealed that there was a reliable effect of age on performance in the distractor-salient condition but not in the target-salient condition [t (41) = -2.11, $p = 0.041$ and t (41) = 0.51, $p = 0.510$ for distractor-salient and target-salient conditions, respectively]. Thus, the old group was characterised by larger congruency effect compared to the young group but only when low saliency targets had to be selected while high-salience distracters ignored (e.g. select the local element in a stimulus weighted to the global level). Importantly, the increased congruency effect was not specific to a particular level of processing (local or global) and therefore indicated a general problem in suppressing high saliency distractor irrespective of the level of form involved.

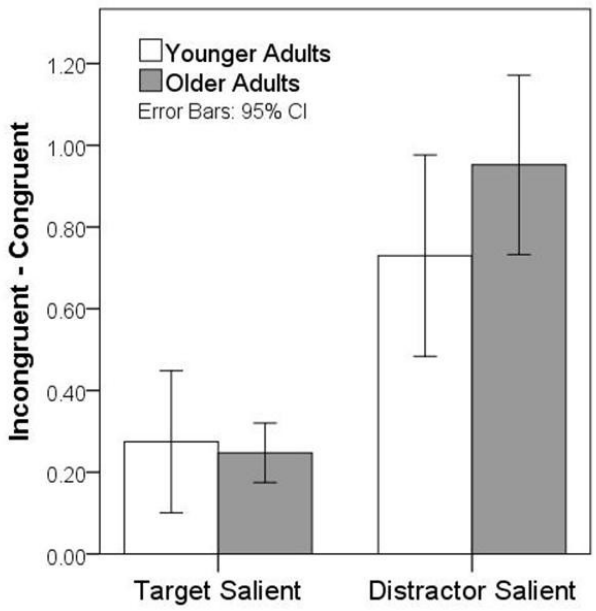

Figure 3.3. Congruency cost calculated from the Z-score difference between incongruent and congruent trials for target salient and distractor salient conditions separately for young and older adults.

Separate from the 3-way interaction between saliency, congruency and age, there was an overall effect of task and a reliable interaction between task and age (Table 3.1). The main effect of task occurred because, overall, responses to global targets were more efficient than responses to local targets. However, this effect varied with age (Figure 3.4a). Relative to the overall average of performance for their age group, the young participants showed a larger difference between the global and local tasks (relatively fast for global and relatively slow for local, a Z difference of 0.67 in efficiency), when compared with the older participants (a Z difference of 0.27 in efficiency) [$F(1, 41) = 5.35$, $p = 0.026$]. Interestingly, the contrasting variation in performance across the age

groups as a function of the task (Figure 3.4a) went in the opposite direction to their respective variation as a function of stimulus saliency (Figure 3.4b). We take this point up in the Discussion section below.

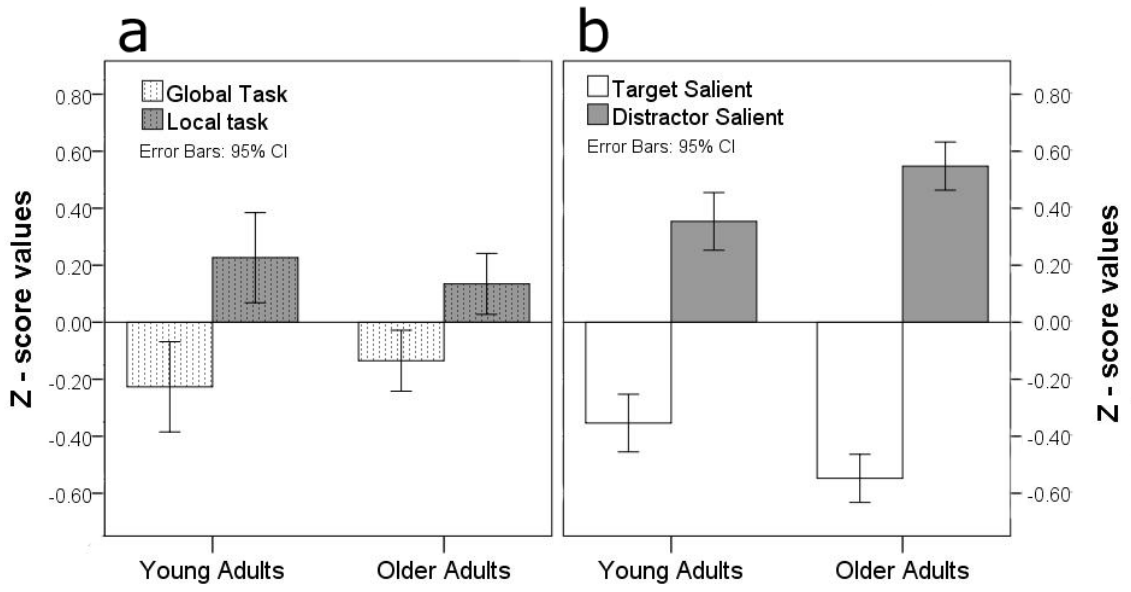

Figure 3.4.Mean Z-score values (± 95% confidence interval) as a function of task (Global / Local, plot a) and as a function of target saliency (Target-salient / Distractor-Salient, plot b) for young and older adults

3.5. Discussion

The main finding was that, relative to young adults, older adults were more affected by salient incongruent distractors (producing higher congruency costs in the distractor-salient condition), even with the analysis scaled for the effects of aging on overall efficiency (using Z transformations). This age-related decline in ability to select a

low saliency target in the presence of a high saliency distractor held for both levels of target identification, both with local and global stimuli (respectively when the global or the local saliency of the distractor was high). Importantly, this increased congruency effect in distractor salient displays cannot be attributed to generally heightened susceptibility to salience in old age. If heightened sensitivity to salience was driving the effect, then our old participants should have also shown a difference in performance in the target salient conditions (e.g., an increased congruency effect when salient targets were reported, since older adults would be less sensitive to target saliency). We therefore conclude that performance in the old group most likely represents reduced down-regulated inhibition of saliency, encountered particularly under conditions where distractors are salient. We note that this result mimics the effects of TMS suppression reported by Mevorach et al. (2010), where the loss of inhibitory control was most evident with salient distractors.

Our findings are concordant with the *inhibition deficit theory* (Hasher & Zacks, 1988), which posits that older adults are generally less able to inhibit unwanted information – though here we show for the first time that this applies to non-spatial selection of local and global forms. According to the inhibition deficit framework, early bottom-up responses to salient, exogenous stimuli require inhibitory mechanisms to limit processing when the stimuli are irrelevant (i.e. to ignore the conversation of nearby passengers while reading a newspaper in a train). Deficits in the efficiency of inhibiting irrelevant distractors may disrupt the ability to focus attention on stimuli of interest,

resulting in the dilution of selection across distractors as well as targets. As noted in the Introduction, deficits in filtering out distractors have been observed across a range of conditions, with different types of stimuli (e.g., in reading (Carlson et al., 1995), language comprehension and production (Burke & Mackay, 1997; Burke, 1997; Tun et al., 2002), visual memory (Gazzaley et al., 2005) and spatial visual selection (Watson & Maylor, 2002; Schlaghecken et al., 2012). To our knowledge this is the first study showing that age-related deficits in inhibition in non-spatial selection[5].

In addition, our findings link the *inhibitory deficit theory* with observations from neuroimaging. There is a striking parallel between prior studies in which TMS was applied to the left PPC, to reduce its influence on suppressing perceptual representations of distractors (Mevorach et al., 2010; Mevorach et al., 2006b). Mevorach et al. report that, across both local and global levels, low saliency targets became difficult to select after the left PPC received TMS, and this was associated with increased activation in early occipital cortex. These data are consistent with low saliency targets being selected through modulated inhibition of high saliency distractors via the left PPC, and with this top-down selection process being compromised with age. The data also fit with the Posterior-Anterior Shift with Aging (PASA) model, which posits an age-decline in the occipito-parietal networks involved in attention (Cabeza et al., 2004; Davis et al., 2008). We may speculate that the age-related decline in the suppression of salient information

[5] Note that the selection of a global stimulus, and ignoring of a local stimulus, cannot be explained in terms of spatial selection, since any 'fitting' of a spatial window of attention on a global stimulus would also lead to local stimuli being selected.

may be due to age-related decreases in the effectiveness of connection between occipital and parietal cortices. Irrespective of this, our behavioural data suggest that altered control of attention to low saliency signals may be a critical factor in cognitive ageing and, at least in our results, something that is more important than alterations in the selection of local and global targets.

Problems in selecting low saliency stimuli may have been critical to findings from studies using distraction as a measure of top-down attentional control in ageing. The inhibition of task-irrelevant information in ageing has been assessed from responses to task-irrelevant abrupt onsets (Kramer et al., 1999) and the inhibition of cued information in top-down visual search (Madden et al., 2004a; Madden et al., 2007). Although there are results suggesting that there is preservation of top-down attentional control with ageing (Kramer et al., 1999; Whiting et al., 2007; Whiting et al., 2005; Madden et al., 2004a), this has not been established in cases where distractors have relatively high saliency (compared with targets) (Kramer et al., 2000; Madden et al., 2004a). The current results go beyond these data by clearly demonstrating impairments in rejecting high saliency distractors at different levels of stimulus representation. It is perfectly possible that other forms of top-down processing, such as the guidance of attention from positive expectancies of targets, remains intact.

One somewhat different account of the present results can also be put forward. This is that older adults suffered more interference from salient distractors because they had more efficient parallel processing of both levels of the hierarchical forms. This more

efficient parallel processing would mean that distractors are processed more deeply and thus create more interference. However, on this account we would expect that RTs on congruent trials would be notably fast for older adults, since they would gain more from redundancy at the distractor level. There was no evidence for this (not shown here).

Aside from the effects of saliency, the old and young age groups differed in how their performance varied in the global and local identification tasks. The young participants showed relatively large difference in performance in the global compared to the local task, when compared with their overall performance. The older participants showed relatively small changes between the global and local tasks, compared with their overall performance. On the other hand, the older participants showed larger variation than the young participants as stimulus saliency changes (Figure 3.4 a and b). These data suggest that, for older but not for young participants effects of saliency produce stronger shifts in performance than effects of task (global vs. local). Our results, stressing the effects of saliency across different levels of form, also help to explain previous inconsistencies in the literature, where opposite effects of ageing have been reported under conditions where the saliency of the local and global forms was varied (cf. Muller-Oehring et al., 2007; Roux & Ceccaldi, 2001).

Finally, the advantage of left over right visual field was unsurprising. Prior studies have reported a left visual field advantage (Orr & Nicholls, 2005) most probably reflecting right hemisphere dominance for attentional processing (Siman-Tov et al., 2007). Critically this did not interact with age. Furthermore, there were no interactions

between visual fields (centre vs peripheral) and age, which provided evidence that the results cannot be explained with loss of visual acuity in the periphery. We conclude that age has a selective effect on rejecting high saliency distraction, an ability associated with the left PPC in our task.

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CHAPTER 4.
Dissociating effects of stimulus identity and load on working memory attentional guidance: Lengthening encoding time eliminates the effect of load but not identity

4.1. Abstract

Effects of the identity and load of items in working memory (WM) on visual attention were examined. With a short interval between the WM item and a subsequent search task, there were effects of both load (slowed overall RTs in a WM condition relative to a mere repetition baseline) and identity (search RTs were affected by re-presentation of the item in WM in the search display). As the time to encode the initial display increased, the effects of load decreased while the effect of identity remained. The data indicate that the identity of stimuli in WM can affect the subsequent deployment of attention even when time is allowed for consolidation of the stimuli in WM, and that the WM effects are not causally related to the presence of cognitive load. The results are consistent with the identity of stimuli in WM modulating attention post the memory consolidation stage.

4.2. Introduction

There is now considerable evidence that working memory (WM) interacts with perception when we select information for action that is consistent with the current goal set. For example, findings from brain imaging studies demonstrate that there are shared neuronal circuits between WM and attention (Cabeza & Nyberg, 2000; Kastner & Ungerleider, 2000). In behavioural studies there is evidence that having the appropriate goal-setting in WM critically determines the efficiency of search (Anderson et al., 2008), and the ability to filter out irrelevant distraction (Watson & Humphreys, 2000). There are also data indicating that stimuli in WM can automatically guide attention to matching stimuli. Downing (2000) had participants hold an initial item in memory and then search for a different target. Prior to the target's exposure, the WM stimulus could re-appear. Downing reported that responses to the target were facilitated when the WM stimulus appeared at the location of the search target relative to when it appeared at a different location. This basic effect has now been replicated across several different laboratories (Soto et al., 2005; Olivers et al., 2006; Han & Kim, 2009). The effect of the WM stimulus is found even when this stimulus never validity cues the target, it affects the first eye movement made in search (Soto et al., 2005) and it affects perceptual sensitivity to targets (Soto et al., 2010). Similar effects are not found when participants see but do not have to hold the initial item in memory (the "mere repetition" condition). The results suggest that there is automatic attraction of attention to stimuli that match the contents of WM, and these effects differ from those of bottom-up priming reflecting the mere appearance of the initial stimulus. This

argument is supported by results from functional imaging studies that show that the WM effects and the effects of mere repetition are supported by different underlying neural mechanisms (Soto et al., 2007).

Although there are robust effects of items in WM on attention, the conditions under which these effects arise remain unclear. For example, in at least some studies the WM has been presented close in time in relation to target displays (e.g. Soto et al., 2006), so that performance could be influenced by stimuli being consolidated into WM rather than being represented in WM in a stable form (Olivers et al., 2006). Soto and Humphreys (2008) varied the interval between the initial WM cue and the subsequent target and found a sustained WM effect across intervals from 181 to 1018 ms. Dombrowe et al. (2010) assessed performance with intervals up to 3.5s. They found that there were strong effects of the WM cue up to an interval of 1 s between the cue and the target, but that the cueing effect decreased at the longer cue interval (3.5s). These data suggest that effects from WM can arise even when there is sufficient time to allow consolidation to take place (at least 1s or so), but the argument is based on the inference that consolidation would be complete across the intervals examined. However this assumption may not hold. Dombrowe et al. (Dombrowe et al., 2010) for example had participants remember the exact shade of the WM stimulus and the precise encoding of colour may require time. If consolidation of the precise colour was prolonged over a second, then the strong effects up to this interval could be due to the consolidation process still continuing; the decreased influence at the longer interval could then reflect the completion of the consolidation process and the possibility that stimuli encoded into WM no longer guide attention. These arguments about the relatively long time that

might be taken to encode stimuli into WM are supported by studies on the 'Attentional Blink', where there can be poor report of a second target some hundreds of milliseconds after a first target when the first target is being consolidated in memory (Raymond et al., 1992). A better test of whether WM effects arise only under conditions where items are being encoded in WM would be to include a further manipulation which would provide independent evidence for consolidation being completed, and also for comparisons to be made with any priming effects. This was done in this chapter by including a "mere repetition" baseline across a set of intervals separating an initial cue from a subsequent search display. The interest in the "mere repetition" (MR) baseline was as follows. A little studied aspect of studies contrasting top-down cueing from WM with the effects of mere repetition from a cue (WM vs MR condition) is that there is typically an overall worsening in performance in the WM condition (e.g. , an RT cost is found even on neutral trials, when the initial cue does not re-appear in the search display). This worsening of performance can be attributed to the extra load, from the item(s) in WM (Soto et al., 2005). This overall cost, however, may provide an index of memory consolidation, rather than a cost from the memory load per se – at least when a minimal memory load of a single item is used. If this is the case, then the overall cost in the WM condition may decrease as the interval between the cue and the search display increases (e.g., as indexed on neutral trials). We assessed this, while testing at the same time whether there was any variation in the strength of the cueing effects from WM. If there is no evidence for a decreased cueing effect, but concurrent abolition of the overall WM cost, this would provide strong evidence that cueing effects do not stem from the WM stimulus still being consolidated.

There is one other point about the overall WM cost, which is that it may reflect the WM condition imposing a greater "cognitive load" on performance (Lavie, 1995). Under conditions of cognitive load, participants often find it more difficult to ignore distracters compared to when the cognitive load is reduced (Lavie, 2005). It is possible, then, that the extra cueing effects from WM are caused by the increased cognitive load from holding an item in memory, rather than from the identity of the cue in WM directing search. For search to be affected it may be enough that there is bottom-up activation of a representation of the cue; what the WM condition does, then, is to allow this bottom-up activation to influence search by lessening cognitive control of selection. This "cognitive load" interpretation can be tested if the load effect (the overall WM cost) decreases as the cue-search interval increases, when memory consolidation is completed. If the load effect is necessary to generate the differential cueing effect in the WM and mere repetition conditions, then the cueing effects should be eliminated when the load effect disappears.

4.3. Method

4.3.1. Participants

Twenty-four young adults (eleven male, mean group age 21 years) participated in exchange for course credits or cash (£6 per hour). The recruitment was carried out through an online experiment management system (Research Participation Scheme, University of Birmingham). All participants had normal or corrected-to-normal vision.

4.3.2. Stimuli, Task and Procedure

Trials began with a fixation cross for 500ms. This was followed by the presentation of two consecutive cues (100ms and 500ms, respectively), separated by a 100ms fixation display. The cues could be a circle, a triangle, a square, a diamond, or a hexagon in one of five possible colours (red, blue, green, yellow or pink). An inter-stimulus interval (ISI) with three different durations (250ms, 1000ms and 3000ms) separated the cue and a subsequent visual search display. The search display contained four objects, each different in shape and colour from the others. The four stimuli fell in separate quadrants of the display and the distance between the centres of the objects was 18 degrees. The centre of each object contained a black line whose length was 0.88 degrees and whose width was 0.12 degrees. One of the lines was tilted (26 degrees) to either the left or right, whereas the remaining lines were vertical. The search task required participants to distinguish the orientation of the tilted line within a time window of 1000ms by pressing either the left or right mouse button. There were two cue conditions. In the WM condition (Figure 4.1a), participants were asked to hold the shape and the colour of the cue for a memory recognition task after the search task. In the mere repetition (MR) condition (Figure 4.1b), participants were required to merely compare shapes and colours of the initial two cue objects. If the objects matched, participants were asked to carry on with the search task. If they did not match (on 20% of the trials) participants did not carry out the search task. This match procedure was introduced in order to ensure that participants processed the cue in the MR condition (Soto et al., 2007). In the WM condition, a memory test followed 500ms after the response in the search task. A single coloured shape was presented and participants were required to indicate whether the new

stimulus was the same or different in colour and shape relative to the cue object. On mismatch trials, the colour or the shape alone could match the initial cue.

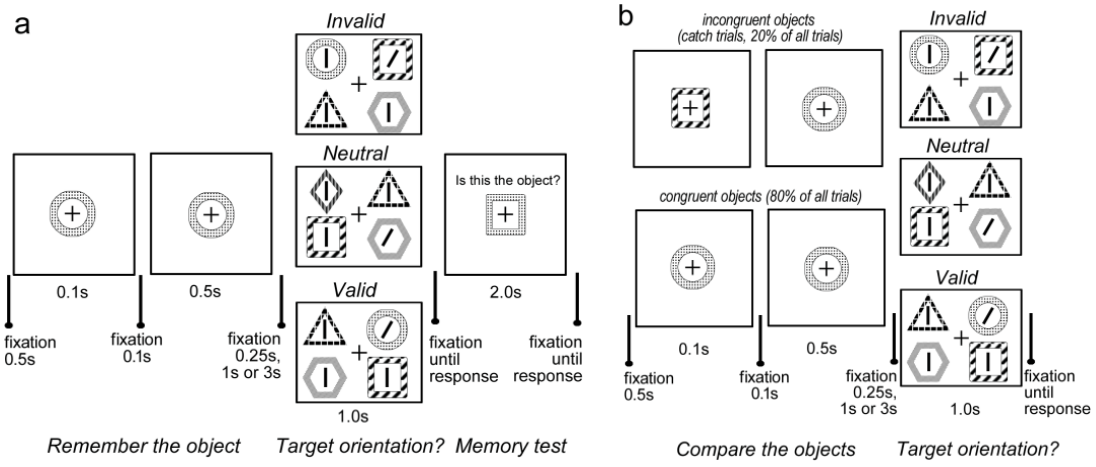

Figure 4.1. Example of the display sequences used in (a) working memory and (b) mere repetition conditions.

The search task included three different conditions determined by the validity of the cue (valid, invalid and neutral). On valid trials the cue was re-presented in the search display and shared the same spatial location as the target (the tilted line). In the invalid condition the cue was again re-presented but it occupied a different location to the target and it contained a vertical (distractor) line. In the neutral condition the cue was not presented in the search display and no other stimulus in the search display had either the shape or the colour of the cue.

Search trials were randomised and counterbalanced across quadrants and validity conditions. The experiment was split in blocks across memory conditions. Each block started with a prompt on the screen followed by two catch trials to minimise task-switching effects from the previous block. This resulted in 6 randomly selected blocks (3 x WM and 3 x MR) with a

total number of 576 trials (3 ISIs x 4 quadrants x 3 validity conditions x 16 repetitions) lasting around 50 minutes. The stimuli were created and presented using the Psychophysics Toolbox for Matlab (Kleiner et al., 2007).

Observers were encouraged to respond as quickly as possible while still trying to maintain a high level of accuracy. Each participant performed a practice trial in each memory condition before the start of the experiment. Response times and performance accuracy were collected from subjects on search task trials in both memory conditions, and on memory performance in the WM condition. Incorrect responses were excluded from the analysis. In the WM condition, correct RTs included only trials with correct responses to both, search and memory tasks. As all responses to the task in both conditions were not time-windowed, the RT data were screened for outliers by rejecting all RTs beyond three standard deviations from the mean of each experimental cell (cf. Van Selst & Jolicoeur, 1994).

4.4. Results

Overall accuracy levels were very good. Performance in the WM condition (89%) was less accurate compared to the MR condition (96%). The screening procedure for outliers (see Methods) excluded on average 0.3% and 0.4% of all trials for the WM and MR conditions respectively.

A three-way ANOVA on the mean search response times was created with the within-subject variables of Task (WM vs MR), Cue validity (valid, invalid and neutral) and inter-

stimulus interval (ISI - time between cue and search display: short, medium and long with a duration of 0.25, 1.00, 3.00 seconds, respectively). Greenhouse-Geisser correction was used where necessary. We observed a main effect of task, F (1, 23) = 10.32, p = 0.004, with slower responses to the WM trials compared to the MR trials. The main effect of ISI was also reliable, F (2, 46) = 9.67, p < 0.001; faster responses with longer ISIs. We observed a main effect of validity, F (1.20, 26.54) = 100.89, p < 0.001. Compared to the neutral condition (no cue in the search display), response times were slower on invalid trials, mean difference 58ms (p < 0.001), and faster on valid trials, mean difference 72ms (p < 0.001).

There was a reliable two-way interaction between Cue validity and Task, F (2, 46) = 30.35, p < 0.001, and between ISI and task, F (2, 46) = 3.48, p = 0.039. The 3-way interaction was significant (Task x Validity x ISI), [F (4, 92) = 3.17, p = 0.017] (Figure 4.2).

The 3-way interaction suggested that validity effects may change across the ISIs differentially for the WM and MR conditions. To test this we decomposed the 3-way interaction to two 2-way ANOVAs (one for each memory condition) including the within-subject factors of ISI and validity.

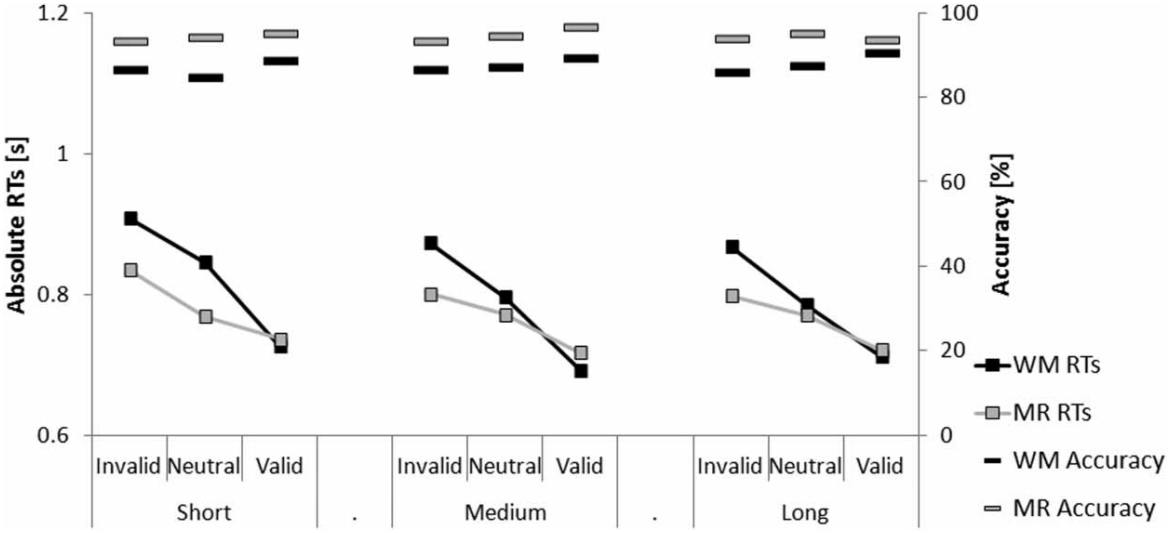

Figure 4.2. Response times (RTs; lines on the left axis) and accuracy (bars on the right axis) for short (0.25-s), medium (1-s) and long (3-s) interstimulus intervals (ISIs) in working memory (WM, black colour) and mere repetition (MR, grey colour) conditions.

Analysis of WM trials showed that the main effects and their interaction was still valid – F (1.45, 33.26) = 9.25, p = 0.002; F (1.33, 30.47) = 165.07, p < 0.001; and F (4, 92) = 3.12, p = 0.019 for ISI and validity main effects and for the ISI x Validity interaction, respectively. Functionally, the validity effect has been related to two different processes – the benefit from cueing, where the cue item and the target share common properties, and the cost from cueing, where the cue item and a distractor share properties (Rotshtein et al., 2011). To further investigate how the validity effect changed across ISIs, we decomposed the validity effect into (i) the benefit from cueing ((neutral trials – valid trials) / (neutral trials + valid trials)) and (ii) the cost from cueing ((invalid – neutral) / (invalid + neutral)) (Figure 4.3). An initial two-way ANOVA with the within-subject factors of cueing effect (Benefit effect vs. Cost effect) and ISI (short, medium and long) revealed a main effect of cueing, F (1, 23) = 9.61, p = 0.005 and a

significant interaction between cueing and ISI, F (2, 46) = 3.59, p = 0.036. Further decomposition of the two-way ANOVA into independent one-way ANOVAs showed that the benefit effect decreased significantly as the ISI lengthened, F (2, 46) = 5.35, p = 0.008 (76ms, 66ms and 49ms for short, medium and long ISI, respectively). In contrast, the cost from cueing tended to increase with longer ISIs (37ms, 48ms and 50ms for short, medium and long ISI, respectively), though this trend was insignificant, F (2, 46) = 1.23, p = 0.302 (37ms, 37ms and 52ms for short, medium and long ISI, respectively). For MR trials the validity effect did not vary across ISIs, F (4, 92) = 2.09, p = 0.089, although there was a trend for the cost from cueing effect to be larger at short ISI (42ms) when compared to medium and long ISIs (19ms and 18ms for medium and long ISI) [Cueing cost x ISI: F (2, 46) = 3.12, p = 0.062].

The overall contrast between the WM and MR conditions across the ISIs is illustrated most clearly by taking the data for the neutral trials (uncontaminated by the effects of cue validity). We assessed the change in the neutral condition across the ISIs with RT values normalised to the sum of the neutral conditions in the two tasks (($WM_{neutral}$ - $MR_{neutral}$) / ($WM_{neutral}$ + $MR_{neutral}$)) – to extract out any general effects of task difficulty across the ISIs.

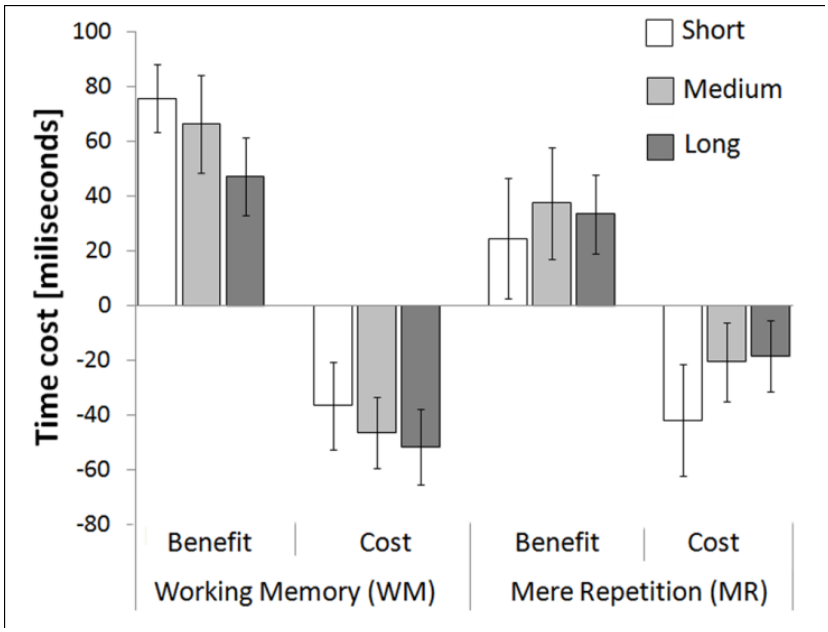

Figure 4.3. Magnitudes of the benefit effect (neutral – valid, shown as a positive value) and the cost effect (invalid – neutral, shown as a negative value) as a function of time for working memory (WM) and mere repetition (MR) conditions.

A repeated-measures ANOVA with the within-subject factor of memory cost (as a function of ISI) revealed a main effect of memory cost, F (2, 46) = 6.08, p = 0.005, driven by larger differences between performance in the WM and MR conditions at the short ISI (mean difference 78ms), when compared to the longer intervals (25ms and 14ms for medium and long ISIs, respectively). Furthermore, the absolute reaction times revealed that the only significant difference between performance in the WM and MR conditions was at the shortest ISI, t (23) = 3.86, p = 0.001. At the longer intervals (1 and 3s) there were no differences between WM and MR trials in the neutral condition, t (23) = 1.54, p = 0.138 and t (23) = 1.20, p = 0.243 for 1 and 3 seconds, respectively (see Figure 4.2).

4.5. Discussion

The current study investigated the time-course of cueing effects on attention from working memory (WM) and from mere stimulus repetition (MR). We introduced three intervals (250 ms, 1000 ms and 3000ms) between the initial cue and the subsequent visual search display. Overall, search time was reduced on invalid trials (when the memory cue was re-presented at the location of a distractor), relative to neutral trials, while performance was boosted when the cue was valid (appearing at the location of the target). The overall validity effect was larger in the WM condition than the MR condition. This general pattern of results replicates prior findings showing that the contents of WM drive visual selection (Soto et al., 2007; Soto et al., 2008; Olivers, 2009; Olivers et al., 2006).

In addition to this we found that the validity effect varied as a function of time, with benefit from cueing on valid trials reducing as the time between memory cue and the search display increased up to 3 seconds, although it remained significant throughout. In contrast, the effect of cost from cueing (when the WM cue fell at the location of a distractor) was robust across the time intervals. In the MR baseline, the validity effect was small and did not vary as the interval lengthened. The finding that cost of cueing in WM condition remained sustained for at least 3 seconds has been reported previously (Soto & Humphreys, 2008), whereas the findings on the beneficial effects from cueing replicated partially those reported by Dombrowe et al. (Dombrowe et al., 2010) (though in Dombrowe et al., the beneficial effect of WM guidance was no longer reliable at a similar interval [1000ms presentation + 2500ms blank field]). There may

be various reasons for our beneficial effect remaining. One possibility is that we had WM stimuli match both the shape and colour of the items in the search display whereas Dombrowe et al. only had items match in colour. Soto et al. (2005) reported that the effects of matching from WM were stronger when a conjunction of features matched relative to when single features matched. Again we would expect stronger cueing effects under our conditions. Interestingly, there was also a decrease in cueing as the interval between the cue and the search display increased in the MR condition. This effect was observed on the cost from cueing effect on invalid trials. Possibly passive activation of the cue's representation has a transitory influence on subsequent attentional orienting, which emerges most strongly on invalid trials due to the cost of participants then having to disengage attention. However this effect decreases across time unless the cue is maintained in WM, when it continues to exert a reliable effect on performance. Unlike the effect of benefit from cuing in the WM condition, the cost from cueing effect was robust across time (even tending to increase). Again this may reflect the cost of having to disengage attention when it is focused initially on a distractor rather than the target, and this cost may even increase as the WM item is better consolidated in memory – note that, as the ISI increases, such increased costs of disengagement may trade against a lower likelihood of attention being cued to the distractor as memory consolidation takes place. That is, memory consolidation may gradually reduce the power of a WM cue from attracting attention (indexed by the reduced beneficial effects across time) but there is a greater difficulty in disengaging attention from the cue, once attention has been draw to it (indexed by the robust cueing cost effect across time).

The argument that the WM was consolidated into memory over time is indicated by finding that there was an abolition of the memory load effect between MR and WM as the time between the cue and search increased – measured here by the relative decrease in the task effect on neutral trials, with performance normalised for overall response time changes across the ISIs. We suggest that the load effect between WM and MR at the shorter ISI reflects memory consolidation, with the effect reducing as the ISI increases. There then remains an effect of the item that is maintained in WM, which can continue to cue attention and generate costs on performance when the cue is invalid. A critical result to note however is that the effect of WM cueing remained even when the effect of memory load (WM vs. MR, on neutral trials) disappeared. This finding provides the first evidence that the enhanced cueing effects from WM, found here and in other studies in the literature, cannot be due to the greater load in the WM condition (cf. Lavie, 1995). There appears to be a specific effect of items maintained in WM, not load.

It is also possible to make a rather different argument about the effects of the ISI on the memory load and validity effects. This is that, since items are only being consolidated in WM at the short intervals, then one might expect WM effects to increase at the longer interval (once items are consolidated in WM). This argument, however, needs to be reviewed in the context of prior results on attentional guidance from WM. Olivers (2009) discusses the conditions under which the WM effects are maximized, which include cases where there is a single item that is 'at the forefront' of WM. We suggest that, during consolidation, an item is held at the forefront of WM, and this leads to large effects of memory guidance. Once the item is encoded, though, it

may be possible for participants to weight more strongly other representations in WM – such as those specifying the target for the search task. When the target is strongly represented, the magnitude of the WM effect decreases (while remaining reliably larger than effects in the MR condition).

One other point pertains to the nature of the information that is maintained in WM. There are studies showing that there are effects of verbal cues on the guidance of visual attention (Soto & Humphreys, 2009), consistent with visual attention being directed by verbal as well as visual representations of stimuli. It is possible then that, at longer time intervals between the cue and the search displays, the cue could be coded in a non-visual format in WM. However, even if this was the case, the point is that the effect of WM load disappeared at the longer interval, yet effects on memory guidance remained. This demonstrates that the guidance effects cannot be due to search generally being vulnerable to the increased load under WM conditions – which is the main point of the paper.

4.6. Conclusion

In sum, these results point to there being two separate factors influencing WM and WM-based guidance of attention. First, there is a short lasting process of WM consolidation lasting for limited period of time after cue presentation. This leads to an overall cost in performance under WM conditions compared to conditions of mere repetition, as measured even when the cue is not re-presented in the search display. Second, there is a robust effect of WM on attentional selection,

based on top-down guidance from items consolidated and maintained in WM. The important result is that the load effect is not critical to WM guidance on attention, which remains even when load effects are eliminated. The current data suggest having memory consolidation ongoing when the search display appears is not necessary for validity effects to occur (relevant to Olivers et al., 2006 argument) and points to the overall memory costs being different from the WM cueing effect.

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CHAPTER 5.
Attentional Guidance from WM: Age Dissociates Effects of Overall Load and Attentional Guidance

5.1. Abstract

We report data on the effects of cognitive ageing on attentional guidance under conditions of bottom-up stimulus priming and top-down activation of working memory (WM). Young (mean age 24 years) and older adults (mean age 76 years) were asked to hold an item in WM while searching for a different stimulus. Relative to the younger participants, older individuals were detrimentally affected by having to hold a first item in WM compared with a condition where they had only to identify the same item, even when the item did not appear subsequently in the search display. This occurred even with the effects scaled by overall reaction times. However, older participants showed weaker effects of guidance from irrelevant items in WM when compared with the younger participants. That is, there was a double dissociation between the effects of load and of attentional guidance. We discuss the results in terms of older participants having to devote greater resources to maintaining the task template, leaving fewer resources available for irrelevant items in WM to modulate attention.

5.2. Introduction

There is accumulating evidence that attentional selection is influenced by items held in WM, even when the items are irrelevant to the goals of the attentional task (Soto et al., 2005; Soto et al., 2006; Olivers et al., 2006; Soto & Humphreys, 2007; Soto & Humphreys, 2008; Olivers, 2009). Downing (2000), for example, had participants hold a stimulus in memory and then search for a different target. A pair of stimuli appeared just before the search target, one of which could be the item held in memory. RTs to the search target were faster if it fell at the location where the memory item appeared (valid trials) compared with when the target fell at the location of the other item (i.e. invalid). In contrast to the effects of an item coded in WM, there is reduced memory guidance if the initial cue has to be identified but representation in WM is not required (Soto et al., 2007; Tsvetanov et al., 2012a). The results indicate that under some conditions there is automatic capture of attentional selection by stimuli matching the contents of WM. There remains the question though of how these capture effects come about, and in particular how attentional capture relates to more general effects of cognitive load that can vary across task conditions. Ageing has been associated with increased processing load in WM to compensate for the decline in cognitive function (Cabeza, 2002; Davis et al., 2008). In this paper we take ageing as a model to test how the guidance effect is affected as overall load increases. We evaluated the effects of ageing on (i) the general effects of memory load on search, found when memory items do not re-appear in the search displays, and

(ii) the guidance of attention by WM specific cueing effects. Does ageing influence these effects in the same or in opposite ways?

Having to hold an item in WM, as opposed to merely identifying it, increases the overall cognitive demands on performance. For instance, RTs are typically slower overall under memory load compared to visually matched identification conditions (Soto et al., 2005). It is possible then that non-specific cognitive load may modulate the interaction between memory and selection, rather than the specific memory load. Lavie (1995), for example, has argued that the effect of carrying a greater cognitive load is to decrease selectivity in information processing, increasing effects of irrelevant distractors. Here it could be argued that the general decrease in cognitive control, when there is a memory load, allows irrelevant distractor in memory to be selected when they re-appear in the search display. Tsvetanov et al. (2012) presented some evidence against this. In this study increasing the time between the onset of the WM cue and search display to above 2sec diminished non-specific cognitive costs but had only minimal impact on the interaction between WM and selection (Chapter 4). Tsvetanov et al. suggested that the non-specific cognitive costs arise from the process of encoding the initial cue into WM, but WM-based guidance occurs even when items have been consolidated. That is, general effects of load and attentional capture may dissociate.

There is also evidence indicating that working memory does not always guide selection (Downing & Dodds, 2004; Houtkamp & Roelfsema, 2006; Woodman & Luck, 2007). Notably, guidance from WM can decrease when more items are memorized,

especially when participants also engage in articulatory suppression (Soto et al., 2007; Soto et al., 2012a). In addition, the robust guidance effects that occur when the search items are constant across trials (Soto et al., 2007) decrease when the search items vary (Downing & Dodds, 2004; Houtkamp & Roelfsema, 2006; Woodman & Luck, 2007). Olivers and colleagues (Olivers et al., 2011; Dombrowe et al., 2010) have hypothesised that a varied selection target must be kept 'at the forefront of WM', and, if only one item can be actively represented in the forefront (Cowan, 2001; Cowan, 2005; Oberauer, 2002), then the influence of the WM item will be reduced. In contrast, when the search item is constant, a search template can be automated without active maintenance in WM (Rossi et al., 2007). In this case the irrelevant memory cue may be maintained at the forefront of WM, and this guides attention when the cue re-appears in the search display. These results suggest that an increased memory load and variable search targets both decrease attentional capture from WM by reducing the likelihood that repeated memory items are at the forefront of WM.

There is strong evidence that, as we age, so our ability to update and manipulate the content of working memory is reduced (Hasher & Zacks, 1988; de Beni & Palladino, 2004; Babcock & Salthouse, 1990). In particular reduced WM capacity with age is associated with a poor ability to 'delete outdated' information irrelevant to current goals (Park et al., 2002; Hedden & Park, 2001) and to automate memory processes (Park & Reuter-Lorenz, 2009). A consequence of this is that higher demands are placed on cognitive resources to maintain performance, potentially by keeping task goals and

targets as active memory representations (Park & Reuter-Lorenz, 2009). It follows that we can make a counter-intuitive prediction. If older participants find it more difficult to automate a representation of the task template, then they may need to maintain it at the forefront of WM to accurately perform the primary search task. Consequently, weaker effects of WM-based guidance might occur. Note that this might even arise when individuals show stronger effects of the overall task load (in the WM condition vs. the mere identification baseline), due to slower memory consolidation and/or the greater processing demands involved in holding items even in the background in memory. The effects of ageing may doubly dissociate general load effects from attentional capture effects. These proposals were tested here.

We conducted two experiments. In Experiment 1, we tested effects of age on the interaction between WM and selection using the memory-search dual task paradigm (Soto et al., 2007). In Experiment 2, we tested whether age related effects on WM-based selection were related to the non-specific cognitive load effect associated with encoding an item into WM (Tsvetanov et al., 2012). Participants searched for a tilted line falling within one of four objects (coloured geometric shapes). Prior to the search display, participants saw an initial object which could match one of the objects in the subsequent search array. In the working memory (WM) condition, participants were asked to hold the initial item in WM for a later recognition task. In the 'mere repetition' (MR) condition, participants had to identify the initial shape but not hold it in memory. The initial item, when it re-appeared in the search display, could be a valid or invalid cue to

the search target (according to whether the re-presented cue shared the spatial location of the target or a distractor line). On neutral trials, the initial cue did not re-appear in the search display. Neutral trials measure the general effects of the WM load. Previous results indicate an overall slowing of RTs on neutral trials in the WM condition compared with the priming baseline (Soto et al., 2005), though this effect decreases when the time between the WM cue and the search array is increased (Tsvetanov et al., 2012). We anticipated that non-specific load effects in the WM condition would be larger in the elderly participants due to their slower consolidation/reduced cognitive resources. At the same time, the effects of the WM item on the guidance of search may reduce, since older participants may have to hold the search target at the forefront of WM to maintain search performance. As a consequence older adults will show a weaker modulation of visual selection by irrelevant items in WM (Experiment 1). In Experiment 2, we tested whether age related effects on WM-based attentional guidance reflected slower memory consolidation. Here we manipulated the time between encoding the memory item and performing the search task (see Tsvetanov et al., 2012). We asked whether older participants showed a slower reduction in overall memory costs over time, due to slowed consolidation of the memory item. On the other hand, if age-related differences in WM-based guidance are due to whether the search template is held at the forefront of WM, then effects of the validity of the WM stimulus should be maintained irrespective of the consolidation time, dissociating the effects of overall memory cost on distractor effects from WM.

5.3. Experiment 1: Age effects with a single encoding time

5.3.1. Methods

5.3.1.1. Participants

Twenty-four young (12 male with mean age of 24 years, 12 female mean age of 23 years) and nineteen (9 male with mean age of 74 years, 10 female with mean age of 76 years) senior healthy volunteers participated in the study for exchange of course credit or cash (£6 per hour). The participants were recruited by advertisements in local communities, word-of-mouth information and advertisements on an online experiment management system (Research Participation Scheme, University of Birmingham). All the subjects had normal or corrected-to-normal vision and were healthy with no history of psychiatric or neurological disease.

5.3.1.2. Stimuli, Task, and Procedure

Trials began with a fixation display for 500 ms followed by two cue displays (100 ms and 500ms respectively) with a 100 ms blank interval in between. The cue shape could be a circle, square, triangle, diamond, or hexagon. The possible colours of the cue were red, green, blue, yellow, or pink, displayed on a gray background. After the second cue there was a blank interval for 250 ms before the search display. The search display contained four unique objects, one for each quadrant. The distance between the centre of

the objects was 18°. The target, a black line (26°) tilted to either the left or right with length 0.88°, was displayed in the centre of one of the objects. The other three objects contained a distracter, a vertical black line (length 0.88°). The task was to discriminate the orientation of the target in a time window of 1000 ms by pressing either the left or right mouse button. In the working memory (WM) condition, the subjects were asked to hold in their memory the colour and the shape of the cue. In the mere repetition (MR) condition, the subjects were asked to compare the cues in terms of their shape and colour and withhold a response to the search display if cues did not match.

To ensure that participants performed the first task (remember (WM)/ compare (MR)) correctly we introduced memory test trials in all WM trials and catch trials on 20% of the trials in the MR condition. In the WM condition, a memory test followed 500ms after the response to the search display. Participants were required to indicate whether the new object was the same or different in colour and shape to the cue object. In the MR condition, 20% of the trials contained two cue objects with different shapes and colours; on these trials participants had to withhold their response in the search task. Catch trials in the MR condition were excluded from the analysis

There were 3 types of search trial. On valid trials the target (a slanted black line) was present in the centre of the cue object and the other three distractor lines were presented in three other objects. On invalid trials the cue object appeared in a quadrant opposite to that containing the target. On neutral trials neutral the cue did not re-appear (objects surrounding the target and distractor lines shared neither the shape nor the colour

of the cue). Search trials were randomized and counterbalanced across quadrants, repetitions and validity conditions.

Each block started with a 3-second prompt on the screen followed by two catch trials to minimize task-switching effects. A dot in the middle of the screen was used for fixation throughout the whole experiment. All this resulted in 18 minutes of four randomly selected blocks (2 x WM and 2 x MR conditions), each with 60 trials (5 trials x 4 quadrants x 3 validity conditions). The stimuli were created and presented in MATLAB R2009b.

Observers were encouraged to respond as quickly as possible while still trying to maintain a high level of accuracy. Each participant performed a practice trial for each task before the start of the experiment.

Data analysis: Response times and accuracy performance were collected from subjects on search task trials in both conditions and catch trials in the WM condition. Incorrect responses were excluded from the analysis. As all responses to the tasks in both conditions were not time-windowed, RT data were screened for outliers by rejecting all RTs beyond 3.5 standard deviations from the mean of each experimental cell (cf. Van Selst & Jolicoeur, 1994).

Ageing may have a generalised effect on the speed of information processing, without disrupting additional processes (such as distractor inhibition; Salthouse, 1981). If there is just generalised slowing, then the *relative* effects of the initial cue will be the same across older and younger participants, even if any cueing effects are increased in

absolute terms for older participants (e.g., if the validity effects are scaled by the RT). In the latter case, then the *relative magnitude* of the effects of attentional guidance should be equal in older and younger observers (e.g., when the validity effect is scaled by the mean RT). In Experiment 1 performance was examined with a single interval between the cue and the search display. In Experiment 2 performance was assessed with a wider range of intervals, to enable items to be better encoded prior to the start of the search task.

5.3.2. Results and Discussion

The data for three subjects were excluded from the analysis. These participants (1 young, 2 older) performed poorly in the MR condition suggesting they did not fully follow the instructions.

5.3.2.1. Memory Accuracy

The response accuracy on catch trials in the WM condition was 94% and 88% for younger and older participants, respectively. Similar performance was maintained on catch trials in the MR condition (88% and 95% for young and old participants, respectively). Catch trials (20% of all trials) showed that participants performed the task correctly and they were excluded from further analysis. See Table 5.1 for the accuracy data.

5.3.2.2. Search Accuracy

The accuracy of performance on search trials in the MR condition was higher for both age groups (97% and 94% for younger and older participants, respectively)

compared to the accuracy of performance on the same search trials in the WM condition (92% and 82% for younger and older adults, respectively). Search accuracy differed significantly between the WM and MR conditions for both age groups; $t(23)= 6.77$, $p < .001$ and $t(18)= 5.73$, $p < .001$ for younger and older participants, respectively. These results are consistent with the WM condition imposing a higher task load. Neither the main effect of validity, nor its interaction with task or age were significant [Validity: $F(2, 82) = .635$, $p = .533$; Validity x Age: $F(2, 82) = 1.11$, $p = .336$; Validity x Task: $F(2, 82) = 1.22$, $p = .300$; Validity x Task x Age: $F(2, 82) = .248$, $p = .781$;]. All trials with an incorrect response were excluded from further analysis.

			Accuracy Catch [%]				Accuracy Search [%]				RTs Search [millisec.]			
			Exp. 1		Exp. 2		Exp. 1		Exp. 2		Exp. 1		Exp. 2	
	Condition		Y	O	Y	O	Y	O	Y	O	Y	O	Y	O
Working Memory	Short	Invalid	93	87	93	96	98	92	89	90	872	1202	908	1217
		Neutral	93	87	93	96	97	92	88	92	812	1124	846	1119
		Valid	95	90	95	97	98	93	91	96	704	1006	726	1024
	Medium	Invalid			94	98			88	92			874	1154
		Neutral			94	97			90	94			796	1060
		Valid			94	98			90	95			692	954
	Long	Invalid			93	98			89	94			867	1070
		Neutral			93	97			90	95			785	1010
		Valid			95	97			92	95			711	915
Mere Repetition	Short	Invalid					98	98	93	95	774	990	834	1007
		Neutral	95	88			98	98	95	96	729	963	768	984
		Valid					98	98	97	97	693	914	736	928
	Medium	Invalid							96	97			800	989
		Neutral			86	95			96	96			771	961
		Valid							96	97			717	908
	Long	Invalid							96	97			798	974
		Neutral							96	98			770	937
		Valid							95	98			721	913

Table 5.1. Mean accuracy of memory in WM condition and catch trials in MR condition (Accuracy Catch) and the mean accuracy, mean RTs in search for each condition separately for young adults (Y) and older adults (O) across experiments (Experiment 1, Exp.1 and Experiment 2, Exp.2)

5.3.3. Search Performance

In order to dissociate task-specific effects from the generalised slowing effects, we transformed the RT data to Z-scores by normalizing each condition cell to the overall performance across all conditions for each subject (see Faust et al., 1999). The Z-transformed RTs are presented in Figure 5.1 (refer to Table 5.1 for the mean RTs themselves). The effects found on the Z-transformed data were replicated in our analysis of the untransformed RTs but, for clarity, we only report the Z-transformation results.

Performance was assessed in a three-way mixed ANOVA over the Z-transformed values with two within-subject variables of task (WM vs MR) and validity (valid, invalid, neutral) and one between-subjects factor of age group (young vs older participants).

Greenhouse-Geisser correction was used to assess the significance of the corresponding *F* where sphericity was violated. *F*- and *p*-values above significant levels for main effects and interactions were reported in (Table 5.2).

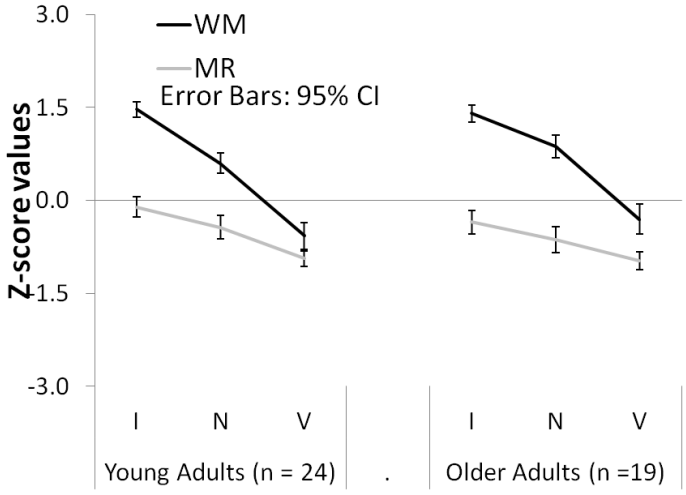

Figure 5.1. Mean Z-score values (right) across different validity conditions (Invalid (I), Neutral (N) and Valid (V)) for tasks when the cue object was held in WM (WM, black coloured line) and when it was merely compared (MR; gray coloured line) for both young and older adults. Error bars represent 95% confidence intervals.

There were significant main effects of task (WM vs MR), F (1, 41) = 215.38, p < .001, and validity (Invalid, Neutral, valid), F (2, 82) = 249.18, p < .001. Compared to the neutral condition, performance on invalid trials was worse [p < .001, indicated by larger Z-scores (slower RTs), see Figure 5.1], and on valid trials they were significantly better [p < .001, indicated by smaller Z-scores (faster RTs), see Figure 5.1]. There was a significant interaction between task and validity [F (2, 82) = 70.23, p < .001], with stronger effects of validity in the WM condition – though the validity effect was reliable

for both the WM and MR conditions [WM: F (2, 84) = 250.33, $p < .001$; MR: F (2, 84 = 56.20, $p < .001$)]. The task effect varied with age [F (1, 41) = 4.42, $p < .042$], driven by a larger z-value contrast between the WM and MR conditions in neutral trials (uncontaminated by the effects of cue validity, $Z_{WM\ Neutral} - Z_{MR\ Neutral}$) for the older group (Z difference = 1.31) when compared to the young group (Z difference = 0.99) [F (1, 41) = 4.14, $p = .048$]. In addition, there was a reliable interaction between validity and age [F (1, 41) = 3.90, $p = .024$]. In this case there was a smaller difference between invalid and valid trials for older adults ($Z_{Invalid\ -\ Valid} = 1.15$) compared to young adults ($Z_{Invalid\text{-}Valid} = 1.48$). The 3-way interaction was not reliable [F (2, 82) =1.81, $p = .170$].

The results replicate prior studies demonstrating an interaction between WM and attention in that they show that the re-appearance of a WM cue in a search display modulated attention to the search target – performance was better when the cue from WM was valid (and fell at the same location as the search target) and it was worse when the cue was invalid (falling at the location of a distractor). These effects were larger when the cue was held in WM than when it was merely identified but not committed to memory (in the MR condition) (see also Soto et al., 2007; Soto et al., 2008). The new result concerns the effects of ageing. When the data were normalised for overall RTs to account for generalised slowing, older individuals were less affected by the WM item than younger participants. With normalised RTs, there was also an interaction between task (WM vs. MR) and age: there was a greater decrement in performance under WM than MR conditions for older compared with younger individuals. That is, there was a double

dissociation between the effects of age on the overall costs from task load (in the neutral condition) and on the effects of cue validity.

This result is important. In nearly all experiments examining the relations between WM and attention it has been found that not only are cueing effects greater in the WM condition, but RTs are also slower – even in the neutral condition where the cue is not repeated in the search display (e.g., Soto et al., 2005). It is not clear whether the greater effect of validity in the WM condition is caused by the general cognitive load of the WM condition, since participants might have fewer resources to control the allocation of attention in this case (Lavie, 2005). The current data refute this account. The older participants showed a generally larger effect of cognitive load in the WM condition (even when relative measures of load effects were taken), but they also had *reduced* cueing effects once the data were normalised for overall response times. The result goes beyond our previous finding (Tsvetanov et al., 2012), which showed that the effects of cue validity could occur even when the overall effects of task load were minimised by using a long cue-search interval. Here we demonstrate that the presence of an increased overall cost can be associated with a reduced validity effect. The presence of a large overall cognitive load is neither *sufficient nor necessary* to generate a greater cueing effect.

The evidence for a relatively smaller effect of WM-based attentional guidance for older participants is also interesting since it is not consistent with the argument for a specific 'frontal' component in cognitive ageing. Soto et al. (2006) found that frontal-lesioned patients showed *larger* cueing effects compared with controls and argued that

the frontal lesions led to problems in inhibiting (and disengaging attention from) cued items that had been selected. This occurred even with normalised measures of performance. Clearly our data for the older participants contrast with this. An alternative is that the older individuals devoted less capacity to the WM item because they had to devote more to the search target to maintain search performance. When less capacity is devoted to the WM stimulus, the effects on attentional guidance reduce (Olivers et al., 2011). A further possibility can be linked to memory consolidation.

Older adults may show *both* a greater cognitive load (as indexed by the WM-MR contrast on neutral trials), and less effect of attentional guidance, because they take longer than younger adults to consolidate the cue in WM. Because they are still consolidating the cue, there is a greater overall cognitive load on performance while the unconsolidated cue may generate less attentional guidance. Tsvetanov et al. (Tsvetanov et al., 2012a) have recently reported that the cognitive load under WM relative to MR conditions disappears if the interval between the cue and the search display is lengthened sufficiently. In Experiment 2 we tested this consolidation account by lengthening the interval between the cue and the search display. If older participants have sufficient time for memory consolidation, do they then show relatively increased effects of the WM cue on attention? Do they show reduced effect of the WM cue in the neutral condition (the general load effect)? By examining the effects of the cue-search interval, we can also assess whether memory consolidation is slowed for older participants (do they show a weaker effect of the time interval, compared with young participants?).

5.4. Experiment 2: Varying the cue-search interval

Experiment 2 replicated Experiment 1 but in this case we introduced a number of different inter-stimulus intervals (ISI) between the first cue and the search task. If the age-related effects in Experiment 1were driven mainly by the longer time required by older participants to consolidate information in WM, then the load effect should disappear at longer ISIs (> 3 seconds).

5.4.1. Methods

5.4.1.1. Participants

Twenty-four young (11 male, group mean age 21 years, age range 21 to 32 years of age) and twenty (11 male, group mean age of 72 years, age range 65 to 82 years of age) senior healthy volunteers participated in the study for exchange of course credit or cash (£6 per hour). The recruitment and the criteria for selection of participants followed those of Experiment 1.

5.4.1.2. Stimuli, Task, and Procedure

The stimuli, the task and the procedure replicated Experiment 1. The only difference here was that we varied the ISI (short, medium and long interval with a duration of 0.25, 1 and 3 seconds respectively) between the memory and search task - in contrast to Experiment 1 which used a single ISI (0.25 seconds).

5.4.2. Results and Discussion

5.4.2.1. Memory Accuracy

Memory test (WM condition) performance was maintained at high levels for both age groups (94% and 97% for young and older participants, respectively). The response accuracy on catch trials in the MR condition followed similar trends (86% and 95% for young and older participants, respectively). Please note that the catch trials (20% of all trials) in the MR condition were only introduced to ensure that participants performed the task correctly (merely observed the cue object, but did not remember) and therefore were excluded from any analysis.

5.4.2.2. Search Accuracy

The accuracy levels on search trials for WM condition were 90% and 94% and for the MR condition they were 96% and 97%, for young and older adults respectively. Both age groups performed at a higher level in the MR condition compared to the WM condition [t (23) = 7.27, $p < .001$ and t (19) = 4.82, $p < .001$]. Trials with incorrect responses or trials falling outside of the screening criteria (> 3 SD the mean RT in a condition) were excluded from further analysis (refer to Table 5.1).

5.4.2.3. Search Performance

The performance of search was assessed in a mixed design ANOVAs over the mean Z-transformations of the RT data (see Figure 5.2; refer to Table 5.2 for raw RTs).

The within-subject factors included the ISI (short, medium and long interval), task (WM vs MR) and validity (invalid, neutral and valid), while the between-subject factor was age group (young adults vs older adults). Significant main effects and their interactions, including their *F*- and *p*-values are shown in Table 5.2.

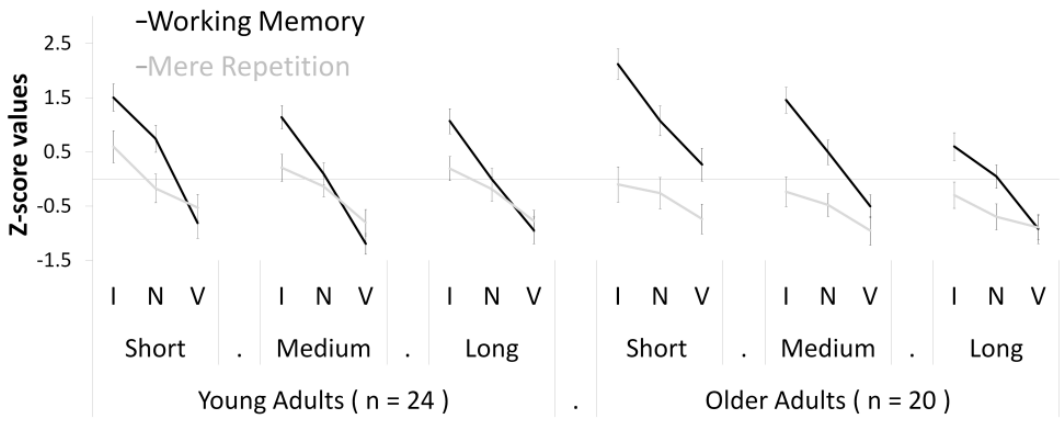

Figure 5.2. Mean Z-score values as a function of task (Working Memory (WM), black colour and Mere Repetition (MR), gray colour), inter-stimulus interval (ISI: short, medium and long with 0.25, 1 and 3 seconds respectively) and validity (Invalid, I; Neutral, N; and Valid, V) for young and older adults.

	Interaction	Experiment 1		Experiment 2	
		F - Value	*p* - value	*F* - Value	*p* - value
Main Effect	ISI			45.39	< 0.001
	Task	215.38	<0.001	86.36	< 0.001
	Validity	249.18	<0.001	260.44	< 0.001
General Interactions	ISI x Task			21.4	< 0.001
	Task x Validity	70.23	<0.001	72.42	< 0.001
Age-related interactions	ISI x Age			7.29	0.002
	Task x Age	4.42	0.042	20.54	< 0.001
	Validity x Age	3.90	0.024	5.62	0.010
	ISI x Task x Age			8.93	0.001
	ISI x Task x Validity x Age			3.89	0.005

Table 5.2. Significance levels (*F*- and *p*-values) for the main effects and interactions involving the factors inter-stimulus interval (ISI), task, validity and age in Experiment 1 and Experiment 2.

Experiment 2 replicated the main effects and the interactions in Experiment 1. In addition, the results were qualified by a four-way interaction.

The four-way interaction was decomposed to two separate ANOVAs, one for each task condition (WM and MR), with the within-subject factors of ISI and validity, and age group as a between-subject factor (in addition, to provide better comparison between both experiments we also analysed the data in Experiment 2 by decomposing the main ANOVA across ISIs[6]). For the MR data all interactions with age were non-

[6] We analysed the data by decomposing the main ANOVA into three separate ANOVAs, one for each ISI, with the within-subject factors of task and validity, and the between-subject factor of age group. The three-way interaction (task x validity x age) was reliable only for the short ISI [F (2, 84)= 4.44, p = .015; F (2, 84) = 0.075, p = .928; F (2, 84) = 1.97, p = .145 for short, medium and long ISIs, respectively]. Task and age interactions was reliable at short and medium ISI [F(1, 42) = 22.69, p < .001, F(1, 41) = 19.60, p < .001, respectively], but not at the long ISI [F(1, 42) = 2.14 , p = .136]. The effects at the short ISI were analysed further through two two-way ANOVAs (one for each task), with the factors of ISI and group

significant. In contrast, for the WM analysis age interacted with the following factors: ISI [F (1.57, 65.36) = 13.01, p < .001], validity [F (2, 84) = 6.24, p = .003] and ISI combined with validity [F (4, 168) = 2.69, p = .033].

To assess the changes in the validity effects across the ISIs within each task we calculated the effect of validity from the difference in performance between the invalid and valid conditions ($Z_{Invalid}$ - Z_{Valid}) within each task. A two-way ANOVA was conducted for the WM data with one within-subject factor (ISI) and the between-subject factor of age. This revealed that the validity effect changed as a function of ISI [F (1.81, 71.52) = 4.29, p = .022; Z-score differences: 2.08, 2.15 and 1.77 for the short, medium and long ISIs]. Post-hoc analysis revealed that the validity effect did not differ across the short and medium intervals [t (43) = 0.44, p = .661], but it declined at the longer interval [short vs long interval: t (43) = 1.95, p = .057; medium vs long interval: t (43) = 3.25 and p = .002]. In addition, the validity effect was overall smaller for older adults when compared to young adults [F (1, 42) = 6.51, p = .003]. However, the interaction between age and validity cost as a function of ISI was insignificant [ISI x Age: F (2, 84) = 0.09, p = .910] (Figure 5.3).

again. For the MR data, the interaction between validity and age was not significant [F (2, 84) = 2.59, p = .081]. For the WM data the interaction was significant [F (2, 84) = 5.58, p = 0.005). The effect of validity tended to be smaller for older participants when compared to young participants ($Z_{Invalid} - Z_{Valid}$, 2.315 and 1.576 for young and older participants, respectively) [F (1, 42) = 4.10, p = 0.049] .

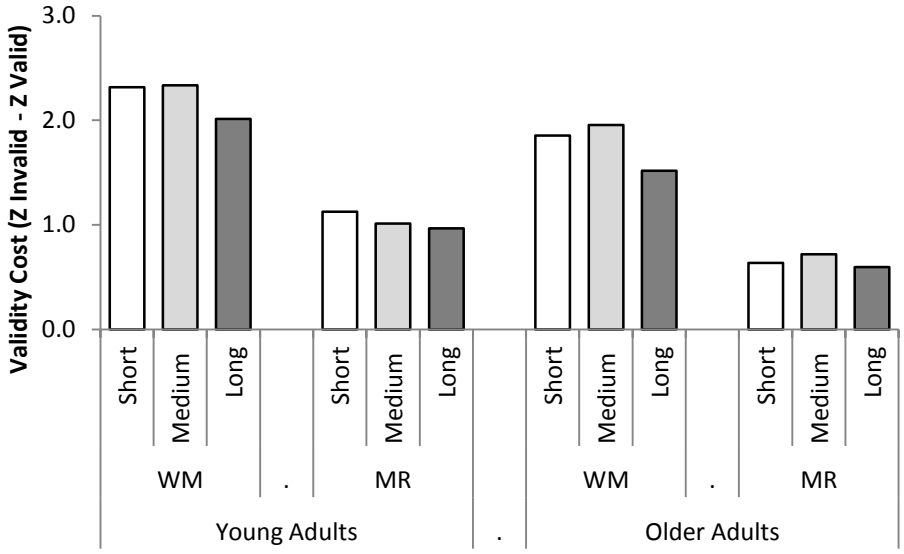

Figure 5.3. Validity Cost as a function of Inter-stimulus interval (short, medium and long for 0.25s, 1s and 3s respectively), task (WM and MR) and age group

In the analysis of the MR data, the two-way ANOVA showed that the main effects of ISI [F (2, 84) = 0.35, p = 0.703] and age [F (1, 42) = 3.31, p = .076] were not significant and their interaction was not reliable [F (2, 84) = 0.29, p = .747].

5.4.2.4. Memory cost as a function of ISI

Along with the four-way interaction (ISI x Task x Validity x Age), we also observed a three-way interaction between ISI x Task and Age, suggesting that the effect of task (memory) load changed as a function of ISI differentially for each age group. To investigate this we calculated the memory cost by contrasting the performance in the WM and MR conditions in neural trials (uncontaminated by the effects of cue validity, $Z_{WM\ Neutral} - Z_{MR\ Neutral}$). A repeated-measure ANOVA was conducted with the within-subject

factor of ISI and the between-subject factor of age. There was a reliable main effect of ISI, as the memory cost declined as the time between cue and search display lengthened [F (2, 84) = 9.02, p < .001]. Overall the performance of older participants was relatively worse in the WM condition compared with the MR condition, when compared to the young adults [main effect of age: F (1, 42) = 12.43, p = .001], see Figure 5.4. The interaction between the ISI and age was not reliable [F (2, 84) = 0.51, p = .602]. Interestingly, for the longest ISI the difference in performance between the WM and MR conditions was negligible for young adults ($WM_{neutral}$ – $MR_{neutral}$: 10ms), whereas for older adults it was still relatively large (37ms) and reliable [t (23) = 1.07, p = .298 and t (19) = 5.90, p < .001 for young and older participants, respectively].

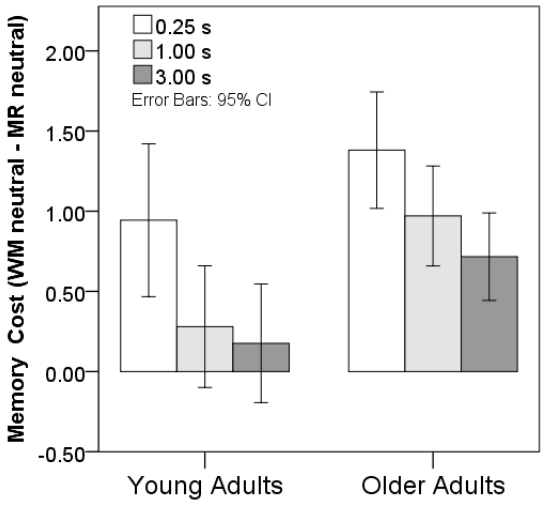

Figure 5.4. Memory cost, calculated from the difference of neutral trials between WM and MR conditions, as a function of inter-stimulus interval (ISI: short, medium and long for 0.25, 1s and 3 seconds, respectively) for young and older participants

As in Experiment 1 we found that there was a greater effect of cognitive load on older relative to younger participants, when we compared the WM and MR conditions on neutral cue trials. Also the older participants showed a relatively small effect of WM cueing. Thus, as before, there was a clear dissociation between general load and WM-based attentional guidance. Both the effects of load and of WM cueing decreased as the ISI between the cue and the search display lengthened. The effects of ISI were the same for both older and younger participants though, for younger participants, the effect of task load disappeared at the long ISI (see also Tsvetanov et al., 2012) while it remained reliable for the older subjects. The absence of load effects for young participants at the long ISI, and the remaining presence of WM-based attentional guidance, also highlights that these two effects can be functionally separated (see also Tsvetanov et al., 2012).

These data are consistent with both the general effects of task load and the effects of memory-based attentional guidance being affected by the consolidation of information in WM: both effects reduce when items are better consolidated. The effects of task load *only* reflect this consolidation process; once information in WM is consolidated, then the main effects of task load are eliminated. However, there remains an effect of WM-based guidance even after consolidation is completed (see the data for the young participants), provided the WM item is held at the forefront of WM. We suggest that young participants are able to do this whilst maintaining the representation of the search target when the target remains the same across trials. In contrast, we propose that older participants must devote more resources to the search target, and this weakens the effect of the WM cue on

attention. Interestingly, there was no evidence here for the rate of consolidation varying with age, as the effects of ISI were additive with age. The rate of consolidation remained the same, but the overall consolidation time was lengthened for older participants.

5.5. General Discussion

In the present two experiments we examined the effects of WM-based guidance on attentional selection in young and older participants. Overall, the findings in both experiments supported previous studies in showing that irrelevant information in WM modulated subsequent attentional selection, and this was not due to the mere presentation of the WM cue (Soto et al., 2007; Soto et al., 2008; Olivers et al., 2006; Olivers, 2009). In addition the WM condition also showed an overall effect of task load (when the WM cue was neutral).

The results provide conclusive evidence that the effects of load and those of WM-based guidance of attention are distinct. For example, in Experiment 2 we found that the effects of load were eliminated at the longest cue-search ISI for young participants, but there remained a reliable effect of the cue on attention. A load effect is not necessary to generate WM-based attentional guidance (see also Tsvetanov et al., 2012). Even stronger than this, we demonstrated a double dissociation between the two factors when we tested older participants. In both experiments here the older participants showed a stronger effect of task load and they were relatively slower than young participants in the WM compared with the MR condition with neutral cues. In contrast, the effects of cue validity

were relatively weaker for older individuals. That is, an effect of load is not sufficient for WM-based guidance. The results strongly indicate that WM-based guidance is not caused by task load weakening the control over attention by the target (cf. Lavie, 1995).

The finding that, for young participants, the load effect (in the WM condition relative to the MR condition, with neutral cues) disappeared as the interval between the memory and search items increased fits with the idea that young participants can successfully consolidate the memory cue into a stable form of WM representation over the interval of 3s (Tsvetanov et al., 2012a). In the older participants, the load effect reduced at the same rate over time period, though it remained significant at 3s. We conclude from this that the rate of consolidation does not vary with age, but there is an overall step increase in consolidation time. Interestingly, despite the greater effect of the memory item on overall RTs, it exerted less influence on the allocation of attention in the older participants. This result contrasts with the data previously reported on the effects of frontal lobe damage on WM-based attentional guidance, where patients with frontal lobe lesions show enhanced not decreased guidance effects (Soto et al., 2006). This argues against an account of cognitive ageing as reflecting a deterioration in frontal lobe function generally, and the inhibitory control of attention in particular (Zacks & Hasher, 1998). Were this the case we would expect effects of WM to be stronger rather than weaker in older participants. The opposite held. The results also cannot be attributed to a general slowing of information processing in the older individuals (Salthouse, 1981) since here we demonstrated effects using a measure of the relative effect of the WM-

based cue. Rather than these accounts, the data do fit with the idea that older individuals need to commit more resources to maintaining the representation of the search target in WM (Grady et al., 2005; Davis et al., 2008). We suggest that older adults committed WM resources to hold the search target at the forefront of WM, while "pushing" the memory item to the "background", reflecting a reduced ability to automate the task template (Park & Reuter-Lorenz, 2009). In contrast, young participants could efficiently automate the task template and store the search target in the 'background' of WM (cf. Cowan, 2001, 2005; Oberauer, 2002), while leaving sufficient capacity to hold the WM cue in the foreground. This foreground cue then disrupts search when it re-appears in the search display (Olivers et al., 2011). Ironically, the data indicate that having less capacity can be protective of search from extraneous interference.

5.6. Conclusion

We conclude that the effects of attentional guidance from irrelevant items in WM are not due to general task load. Furthermore, ageing makes performance more susceptible to the effects of task load by requiring overall more resources to consolidate stimuli in WM. However, older people compensate for the reduction in processing resources by increasing their weighting of a primary task (search), which in turn decreases the effectiveness of the WM cue on attention.

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CHAPTER 6.
Reduced memory guidance of selection in aging: Increased responses in anterior insula and reduced frontal-occipital coupling

6.1. Abstract

We report data on the effects of neurocognitive ageing on attentional guidance under conditions of bottom-up stimulus priming and top-down activation of working memory (WM). In a dual-event fMRI paradigm, young (mean age 24 years) and older adults (mean age 76 years) were asked to remember an item (cue event) and hold the item in WM while searching for a different stimulus (search event). Relative to the young participants, older individuals overrecruited the right anterior insula (AI) / inferior frontal gyrus (IFG) during the encoding of the cue item compared with a condition where they had only to identify the same item. Interestingly, the recruitment of AI/IFG during the cue event was associated with weaker behavioural biasing of attention from WM. Furthermore, functional connectivity analysis demonstrated increased coupling between AI/IFG and occipital cortices in young adults relative to older individuals. We discuss the results in terms of older adults, relative to young participants, having to recruit AI/IFG to

maintain the primary task as a compensatory effect, which helps to protect visual attention from irrelevant information held in the WM.

6.2. Introduction

The biased competition model of attention posits that the information held in working memory (WM) can bias stimulus selection (Desimone & Duncan, 1995). A considerable amount of research, from a number of laboratories, supports this hypothesis. For example, in visual search some stimuli 'pop out' for attention only when participants carry an expectation for specific target properties (Hodsoll & Humphreys, 2001). Also, items held in WM can affect a subsequent search task if they are presented in the search display, even if they are irrelevant to the search task (Downing, 2000; Soto et al., 2005; Olivers et al., 2006; Tsvetanov et al., 2012a).

There are constraints on these effects however. Notably, the effects of irrelevant WM stimuli on attention are diluted under conditions of cognitive load - when several items have to be maintained (Soto et al., 2012a) and when participants undertake articulatory suppression to prevent maintenance through rehearsal (Olivers, 2009; Soto & Humphreys, 2008). This may be because any given stimulus is less likely to be 'at the forefront' of WM under load conditions (Cowan, 2001; Cowan, 2005; Oberauer, 2002). In addition, attention is less likely to be captured by a task irrelevant WM stimulus if the search target is varied across trials, so that the search target rather than the memory item is highlighted in WM (Olivers, 2009).

WM-based guidance of attention changes with age, consistent with changes in the cognitive load of tasks as we age (Park & Reuter-Lorenz, 2009; Tsvetanov et al., 2012b). Tsvetanov and colleagues (2012b) tested WM-based attentional guidance in young and older adults. Participants carried out a search task for an oriented line target, which was preceded by the visual presentation of a coloured shape. Trials where the initial item had to be held in WM (the WM condition) were contrasted with those when the initial item was identified but not maintained (the mere repetition condition; MR). The initial item could re-appear in the search display surrounding either the target or a distractor line. Previous work indicates that, in addition to affecting where search is directed, the memory item also exerts a general cost on performance relative to the MR baseline, found even when the memory item is not re-presented in the search display (Soto et al., 2005). This non-specific cognitive load effect diminishes as the time between the presentation of the WM cue and the search display is extended (Tsvetanov et al., 2012). Relative to young participants, older participants showed a larger non-specific effect cognitive load effect and the effect persisted even with an extended delay between the WM cue and the search display. This is consistent with the idea that older participants are more strongly affected by WM load (Cappell et al., 2010). On the other hand, young adults showed stronger effects of the WM cue on selection, compared to elderly participants. Tsvetanov et al. propose, due to their having reduced processing resources, older participants had less efficient coding of the representation of the search target (Vallesi et al., 2011; Park & Reuter-Lorenz, 2009). As a consequence there was increase

competition between the search target and the WM item for representation at the forefront of WM (Olivers, 2009), lessening the impact of the task irrelevant WM item on subsequent search. In the current study we ask what changes in the neural functions in the elderly are associated with the general effects of task load and the age-based modulation of memory-based guidance of attention.

Soto and colleagues (2005; 2007, 2008, 2012a,b) have investigated the neural correlates of the interaction between WM and selection in young adults. Their neuroimaging data suggest that the effects are associated with two neuro-cognitive effects (Soto et al., 2007; Soto et al., 2008; Soto et al., 2012a; Soto et al., 2012b). The first is a re-appearance effect. The re-appearance of the WM item in the search display is associated with an increased response in several brain structures compared when it does not re-appear. This effect is reported in the superior frontal gyri, supra-marginal gyrus, medial temporal structures (Soto et al., 2007, 2012a) and the precuneus (Soto et al., 2012a,b). In addition, studies using single cell recording indicated increased neural responses to the re-appearance of an item held in WM in similar brain regions (Desimone, 1996; Chelazzi et al., 1993). The second effect reflects the relation between the WM item and the search target – a cue validity effect. Here, increased activity has been noted on valid compared to invalid trials within a fronto-thalamic-occipital network including regions in the: prefrontal cortex (Soto et al., 2007), inferior frontal gyrus and anterior insula (Soto et al., 2012b), superior temporal gyrus, thalamus (Soto et al., 2007, Soto et al., 2011; Rotshtein et al., 2011) and lateral occipital cortex (Grecucci et al.,

2009). It is worth noting, that while this validity effect has been replicated across many studies, it is an a-typical neural response - most studies investigating the neural correlates of selective attention have reported the opposite pattern (i.e., larger responses on invalid compared to valid trials) (Botvinick et al., 1999; Floden et al., 2011; Kelley & Lavie, 2011). Furthermore on the invalid trials in the WM-selection paradigm, the search array contains conflicting information with respects to the tasks goals: the to-be-remembered item has to be ignored in the search display in order for participants to execute the search task efficiently. Typically, stimuli that contain conflicting information elicit stronger activation in the ACC and anterior insula (Floden et al., 2011; Crottaz-Herbette & Menon, 2006; Brass et al., 2005; Roberts & Hall, 2008).

As we have noted, the interaction between WM and selective attention diminishes under conditions of cognitive load (Olivers, 2006; Downing 2010; Cowan, 2001; 2005; Oberauer, 2002; Soto et al., 2012, Soto and Humphreys 2007). In young adults tested under WM-search conditions there is evidence for weaker modulation of posterior visual cortex by cue validity under high WM load conditions while regions in the prefrontal cortex show a stronger response when the WM load is high. In addition, the effects of WM cue validity are stronger under low-load conditions in the left inferior frontal gyri (IFG), anterior insula (AI) and thalamus (Soto et al., 2012b). There is increased positive coupling between the AI/IFG and the visual cortex during low compared with high WM load conditions (Soto et al., 2012b). These data suggest that WM-guidance of selection depends on the interaction between the AI/IFG and the visual cortex, with information

from the AI/IFG projected back to biasing sensory processes (Soto et al., 2012b). When the AI/IFG is pre-occupied with 'managing and maintaining' multiple items in memory (high load > low load), processing in these regions are 'not free' to affect selection.

Interestingly, increased activity within the AI has previously been associated with inefficient memory encoding in older compared with young adults (Cabeza et al., 1997; Salami et al., 2012). This has implications for how ageing might modulate WM-based attentional guidance, when memory and search tasks are combined. In particular, we hypothesised that activity in the AI may be increased in older adults relative to young participants in order to compensate for reduced WM capacity during encoding of the WM stimulus and the search target. As a consequence the coupling of AI/IFG with occipital cortex may be reduced, diminishing the memory-based guidance of selection. It has been further suggested that responses in the thalamic-occipital network are associated with maintaining the WM item in the presence of conflicting information (Rotshtein et al., 2012). Thus we expect that the reduction of WM resources in older participants may lead to the greater recruitment of this network.

We tested these hypotheses using the combined memory and search paradigm (Soto et al., 2007; Tsvetanov et al., 2012). As described above, this paradigm includes a WM task within which a search task is embedded. A control condition (MR) uses identical visual displays, but does not require participants to hold items in WM during the search task. The effects of WM on search are measured by manipulating the relations between the WM item in the search display and the search target (see Figure 6.1a and

methods for details). Importantly, as opposed to previous imaging and behavioural studies using this paradigm, here we increased the interval between the memory item and the search display, to an average of 3 seconds (2-4 seconds)(previously intervals of less than 1 sec have been used; Soto et al., 2005, 2007, 2012a,b). This change was implemented for two reasons: i) to facilitate the separation of brain activity linked to encoding the stimuli in WM (the cue event) from activity linked to visual search (the search event), as it is hypothesised that the encoding phase will be more taxing with older age; and ii) the relatively long interval was shown in young adults to minimize the effects of a non-specific cognitive load, when comparing the WM with the MR conditions (see Tsvetanov et al., 2012). The increased interval will enable greater time for encoding, so reducing non-specific load effects.

We hypothesized that the involvement of prefrontal regions during the working memory task will be affected by age, an effect that will be linked to behavioural responses. This effect will manifest itself as increase responses in the AI/IFG during the WM condition. We further predicted that, in older relative to young adults, the coupling between the prefrontal regions and sensory cortices will be less affected by cue validity, since increased AI/IFG activity reflects greater competition for resources between the WM items and the search target which lessens any highlighting of the WM stimulus in WM.

6.3. Methods

6.3.1. Participants

Data were collected from fifteen young (mean age 26 years, age range 21 to 32 years, 6 males) and thirteen older participants (mean age 75 years, age range 68 to 82 years, 7 males) for exchange of course credit or cash. All participants were recruited by advertisements in local communities, word-of-mouth and advertisements on an online experiment management system (Research Participation Scheme, University of Birmingham). All the subjects had normal or corrected-to-normal vision and were healthy with no history of psychiatric or neurological disease. The experiment was approved by the local ethical committee. One male participant in the older age group performed at chance level in the catch trials (see Methods) suggesting he did not fully follow the instructions. This participant was excluded from further analysis.

6.3.2. Stimuli, Task, and Procedure

A full factorial design was used with age group as between subject factor and memory type (WM, MR) and trial type (valid, neutral, invalid) as the within-subject factors. Memory type was manipulated across blocks while search-by-memory interaction was manipulated across trials. In the working memory condition, subjects were asked to hold in memory the colour and the shape of the cue. In the MR condition, participants had to compare the presentation of the first cue and second cue (see below and Figure 6.1). If the cues differed, participants were required to withhold their

responses in the search trial. Thus, in this case, the cue had to be identified but it did not have to be held in memory during the search event. Given that the visual display was identical across both memory types blocks, to ensure that participants know which task they have to perform, each trial began with a fixation letter that provided the instruction for each trial ("C" for compare the stimulus, in the block of MR condition, or "R" for remember, in the block of WM condition). This letter was presented for 300 ms and followed by two cue displays (100 ms and 500ms respectively) with a 100 ms blank interval in between. The cue shape appeared in the centre of the screen and could be one of the following shapes: circle, square, triangle, diamond, or hexagon, and colours: red, green, blue, yellow, or pink. The shape appeared on a gray background. In the interval between the second cue display and the search array a blank screen was exposed for a variable duration (2-4 seconds, averaged 3 seconds). As mentioned above, this was done to facilitate the separation of the cue from the search events and to allow sufficient time to encode the WM item or to perform the MR task. The search display contained four unique objects, one for each quadrant, where the distance between the centres of the objects was 18 degrees. Each object contained one of the lines from the search task (3 vertical, 1 tilted 26 degrees left or right, all black with height of 0.88 degrees and width of 0.12 degrees). The target was a black line tilted to either the left or right and was displayed in the centre of one of the objects. The task was to discriminate the orientation of the target in a time window of 1000 ms by pressing either the left or right mouse button. Inter-trial intervals (ITI) were jittered from two to four seconds.

In the search displays the initial cue could be re-presented surrounding the search target (on valid trials), surrounding a search distractor (on invalid trials) or not presented in the display (neutral trials). Search trials were randomized and counterbalanced across quadrants, repetitions and validity conditions.

Catch trials were introduced on 20 % of all trials. On these trials in the WM condition, a memory test (another single coloured shape) followed 500 ms after the offset of the search display. Participants were required to indicate whether the new object matched the colour and the shape of the cued object. In the MR condition, the catch trials contained two cue objects with different shapes and colours and participants had to withhold their response in the search task. Catch trials were excluded from the analysis.

Each block started with an 8-second task-prompt on the screen in which the instructions were presented for the following block (WM:"Remember Colour and Shape", MR:"Compare Colour and Shape"). Each block started with two catch trial events, used to ensure that task instructions were adhered to and to minimize task-switching effects (from WM to MR trials and vice versa). A cross in the middle of the screen was used for fixation. In total there was 8 minutes and 25 seconds of two randomly selected blocks (WM and MR conditions) with 30 trials each (2 repetitions x 4 quadrants x 3 validity conditions + 6 catch trials). We repeated this experimental session 6 times, counterbalancing the order of the memory blocks and randomising the order of the trial type within each block. In total there were 48 trials per condition.

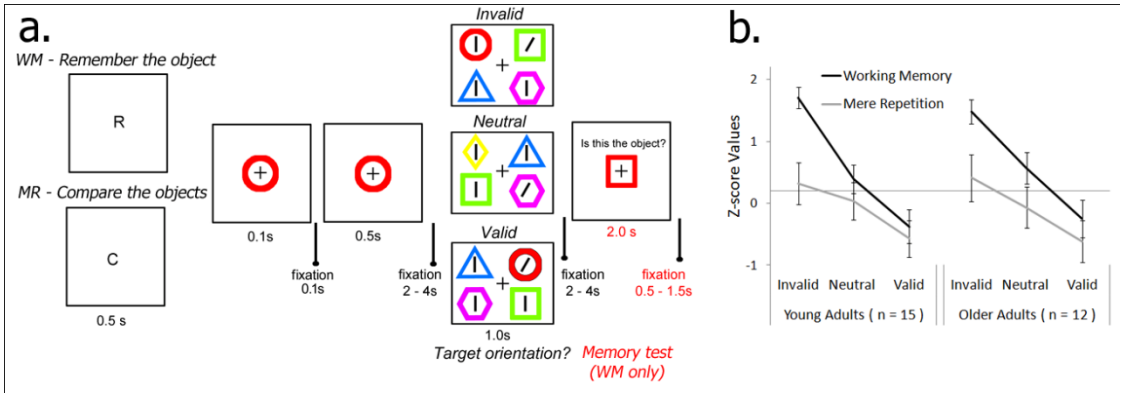

Figure 6.1. (a) *Paradigm:* Example of the trial sequence in the working memory (WM) and mere repetition (MR) conditions. (b) *Behavioural performance:* Z-score values as a function of task (Working Memory, black colour and Mere Repetition, gray colour), validity (invalid, neutral and valid) and age group. Bars denote the 95% confidence interval for each condition

6.3.3. MRI acquisition

Data were acquired using a 3T Phillips Achieva scanner using a phased-array coil, hosted in Birmingham University Imaging Centre. A 3D-structural MRI was acquired on each subject using T1-weighted sequence (Inversion Recovery – Turbo Spin Echo (IR-TSE); Echo Time (TE) = 3.8ms; Repetition Time (TR) = 8.6ms; 175 coronal slices; flip angle α=8°; turbo factor 119; matrix size 256 x 256; field of view (FOV) 232 x 256; slice thickness 1mm; in-plane resolution 1 x 1 mm2) with acquisition time of 284 seconds.

For all fMRI measurements, gradient-echo EPI data were acquired from 32 slices, 3mm thickness with no gap (whole brain coverage excluding the temporal lobe and cerebellum; TR = 2000 msec; TE = 35 msec; flip angle α=80°; voxel size 3 × 3 × 3 mm^3). The eight-channel phase array coil was used with a sense factor of 2.

The preprocessing and the analysis of the data were carried out in SPM8. The preprocessing steps included i) spatial realignment and unwarping to correct for head movement and movement by distortion interactions, ii) temporal realignment of all slices to the middle slice, iii) co-registration the EPI to the high-res T1 anatomical scan, iv) using the unified-segmentation algorithm to normalize the T1 image to the MNI template that was later applied to the functional data and vi) an application of a filter with an FWHM of 8 Hz to spatially smooth the data to accommodate the continuity assumption of the random field theory and to account for residual inter-subject structural variability.

6.3.4. fMRI analysis

For each participant we estimated the effect size for each condition averaged across sessions, using the general linear model framework. Each model included regressors for the onset of the two cue types (WM, MR) and the onset of each validity condition per memory type (i.e. valid, neutral, invalid). Regressors of no interest included the catch trials, error trials, instruction trials, head motion and harmonic regressors that capture low frequency changes (1/128 Hz) in the signal typically associated with scanner and physiological noise. The events in the trials were modelled using Finite Impulse Response (FIR) and hence no assumptions about the shape of the haemodynamic response (HRF) were made. The choice of FIR analysis was based on this method being robust when accounting for potential differences in the HRF response in particular tasks and brain areas in young and older adults (D'Esposito et al., 2003). The modelling of the FIR set consisted of eight 2-second bins from the onset of each event with a total window

length of 16 seconds. The 2nd and 3rd bins depicted brain response corresponding to 4-6 seconds post-event onset (Padmala & Pessoa, 2011), an interval characteristic of the maximum activation of the BOLD response after the onset of a stimulus. An averaged contrast between 2nd and 3rd bins of each event (cue and search events) was used to generate two separate whole brain analyses, one for each event (i.e. memory cue and search). Importantly, the cue and search events across all conditions were only weakly correlated, $r < .14$, ensuring that we can reliably estimate each event despite their sequential nature (i.e. memory always preceding the search task). In each of these second level models participants were treated as a random variable. In the cue event, a two-way ANOVA, with the within-subject factor of task (WM vs MR condition) and the between-subject factor of age group, was used to test for between task effects as a function of age. In the search event, we calculated a three-way ANOVA with the within-subject factors of task (WM vs MR conditions) and validity (invalid, neutral, valid), and the between-subject factor of age.

Based on the previous literature and our hypotheses, we were interested in the following contrasts:

i) the effects of task: WM vs. MR; this was computed for the cue and the search event separately;

ii) the effect of cue re-appearance effect under WM relative to MR conditions, computed on the search event: WM (valid + invalid)/2 > neutral & MR (valid + invalid)/2 < neutral; and

iii) the validity effect during WM computed on the search event: valid vs. invalid.

The analysis focused on identifying common and dissociated effects across the two age groups. Common effects were evaluated using conjunction analysis to test against a global null hypothesis (Friston et al., 1999; Friston et al., 2005), assessing whether effects were reliable across both age groups. Dissociation effects were tested using combinations of simple effects and inclusive masking by the interaction contrast. For example: to test for regions that show a larger validity effect in young compared with old participants, we tested for reliable validity effects in young participants, inclusively masking this contrast with an interaction of validity-by-age. This ensured that the regions observed showed a reliable dissociation between the groups that was driven by one group. We note that the main focus of the current study was to identify common and dissociated effects across age groups, hence the stringent statistical tests specified above. However part of the network that responded to the above conditions had a response pattern that did not reliably dissociated between the age groups, nor was it reliably activated by both groups. To provide a complete description of the data we mention in the text regions that also showed reliable effects when responses of the two groups were averaged (i.e. main effects). For post-hoc additional analyses, the data were extracted from a 10mm-radius sphere around the peak of clusters of interest. These data were used to further compute post-hoc tests using SPSS (IBM SPSS Statistics 20, IBM SPSS, Tokyo, Japan), and also for computing behaviour-brain correlations focusing on

correlations between prefrontal responses and the validity effects, and for computing correlations between brain regions (notably the prefrontal and sensory cortices).

We focused on results that were FWE-corrected ($p < 0.05$) at the cluster level with voxel reliability of $Z > 2.33$, and on findings that were driven by our a-priori hypotheses (see introduction). For completeness we report in the tables all clusters that have at least 200 voxels and reliability of $Z > 2.33$.

6.3.5. Psycho-physiological interaction (PPI) analyses

Additional tests in support of the full brain analysis included "effective connectivity" analysis using psychophysiological interaction (PPI) approach (Friston et al., 1997; Gitelman et al., 2003). Here we defined the right AI/IFG (33 8 4) as a seed (with 3mm sphere) within each participant, to test specifically whether the right AI/IFG was functionally connected to visual cortices in young, but not older adults. For this purpose we extracted the time course of the seed regions, which was later convolved with a model representing the validity effect (invalid - valid). Next, we estimated a new first level-analysis with regressors used in the initial first level analysis (please see above) including two regressors, the raw BOLD time series of the seed regions and the PPI factor. At the group level, t-statistics were applied to identify regions of effective connectivity with the seed regions using an uncorrected voxel-level threshold of $p < 0.05$ for clusters larger than 40 voxels. We used inclusive masking to restrict the analysis to regions showing larger activity during invalid trials relative to valid trials in the WM condition.

6.4. Results

6.4.1. Behavioural Data

6.4.1.1. Accuracy to the 1st Event – analysis of catch trials (WM and MR)

The accuracy on catch trials was high. In the WM condition, the accuracy of memory, measured by the surprise memory test, was 91% and 89% for young and older participants respectively (F (1, 25) = .53, p = .476). In the MR conditions, the accuracy of performance, based on 'not responding' to the search display, was 93% and 95% for young and older participants (F (1, 25) = .37, p = .548).

6.4.1.2. Accuracy to the 2nd Event (Search)

Search accuracy was high and similar for the WM and MR conditions (97% and 97%), t (26) = .106, p = .917. This is consistent with the view that the general effect of task load on accuracy, reflected in the contrast between the WM and MR conditions, is reduced when there is sufficient time between the cue and the search task (on average 3 seconds here; see Tsventanov et al., 2012). Older participants maintained high accuracy (95%), but were overall less accurate in search compared to young participants (98 %), F (1, 23) = 6.1, p= .020. Trials with incorrect responses were removed from further analysis.

6.4.1.3. Latency in the 2nd Event (Search):

We assessed both mean absolute response times and Z-score values. The use of Z scores enables us to account for general slowing on performance due to aging. We transformed the RT data to Z-score values by normalizing each condition cell to the overall performance across all conditions for each subject (Faust et al., 1999). The effects found on Z transformed data were replicated in the analysis of the untransformed RT data. For clarity, we only report the Z-transformation results.

A three-way repeated measures ANOVA was applied with the within-subject factors of task (WM and MR) and validity (valid, invalid, neutral) and the between-subject factor of age group (young vs older participants), using Z scores of RTs as the dependent variable (Figure 6.1 b). In the cases where sphericity was violated the Greenhouse-Geisser correction was applied. We observed an effect of task [F (1, 25) = 34.66, $p < .001$], where WM trials were overall slower than MR trials. There was a reliable effect of trial validity [$F(1, 25) = 121.52$, $p < .001$], with valid trials faster and invalid trials slower than neutral trials. These two main effects were qualified by a reliable interaction between task and validity [F (1, 25) = 10.50, $p < .001$]. The interaction was driven by a significantly stronger validity effect in the WM condition when compared to the MR condition [$t(26) = 4.57$, $p < .001$]. Furthermore, the WM load effect appeared to be driven primarily by the invalid condition (Figure 6.1b) and was not reliable for the neutral and valid trials. There were no significant interactions with age. Based on our previous findings (Tsvetanov et al., 2012b), we tested the effect of age on

the validity effect (invalid – valid trials) in the WM condition. In accordance with our previous study, we observed that the WM-validity effect was reduced in the older participants ($Z_{WM\ Invalfid} - Z_{WM\ Valid} = .91$) compared with the young participants ($Z_{WM\ Invalid} - Z_{WM\ Valid} = 1.32$), $t\ (25) = 1.80$, $p < 0.05$, one tailed.

6.4.2. EPI data

6.4.2.1. BOLD response to Event 1 - Cue Event

Task and age effects: conjunction analysis across age groups showed that midline structures (the cingulate gyrus (CG) and early visual cortex (EVC)) responded more strongly in the relative easier task (MR) compared with the more difficult task (WM). This pattern was also observed in the left motor cortex and left thalamus (Table 6.1). The same brain areas also showed overall a larger modulation of neural activity in the older participants when compared to young participants (Table 6.1). There were no above threshold regions that showed the opposite pattern across both groups when tested using conjunction analysis.

Compared to young participants, older participants are typically reported to show higher activation and the recruitment of additional regions when performing a cognitive demanding task (see the Introduction). We assessed if there were regions that were activated more in the older participants for WM relative to MR conditions, which also did not show a reliable effect in the young (inclusively mask by the interaction). We

observed that in the elderly, the right anterior insula (AI) and the inferior frontal gyrus (IFG) showed stronger responses in the WM condition compared with the MR condition, while the young adults showed an opposite pattern (Figure 6.2).

Interestingly, the extent to which the right AI/IFG was selectively recruited when encoding the items into WM during the cue event (compared to the MR condition) modulated the interaction between the WM-cue and selection in the search task, after controlling for age group (partial correlation, r = - .395, p = .042)). In other words, the change in the activation of the AI/IFG predicted the behavioural validity effect (invalid – valid trials): participants who showed a stronger AI response during WM (vs. MR) also showed a weaker validity effect (Figure 6.2). This is in line with our hypothesis that increased recruitment of the AI/IFG during the WM conditions 'blocks' the role of the region in guiding selection.

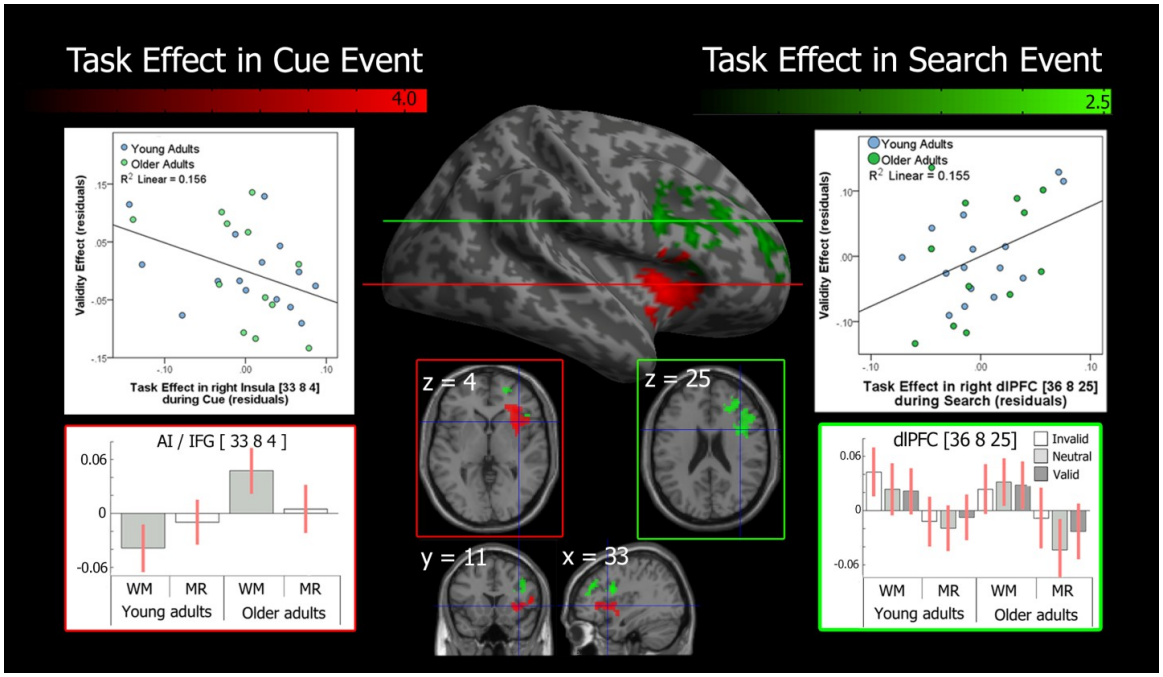

Figure 6.2. Task effects during the Cue Event (red colour) and the Search Event (green colour). ***Red Colour*** **- IFG and AI over-activation in older subjects and under-activation in young subjects in WM condition relative to the MR condition, during the cue event. Scatter plot - Partial correlation between the size of the task effect (the response difference in the WM and MR conditions) in the right AI (33 8 4) during the cue event and the strength of the behavioural validity effect after controlling for age groups (young adults, blue and older adults, green).** ***Green Colour*** **- The interaction between task and age group in the search event. The right dorsal-lateral prefrontal cortex (dlPFC) and the right anterior middle superior frontal gyrus (mSFG) showed increased responses in the WM condition and decreased responses in the MR condition for both age groups. Scatter plot: Partial correlation between (i) the size of the task effect (the response difference between the WM and MR conditions) in the right dlPFC (36 8 25) during the search event and (ii) the strength of the behavioural validity effect after controlling for age groups (young adults, blue and older adults, green).**

Contrast	Anatomical Label	Hemisphere	Cluster size	Z-score	MNI, mm x	y	Z
WM > MR							
	CS	l	875	4.67	-33	-19	43
				4.63	-39	-13	55
				3.76	6	2	58
	EVC	r	1697	4.36	27	-61	-11
				4.20	-24	-73	-5
				4.14	21	-70	-8
	Tha	l	277	3.82	-36	-34	16
				3.82	-9	-19	7
				3.52	-18	-19	10
Young: WM < MR; Older: WM > MR							
	AI / IFG	r	266	3.88	30	32	4
				3.61	33	14	-2
				3.61	33	8	4
Young Adults < Older Adults							
	SFG	l / r	1159	4.21	3	8	43
				4.14	9	-37	46
				4.11	-15	-22	37
	MC	l	647	4.12	-42	-19	37
				3.58	-33	-31	-8
				3.36	-51	-34	22
	EVC	l / r	775	3.83	6	-85	1
				3.76	12	-64	-2
				3.58	-3	-91	10
	CS	l / r	456	3.61	33	8	61
				3.50	42	-25	34
				3.48	27	5	43

Table 6.1. Brain regions for various contrasts during cue event. *CS*, central sulcus; *EVC*, early visual cortex; *Tha*, thalamus; *AI*, anterior insula; *IFG*, inferior frontal gyrus; *ACC*, anterior cingulated cortex, SFG, superior frontal gyrus.

6.4.2.2. Bold response in 2nd Event - Search

6.4.2.2.1. Task effects

Conjunction analysis across age groups for the task effect (WM > MR, independent of cue validity) revealed that the right dorsal-lateral prefrontal cortex (dlPFC) and the right anterior middle superior frontal gyrus (mSFG, BA 10) were more active when search was carried out under WM relative to MR conditions (Figure 6.2), for both age groups. We note that the homologous regions (i.e. left PFC) showed a similar response pattern when computed across groups - though this effect was not reliable when tested on each group on its own, nor was it reliably different between the groups. There were no regions showing the opposite pattern (MR > WM) or a reliable dissociation between the age groups for this contrast.

In addition, activity in the right dlPFC during the search event correlated positively with the size of the behavioural validity cost after controlling for age. However, the trend was in the opposite direction to that observed in the AI/IFG during the cue event- the larger the involvement of the dlPFC under WM relative to MR conditions, the larger the validity effect, r = .394, p = .046 (Figure 6.2). A further correlation analysis showed that the task responses in the right AI/IFG during the cue event correlated negatively with responses to the task during the search event in the right dlPFC, r = -.508, p = .008 . These results suggest that, in the context of the current task, these two regions inhibit each other.

					MNI, mm		
Contrast	Anatomical Label	Hemisphere	Cluster size	Z-score	x	y	z
Task Effect: WM > MR							
	IFG	r	523	3.96	36	8	25
	amPFC	r		3.93	30	47	16
	dlPFC	r		3.73	36	35	31
Reappearance Effect: Young:WM I, V > N; MR I,V < N							
	PPC	r	404	4.48	30	-55	40
				4.06	12	-61	43
				2.89	24	-61	31
Validity Effect: WM I > V, conjunction							
	EVC	l	2812	5.56	-21	-61	4
				5.36	-12	-67	4
	ACC			5.40	6	11	64
Validity Effect: WM I > V, Young Adults > Older Adults							
	EVC: CalS/LG	l / r	466	4.55	-9	-67	1
				4.16	9	-64	4
				3.29	21	-55	-2
	EVC: POS/PreC	l / r	226	3.56	6	-82	19
				3.06	-3	-79	25
				2.97	6	-79	37
	ACC	l, r	2271	4.43	0	14	46
				4.22	6	23	52
				4.22	3	20	40
	AI	l	211	3.87	-45	17	-2
				3.14	-45	5	10
				2.86	-30	23	-2

Table 6.2. Brain regions for various contrasts during the search event. *WM*, Working Memory; *MR* – Mere Repetition; *I*, Invalid; *V,* Valid; *N*, Neutral; *IFG*, Inferior frontal gyrus; *amPFC*, anterior medial prefrontal cortex; *dlPFC,* dorsolateral prefrontal cortex; *PPC*, posterior parietal cortex; CalS, Calcarine sulcus; *LG,* Lingual gyrus, cortex; *POS,* parietal occipital sulcus; PreC, precuneus; *ACC,* anterior cingulate cortex; *AI,* anterior insula

6.4.2.2.2. Reappearance Effect

We examined the neural mechanisms related to the re-presentation of the cue by contrasting the neural response on trials where the cue re-appeared in the search display (averaging across invalid and valid trials) and trials where the cue was not present in the search display (neutral trials). To examine whether the reappearance effect is susceptible to ageing, we looked for regions showing a stronger reappearance effect in young relative to older adults (reappearance x task in young (Young adults: $WM_{Invalid\ +Valid} > WM_{2\ x\ Neutral}$ & $MR_{Invalid+\ Valid} < MR_{2\ x\ Neurtal}$) , masked by reappearance x task x age interaction (Young adults: $WM_{Invalid\ +Valid} > WM_{2\ x\ Neutral}$ & $MR_{Invalid\ +\ Valid} < MR_{2\ x\ Neurtal}$; Older adults: $WM_{Invalid\ +Valid} < WM_{2\ x\ Neutral}$ & $MR_{Invalid,+\ Valid} > MR_{2\ x\ Neurtal}$). In accordance with previous studies (Soto et al., 2012a), the right PPC showed a positive reappearance effect (invalid, valid > neutral) in the WM condition and the opposite effect (invalid, valid < neutral) in the MR condition, but this effect was specific to young adult participants (Table 6.2). There were no above threshold responses that showed a reliable re-appearance effect for the WM condition across both groups tested using conjunction, or main effect analyses.

6.4.2.2.3. Validity Effect

We next identified the neural mechanism selective to the effects of cue validity on search. In agreement with previous studies (Soto et al., 2007; Rotshtein et al., 2011) we observed increased responses to valid compared with invalid trials within the fronto-thalamic-occipital network. However, this pattern was stronger in the elderly (elderly, WM: Valid > Invalid; inclusively masked by the interaction with age) and had relatively

weak reliability (i.e. uncorrected for FWE): the right thalamus (MNI: 18 -13 1; Z_{Peak} = 3.23; with 58 voxels reliable at Z > 2.33), the left thalamus (MNI: -21 -25 -2; Z_{Peak} = 2.58, with 20 voxels reliable at Z > 2.33), the left lateral occipital cortex (MNI: -33 -73 -5; Z_{Peak} = 2.94, with 6 voxels reliable at Z > 2.33), the right BA10 (MNI: 15 56 13; Z_{peak} = 2.87, with 9 voxels reliable at Z > 2.33) and the left BA10 (MNI: -18 53 7; Z_{peak} = 2.78, with 6 voxels reliable at Z > 2.33) (not shown). No above threshold responses were observed using conjunction analysis across age group, though there was a reliable increase for valid vs. invalid trials in the WM condition across both groups, when the groups were averaged (i.e., a main effect analysis) in the left superior frontal gyrus (MNI: -15 53 25; Z_{Peak} = 3.25, with 11 voxels reliable at Z > 2.33 and MNI: -18 29 55; Z_{Peak} = 2.97, with 12 voxels reliable at Z > 2.33), and the left PPC (MNI: -45 -58 25; Z_{Peak} = 3.25, with 123 voxels reliable at Z > 2.33).

Interestingly in contrast to previous studies (Soto et al., 2007; Rotshtein et al., 2011) we observed reliable effects for the reverse contrast, with larger responses for invalid compared with valid trials. Conjunction analysis across groups for the interaction between task and validity (conjunction of WM: Invalid > Valid for both groups, masked inclusively by the two-way interaction, Task x Validity – WM: Invalid > Valid & MR: Invalid < Valid) identified a number of brain areas including: the early visual cortex (EVC) and the anterior cingulate cortex (ACC) amongst other regions (Table 6.2, Figure 6.3, green colour). In these regions, both groups showed a reliable validity effect (invalid > valid) in the WM condition, but not the MR condition.

To assess the dissociation between the groups, we specifically investigated which brain regions showed a greater validity effect in the WM condition for young but not older participants (Figure 6.3 red and orange colour). This was computed using the interaction contrast in the young (WM: Invalid > Valid & MR: Invalid < Valid) inclusive mask by the three way interaction contrast (Age x Memory x Validity; Figure 6.3, red colour). The left AI/IFG, the ACC and the EVC showed stronger validity effect in the young compared with the old. Note that the EVC and ACC showed common validity effects across ages, though the effect was larger in the young (Figure 6.3, orange colour), suggesting that these regions were shared across both groups in response to the validity manipulation in WM but with significantly stronger validity effect in young adults relative to older adults (Figure 6.3, orange colour). There were no regions that were involved in the older adults but not in the young participant for this contrast.

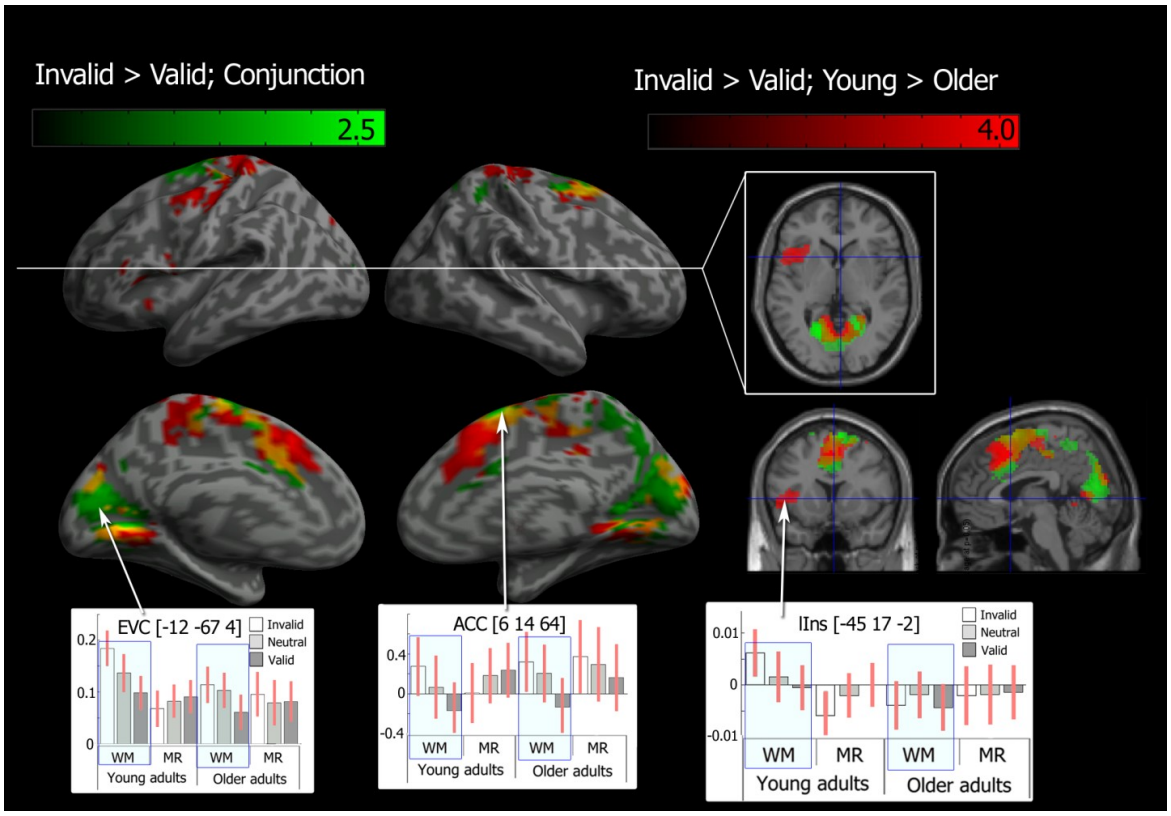

Figure 6.3. The BOLD response to the effects of validity in the WM condition across both groups (green), only in young participants (red) and more in the young relative to the older participants (orange). The threshold was set at p <0.01 for cluster sizes larger than 200 voxels; b) plotted contrast estimates as a function of the effect of validity (Invalid – I, Neutral – N and Valid – V), task (Working Memory – WM and Mere Repetition – MR) and age group (young and older participants) in the EVC and the ACC. The estimates were extracted from a sphere with 10mm radius around the peak of interest.

6.4.2.3. Between region correlations

Finally, to assess whether the neural coupling between prefrontal cortex and sensory cortices is reduced in the elderly participants, we computed a Pearson correlation across participants, separately for each group. The dependent variables were the neural response to validity in the left and right AI/IFG and the EVC. We observed a reliable correlation for young participants (left: r = .687, p = .005, right: r = .630, p = .012), but not for older adults (left: r = .289, p = .361; right, r = -.023, p = .945) (see left and centre

of Figure 6.4, for left and right AI/IFG, respectively). To assess the validity of the inter-subject correlations, we also computed a correlation between the two homologous regions of the right and left insula/IFG. As expected, we found a positive relationship between the validity effect in the right and the left AI/IFG for both the young (r = .892, p < .001) and the older participants (r = .698, p = .012) (Figure 6.4).

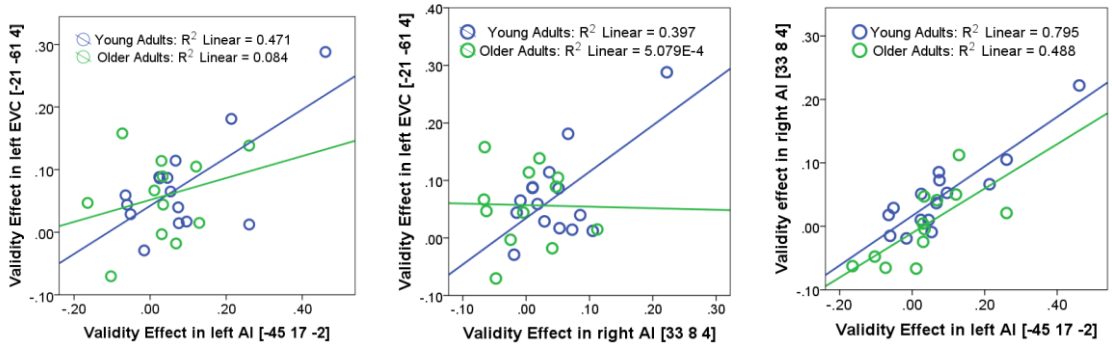

Figure 6.4. Correlations of the validity effect (Invalid - Valid) between the AI/IFG and EVC for young participants (blue) and older participants (green colour): *left* – left early EVC and the left anterior IA/IFG; *centre* – left EVC and the right AI/IFG; and *right* – the right and left AI/IFG.

6.4.2.4. PPI analysis

Effective connectivity analysis was used to examine whether the correlation between the right AI/IFG [33 8 4] and the visual cortex was sensitive to the experimental manipulation. We restricted this analysis to regions that showed a validity effect in both groups (conjunction contrast). Within these regions, we observed that, across both groups, there was higher coupling of the right AI/IFG and the EVC ([9 -88 10] , p = .029) on invalid trials compared with valid trials. This effect was stronger and specifically more implicated in young relative to older adults (EVC [9 -58 13], p = .021). Unsurprisingly, the right AI/IFG also showed increases in functional connectivity for

invalid trials with the ACC across both groups (dACC [6 14 58], p = .001; cACC [9 29 22], p = .002) and specifically for young relative to older participants (dACC [9 11 55], p = .004 and cACC [9 17 37], p = .013). These findings reinforce the correlational analysis (see above) and they suggest that the validity effects in the EVC and ACC are associated with the responses of the AI/IFG on invalid trials. In addition, analyses of the intra-subject (i.e. trial by trial) variability indicate stronger coupling (AI/IFG→ EVC and AI/UFG→ACC coupling) for young relative to older participants. No brain clusters above the set threshold engaged more for older adults relative to young adults.

6.5. Discussion

The current study focused on the effects of ageing on the neural mechanisms implicated in biasing attention from information held in WM. The behavioural analysis showed that the validity effect (RTs for invalid>neutral>valid trials) was stronger under WM relative to MR conditions (see also Soto et al., 2005; Tsvetanov et al., 2012a). In addition, the results replicated previous findings (Tsvetanov et al., 2012b) in showing that the older adults generated weaker validity effects under WM conditions compared to the young adults, indicated by weaker costs on invalid relative to neutral trials.

On a functional BOLD level, we observed an increase response in the right AI/IFG during the encoding period of the WM cue in elderly but not in young participants. Furthermore the degree which the right AI/IFG responded during the encoding period of the WM item correlated negatively with the observed behavioural

validity effect. In young participants the extent to which the right AI/IFG responded to the effect of validity also correlated with the responses of the visual cortex (EVC) to the same manipulation. This frontal-sensory, validity-dependent coupling was not observed in the elderly participants. Finally, we observed that the fronto-thalamic-occipital network was more strongly recruited in the elderly than the young participants. We consider each of the findings in more detail.

6.5.1. Effects of age on memory encoding.

Effects during the cue events: In elderly participants the right AI/IFG was more active in the WM condition relative to the MR condition, whereas young participants showed the opposite pattern. Importantly, the size of the task effect (WM - MR) in the right AI/IFG during the cue event predicted the magnitude of the validity effect on behaviour: the larger the task effect in the AI/IFG, the smaller the behavioural effect of validity. These results indicate that, with age, the use of the right AI/IFG changes. Older adults appeared to rely more on the recruitment of the right AI/IFG for memory encoding, when compared with young participants, presumably to compensate for reduced processing resources (Schneider-Garces et al., 2010; Park & Reuter-Lorenz, 2009). We note though that the recognition test performance did not differ across the age groups, consistent with higher AI/IFG activation during the WM task helping to compensate for decreased WM capacity in the older participants (Grady, 2012; Cabeza et al., 1997; Salami et al., 2012; Park & Reuter-Lorenz, 2009). This observation is consistent with previous reports showing that increased activity within the AI/IFG is

associated with inefficient memory encoding in older adults when compared with young adults (Cabeza et al., 1997; Salami et al., 2012). Interestingly, in Soto et al. (2011), where a similar paradigm was deployed, young participants showed increased bilateral PFC activation (including the right AI/IFG) when there was a high WM load (remember three items) relative to a low WM load (remember one item). There was also a reduced behavioural effect of the WM cue on search in the high load condition. Here we observed reduced activation of the right AI/IFG in the WM relative to the MR condition. The reason for this reduced response is unclear, but it may reflect an attempt to separate the memory cue from the search template and with placing the memory cue at the forefront of WM. This fits with the behavioural data indicating a stronger validity effect in the younger participants.

In support of the idea that increased activation of the right AI/IFG during WM is linked to WM biases on selection, we found that high activity in the right AI/IFG during the cue event predicted a low cue validity effect on behaviour. We propose that the increased activation of the AI/IFG reflects participants keeping both the WM cue and the search target in WM, which means that the effectiveness of the WM cue for selection decreases (see also Olivers, 2009).

Effects during the search event: Mirroring the behavioural results, the task (WM vs. MR) also affected responses during the search event, independent of the validity manipulation. This task effect was observed in both age groups. Here the right dlPFC and the right BA10 areas (and to a lesser degree the left PFC) were more active in search

under WM compared to MR conditions. Interestingly, the size of the task effect (WM - MR) was positively correlated with the size of the behavioural validity effect - the larger the task effect at a neural level, the slower the response to invalid relative to valid trials. Taken together, these findings suggest that WM-related modulation of the right AI/IFG during the cue event and the right dlPFC during search was essential for a strong validity effect to emerge on behaviour. For example, activation of a WM representation in dlPFC could be key to attention then being allocated to the cue in the search display, provided the AI/IFG is not also strongly representing the search target (see Soto et al., 2007).

6.5.2. The interaction between WM and selection

Reappearance of the cue, irrespective of its validity, was associated with activity in the right PPC in young participants. Under WM conditions cue reappearance increased activity in the PPC, while there was decreased activity under MR conditions. The link between PPC activity and reappearance of the cue has been noted previously (Soto et al., 2012c) .

We also observed effects of validity during the search event, where we identified a brain network sensitive to the relations between the cue and the search target under WM conditions. In line with previous studies (Soto et al., 2007, 2011, 2012), we observed increased responses in the fronto-thalamic-occipital network when the WM cue surrounded the search target compared to when it surrounded a distractor. However, here this effect was primarily observed in the elderly participants and was relatively weak. Relative to prior studies, we used a long interval between the WM cue and the search

display, which has been shown to lessen effects of memory consolidation on search (Tsvetanov et al., 29012a). We suggest that the effects of validity found in the fronto-thalamic-occipital network reflect the ongoing consolidation of the WM item, which we assume may be more extended in the elderly (see Tsvetanov et al., 2012b, for behavioural evidence). The increase in activity in the network on valid trials, and the reduction in activity on invalid trials, acts to protect search from ongoing memory-consolidation processes when the WM item and the search target fall at different (conflicting) locations (Rotshtein et al., 2012).

In contrast to prior studies (Soto et al., 2007, 20011, 2012a,b) here we also observed increased activation for invalid relative to valid trials in WM condition. This was found in the left AI/IFG, the bilateral EVC and the bilateral dorsal and caudal parts of the ACC in young participants. Older adults recruited the same brain regions with the exception of the left AI/IFG. The greater activity on invalid trials suggests that these regions reflected effects of competing activation from the re-presented cue and the search target and/or that they were involved in a specific process of re-engaging attention on the target after mis-direction of attention to an invalid shape.

The pattern of responses linked to the relevance of the memory item during search event fits with the biased competition model of attention (Desimone & Duncan, 1995; Botvinik et al., 1999; Kerns et al., 2004; Kelley & Lavie, 2011) – on invalid trials there is interference between the information held in WM and the current goal (i.e. memory item and target fell on different locations on the screen), where both stimuli compete for

selection. This, results in higher responses in brain areas associated with sensitivity to competition. On valid trial, the memory item and the target are in agreement and no response competition is observed, resulting in low involvement of the brain areas selective to stimulus competition. We note that the brain areas involved here match with previous findings using tasks where response competition was manipulated including the Stroop effect, go/no-go and flanker tasks and oddball detection (Botvinick et al., 1999; Kerns et al., 2004; Cole & Schneider, 2007; Nee et al., 2007; Roberts & Hall, 2008; Kelley & Lavie, 2011; Floden et al., 2011). Our findings suggest that the network involved in conflict resolution may also be implicated under conditions when invalid information from WM needs to be ignored for successful selection of a target.

6.6. Conclusion

Older participants showed increased activity in the AI/IFG during memory encoding which was associated with reduced behavioural effects of WM cues on attention. We link this to reduced partitioning of WM and task template in memory, and this weaker partitioning results in reduced coupling of prefrontal regions to sensory cortices during search. In addition the data also indicate that older participants are more sensitive to memory consolidation processes and the recruitment of a fronto-thalamic-occipito network in order to protect search from ongoing memory consolidation.

CHAPTER 7.
Resting GABA modulates the effects of ageing on memory-dependent BOLD signals in the right Anterior Insula / Inferior Frontal Gyrus

7.1. Abstract

We report data on the relations between ageing and resting GABA concentration in a brain region (right anterior insula (AI)/ inferior frontal gyrus (IFG)) differentially recruited for working memory in young (mean age 24 years) and older adults (mean age 76 years) . The concentration of resting GABA was found to be inversely correlated with the BOLD signal change related to memory encoding – the lower the resting GABA concentration in the right AI/IFG, the stronger the signal BOLD change when a WM item was encoded compared to when the item was merely identified. I discuss the finding with respect to (i) how neuronal inhibition, mediated by GABAergic interneurons, may influence both memory coding and the guidance of attention from information stored in the WM and (ii) how age-related decline in resting GABA might contribute to the over-recruitment of brain areas to support cognitive performance in older participants.

7.2. Introduction

There is a growing literature suggesting that the concentration of the main inhibitory neurotransmitter in the brain, γ-Aminobyturic Acid (GABA), plays a significant role in the modulation of neurocognitive functioning (Northoff et al., 2002; Ferster et al., 1996; Ferster & Miller, 2000; Buzsaki et al., 2007; Allison & Bonds, 1994; Sawaguchi et al., 1988; Crestani et al., 1999; Low et al., 2000; Chen et al., 2005). Furthermore, a number of behavioural and computational studies have suggested that the decline in GABAergic inhibition underlies cognitive deficits observed with ageing (Caspary, Milbrandt, & Helfert, 1995; Caspary, Ling, Turner, & Hughes, 2008; Zhang et al., 2008; Yang et al., 2009; Fujiwara, Zheng, Miyamoto, & Hoshino, 2011). Recent developments in magnetic resonance imaging (MRI) demonstrate that (i) *in-vivo* measurement of GABA is correlated to the BOLD signal measured by fMRI in a number of cognitive tasks (Northoff et al., 2007; Muthukumaraswamy et al., 2009; Michels et al., 2012) and (ii) age is associated with reduced resting levels of GABA within the dorsolateral prefrontal cortex (dlPFC), the orbitofrontal cortex (OFC), the sensorimotor cortex (Grachev et al., 2001), the superior frontal gyrus (SFG) and the superior parietal lobule (SPL) (Gao et al., *submitted*). Therefore, the focus of this study is to examine whether resting GABA concentrations are correlated with the BOLD signal change of a brain region (right anterior insula (AI) / inferior frontal gyrus (IFG)) that is differentially recruited during encoding of a memory item in young and older participants (Chapter 6).

The right anterior insula (AI) / inferior frontal gyrus (IFG) has repeatedly been shown to be differentially activated in older relative to younger adults during encoding of a memory item (Cabeza et al., 1997; Leshikar et al., 2010; Salami et al., 2012; Chapter 6). In Cabeza et al. (1997) the increased activity within the right AI/IFG in older adults was associated with less efficient inhibition of the region and linked to the encoding of irrelevant information. Leshikar et al. (2010) suggested that the observed pattern of activation might be an indication of less efficient inhibition of default activity in older adults. Both views are closely linked to the inhibitory deficit account of cognitive ageing proposed by Hasher and Zacks (Zacks & Hasher, 1997; Lustig et al., 2007). In Chapter 6 the data showed that the control of the right AI/IFG was essential for the modulation of attentional guidance from information held in WM (also Soto et al., 2012a). In young adults, the right AI/IFG remained "silent" (neuronal inhibition) when an item was encoded into the WM but it was then involved in the guidance of attention from WM item during a subsequent visual search task. There was functional coupling between AI/IFG and occipital cortices when the WM item reappeared in the search display (Chapter6; Soto et al., 2012). Older adults, however, recruited the right AI/IFG when they had to encode the cue item in memory (compared with a condition where they had only to identify the same item) and this was associated with (i) a weaker behavioural bias of attention from WM and (ii) a decreased coupling between the right AI/IFG and the early visual cortex (EVC) during search (Chapter 6). The pattern of activity in older adults was similar to that in young adults under high WM load in the same paradigm (remember

three memory items vs. one memory item, Soto et al., 2012a). Taken together the findings suggest that older adults might recruit the right AI/IFG during the encoding of a stimulus in WM item as a compensatory effect of reduced WM capacity. However, this may in turn protect attention from being biased when the item in WM reappears in a search display.

There is almost no direct evidence suggesting what could be the underlying biophysiological mechanism that, as we age, modulates the observed effects in our fMRI findings (Chapter 6). GABA is the main inhibitory neurotransmitter and as a modulator of the GABAergic interneurons it is intriguing to examine whether the decrease of resting GABA concentrations found in older participants (Grachev et al., 2001; Gao et al., submitted) might play a significant role in the compensatory mechanisms observed in ageing. There is evidence that the BOLD response is negatively correlated with the resting GABA concentrations measured by MRS (Northoff et al., 2007; Muthukumaraswamy et al., 2009). For example, Northoff et al. (2007) found that the decrease in the strength of responses in the anterior cingulate (ACC) to emotional stimuli correlated with resting level concentrations of GABA as measured by MRS. Furthermore, Muthukumaraswamy et al. (2009) demonstrated that resting levels of GABA are negatively correlated with the BOLD response to visual stimulation in the young human visual cortex. Taken together, the data suggest that age-related over-activations of the BOLD signal, found across a number of cognitive domains (including findings in Chapter 6), is related to decreasing resting levels of GABA.

We measured the resting levels of GABA in brain regions showing a differential functional recruitment between young and older adults. Regions of interest (right AI/IFG and the EVC) were selected *a posteriori* based on fMRI findings where, relative to young adults, older adults (i) recruited the right AI/IFG during the encoding of a WM item, while (ii) younger adults recruited this region more during a subsequent search event, showing an interaction between memory and selective attention. In addition, the earlier work showed a reduced functional coupling between the right AI/IFG and the EVC during the search event in the older participants relative to the younger subjects (see Chapter 6). I hypothesize that age-related differences in resting levels of GABA are correlated negatively with the memory related BOLD response – in our case low GABA concentrations would be related to higher recruitment of right AI/IFG during WM encoding when compared to a baseline condition, and with less engagement of the AI/IFG in subsequent search.

7.3. Methods

7.3.1. Overview

The aim of this study was (i) to measure GABA concentrations within two regions of interest (EVC and right AI/IFG) based on the findings from fMRI study and (ii) to correlate the GABA concentrations within these two regions with main effects of task (WM - MR) and validity (Invalid-Valid trials, based on the relations a reappearing memory item in the search display and the search target) on both behavioural and brain

activity. For this purpose all participants who volunteered in the earlier fMRI study (Chapter 6) were recalled for a second MRS session.

7.3.2. Participants and procedure

Data were collected from fifteen young (mean age 26 years, age range 21 to 32 years, 8 males) and ten older participants (mean age 75 years, age range 68 to 82 years, 5 males) for exchange of course credit or cash. For recruitment and exclusion criteria please refer to Chapter 6. Two elderly males did not return to this second scanning session. One had subsequently heart surgery and another because he did not want to be scanned again. MRS from the right AI/IFG was not collected from 2 elderly participants and 1 young adult due to misplacement of the voxel where data were recorded.

Detailed explanation of the paradigm was presented in Chapter 6.

7.3.3. MRI methods

We collected two separate MRI datasets on a 3T Phillips Achieva with an eight-channel receive head RF coil. Each participant took part in two one- hour sessions within 6 months of each other.

7.3.3.1. First Session – fMRI study

In the first session we acquired (i) a high-resolution anatomical T1-weighted image and (ii) six EPI measurements. For more details about the sequences refer to Chapter 6.

7.3.3.2. Second Phase – MRS study

Three fast T2-weighted structural images, one for each plane, were acquired to aid localisation of the volume of interests (VOIs).

Based on findings from the fMRI study (Chapter6), we were interested in measuring GABA+ concentrations within the early visual cortex (EVC) and the right anterior insula (AI)/ inferior frontal gyrus (IFG). The first MRS measurement was always collected from the EVC, followed by a measurement in the right AI/IFG (see Figure 7.1). GABA+ concentrations were measured with an edited single voxel Point-RESolved Spectroscopy (MEGA - PRESS) sequence (TE = 68ms; TR = 1800ms; sample data points = 2048; spectral bandwidth = 2150Hz; voxel size = 30 x 30 x 30 mm^3) (See Chapter 2 for more detailed description of the method, Mescher et al., 1998; Edden & Barker, 2007). Forty spectra, each a result of 8 averages, were acquired over to a total duration of 10 minutes. Half of the spectra were acquired with a 16ms Gaussian editing pulses applied at 1.9ppm, whereas other half used an editing pulse at 7.5ppm. Prior to each MEGA-PRESS measurement, a PRESS measurement without water suppression was acquired to be used as an internal reference for estimating GABA concentrations in institutional units. The total duration of the MRS session was about 30 minutes.

7.3.4. Analysis

MRS analysis was performed using a fully automatized processing pipeline in the Gannet software package (available at gamamrs.blogspot.com). For more details please refer to Chapter 2. Concentration measurement of GABA+ was given in institutional

units based on (i) the ratio between GABA+ and water unsuppressed signals and adjusting for (ii) macromolecules, (iii) editing efficiency, (iv) T1 and T2 relaxation times of water and GABA. To ensure high quality of data and proper estimation of the GABA concentration we removed data in which the spectra editing efficiency was larger than 15% . Inefficient subtraction between edited and non-edited spectra might be caused by (i) subject movement during the acquisition, or (ii) frequency drifts of the scanner as a result of heavy EPI acquisition from another research group prior to the MRS session.

GABA+ concentrations were correlated to main effects of task (WM - MR) and validity (Invalid - Valid) from behavioural and fMRI data using non-parametric statistics in SPSS(Spearman correlations). (Note that similar results (albeit weaker) were obtained if we computed linear correlations.)

To control for potential partial tissue volume in the VOI affecting the GABA signal, we re-computed the same correlations using partial-correlation controlling for the tissue type ratio in the VOI (grey matter/ (grey matter + white matter)). To plot the partial correlation, we used partial residual plots to represent correlation between GABA and BOLD signal after accounting for partial tissue type. Data set of residuals for GABA and BOLD was derived from the unstandardized residuals of two independent linear regression models, where GABA and BOLD signal change were used as dependents and partial tissue volume as independent. For more information on the segmentation procedure please refer to Chapter 2.

7.4. Results

Following quality checks of the data (editing efficiency error < 15%), 3 spectra were removed from the EVC region, so the analysis included only 22 spectra from the EVC (13 young adults and 9 older adults); one spectrum had to be removed from the right AI/IFG, leaving 21 spectra altogether for the analysis (13 young and 9 older participants).

7.4.1. Effects of age on GABA+

Inspecting the data using an outlier labelling procedure (Hoaglin et al., 1986) revealed one elderly participant (76 years of age) as an outlier. Interestingly, this participant was also an outlier when inspecting the effect of WM-attention interaction in her fMRI responses. When we exclude this participant from the analysis then the levels of GABA concentration were found to be affected by age, with older adults showing reliably less GABA+ in the right AI/IFG, F (1, 18) = 5.12, p = .036 and also a trend for less GABA+ in the EVC, F(1, 19) = 2.78, p = .058 (single-tailed).

7.4.2. Effects of GABA+ on BOLD signal change

In Chapter 6, I demonstrated that age-related decreases in the guidance of attention from WM was related to (i) increased recruitment by older adults of the right AI/IFG during WM encoding, and (ii) a weaker recruitment of the right AI/IFG by older adults during the search task. I therefore examined the relationship between GABA and changes in the BOLD signal change between WM and MR during the cue event, and

between invalid and valid trials in the search event. Non-parametric analysis showed an inverse correlation, r = -.543, p = .011 (Figure 7.1c) – participants with high concentrations of $GABA_{AI/IFG}$ showed a reduced signal BOLD response during encoding of the memory item in the very same region (right AI/IFG). There were no significant correlations between GABA+ levels in the right AI/ IFG and the main effect of task (WM-MR) on behaviour (r = -.209, p = .363) or on the behavioural magnitude of the validity effect (Invalid – Valid: r =- .362, p = .106).

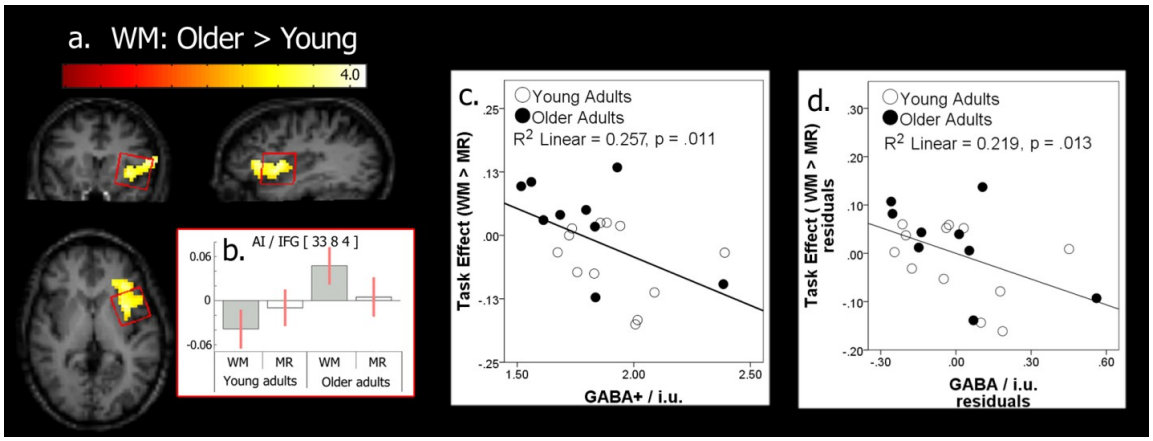

Figure 7.1.Results from right anterior insula (AI)/ inferior frontal gyrus (IFG). (a) T1-image in three planes showing in yellow the right AI/IFG being less activated in young compared with older participants during encoding of a memory item (WM: Working memory condition) compared to a baseline condition (MR: Mere Repetition). (b) bar plot showing the BOLD response in the right AI/IFG (MNI:33 8 4) during WM and MR separately for young and older adults. (c) Correlation between resting GABA+ and task effect (signal BOLD change between WM and MR) in the right AI/IFG. (d) Partial residual plot to demonstrate the correlation between resting GABA+ and the task effect after accounting for partial tissue type (grey matter/ (grey matter +white matter)) within the voxel.

No correlations between GABA+ concentration and signal BOLD change in the EVC were observed.

7.4.3. Effects of grey matter on GABA+

To account for partial tissue volume within the MRS voxel, we calculated the grey matter tissue fraction (grey matter / (gray matter + white matter)). MRS voxels contained significantly less grey matter tissue in older relative to young adults for both measurements (AI/IFG: 73% and 66% for young and older adults, $F(1, 19) = 10.45$, $p = .004$; EVC: 68% and 63% for young and older adults, $F(1, 20) = 21.54$, $p < .001$). This is not surprising as aging is known to be associated with increased atrophy.

Partial tissue volume did not correlate with resting GABA measurements in either the EVC ($r = .284$, $p = .200$) or the AI/IFG ($r = .135$, $p = .559$).

The correlations between GABA and signal BOLD change were reanalysed using partial correlations to control for partial tissue volume. The negative correlation between $GABA_{AI/IFG}$ and signal BOLD change of task effect (WM - MR) remained significant, $F(1, 19) = -.544$, $p = .013$ (Figure 7.1). No correlations with other factors were observed. Accounting for partial volume did not change the effects for the EVC .

7.5. Discussion

The most important finding of this study was that the concentration of resting GABA+ could predict the recruitment of the right AI/IFG during encoding in the cue event in our fMRI experiment. Concentrations of resting GABA were found to be inversely correlated with BOLD change relating to the main effect of task (WM-MR) –

the lower the resting GABA+ concentration in the right AI/IFG the stronger the signal BOLD change during encoding of WM item when compared to a baseline condition in the same region.

7.5.1. Effects of GABA+ on memory related BOLD

The neuroimaging literature supports our functional findings in which the BOLD signal change in the right AI/IFG during memory encoding was negative for young adults and positive for older adults (Cabeza et al., 1997; Salami et al., 2012; Leshikar et al., 2010). In Cabeza et al. (1997) was proposed that the patter of activation in older adults reflected poor ability to inhibit the right AI/IFG to encode irrelevant information, whereas Leshikar et al. (2010) suggested that activity in the right AI/IFG during encoding was an indication of older adults having difficulties to inhibit default activity; both views being in line with the inhibitory deficit theory (Hasher & Zacks, 1988; Lustig et al., 2007).

Negative BOLD responses have previously been associated with decreases in neuronal activity (Shmuel et al., 2006; Shmuel et al., 2002; Devor et al., 2007; Pasley et al., 2007; Schafer et al., 2012). In addition, there is evidence that the BOLD response is negatively correlated with resting GABA concentrations measured by MRS (Northoff et al., 2007; Muthukumaraswamy et al., 2009). These findings are supported by animal and human studies using GABAergic modulation (Crestani et al., 1999; Low et al., 2000; Northoff et al., 2002; Chen et al., 2005). For example, administration of medication to reduce the rate of GABA break down (by blocking GABA transaminase) results in

increased GABA levels coupled with a reduced BOLD response (Chen et al., 2005). Taken together, the data in the present study complement previous findings by showing that individual differences in resting GABA concentrations are correlated negatively with BOLD response – in our case individuals with low GABA concentrations showed higher recruitment of brain areas relative to counterparts with high GABA concentrations who either did not recruit or inhibited the very same region.

7.5.2. Effects of Age on GABA+

We were interested in measuring resting GABA concentrations of brain regions showing age-related changes in brain activity in WM and in WM-based attentional guidance. We found that relative to young adults, the resting levels of GABA in older adults were reliably smaller in the right AI/IFG and marginally smaller in the EVC, even after accounting for partial tissue volume in the MRS voxel. Our findings are in line with previous studies showing age-related decreases in resting levels of GABA within the dorsolateral prefrontal cortex (dlPFC), the orbitofrontal cortex (OFC), the sensorimotor cortex (Grachev et al., 2001), the superior frontal gyrus (SFG) and the superior parietal lobule (SPL) (Gao et al., submitted). Taken together, these findings suggest that GABA concentrations in older adults may be reduced across a number of regions.

It is intriguing whether the rate of GABA decreases across brain regions is uniform, or whether there regions more susceptible to ageing (decreasing their GABA levels at a higher rate) than others. I found that, relative to young adults, older adults had reliably less resting GABA in the right AI/IFG, whereas in the EVC there was a trend

approaching significance. Similarly, Grachev et al. (2001) found that the frontal brain regions were most susceptible to effects of ageing on maintaining GABA concentrations. These findings are in line with neuroimaging finding showing that the age-related atrophy and alteration of function is most noticeable in the frontal areas. Taken together with previous findings the data suggest that three can be a steeper rate of structural and functional age-related decline within the frontal areas relative to other brain regions which is linked to resting levels of GABA. Further work should confirm the direction of these findings, particularly using longitudinal rather than cross-sectional designs.

7.5.3. Effects of Age on GABA+ and memory related BOLD

The most important finding in our study was related to the effect of ageing on the link between GABA concentrations and cognitive function. A number of behavioural and computational studies have suggested that a decline in GABAergic inhibition underlies many of the cognitive declines observed with age (Caspary et al., 1995; Caspary et al., 2008; Zhang et al., 2008; Yang et al., 2009; Fujiwara et al., 2011). The findings suggest that age-related decreases in resting levels of GABA+ 'mediate' a disinhibition of the right AI/IFG, indicated by an increased change in the BOLD signal under WM vs. MR conditions. There is also a reduced behavioural effect of WM-based guidance on attention (Chapter 6 and 7). Based on these two results I propose two possible acocunts of the data. Increased levels of GABA+ might reflect either (1) poor encoding into WM (the neural response having to be greater to achieve memory encoding) and subsequently weaker effects of WM-guidance on attention, or (2) the involvement of the AI/IFG in

holding the task template for the search task as well as the cue being encoded into WM; the maintained activation of the target template as well as the WM item then reduces attentional guidance from WM. This last argument would fit with the idea that older participants are less good at 'partitioning' WM and holding templates for search in the 'background' of WM and the WM cue in the foreground. A similar argument has been made concerning the performance of neuropsychological patients with brain lesions to frontal cortex (Soto et al., 2006).

Possible limitations in the current study were discussed in Chapter 2.

To our knowledge, the current study is the first one to date to explicitly link age-related alterations in neurocognitive function and age-related changes in GABA. The results however should be interpreted with caution. Future studies combining negative BOLD response, GABA measures and more direct measures of neural activity (EEG/MEG) are required to further support the findings.

7.6. Conclusion

The current study provides evidence for the importance of studying age-related decreases of GABA with respect to understanding age-associated functional reorganisation and over activations in wider brain areas related either to compensatory or to dedifferentiation mechanisms. The results show that there is an inverse correlation

between age-related decline of GABA levels and BOLD response related to encoding of a memory item in the right AI/IFG.

CHAPTER 8.
Thesis Summary and Concluding Remarks

The structure of the chapter starts with a summary of the main contributions of the thesis, divided into neuroscientific, conceptual and methodological sections. The neuroscientific section summarizes the findings of all the empirical chapters. Next, the conceptual sections discuss whether the BOLD and GABA findings are in line with the proposed extension of the capacity deficit hypothesis. The concluding section is preceded by a section describing future research directions.

8.1. Findings and significance of work in this thesis

8.1.1. Neuroscientific contributions

During this thesis I have tested the effects of ageing on (i) visual selection by visual saliency and (ii) the interaction between WM and visual selection. I have further examined whether functional and neurochemical changes in the PFC and the early visual cortex mediate the observed behavioural changes in the interaction between WM and attention. I have linked age-related decreases of resting GABA levels within the right AI/IFG to increased levels of BOLD when stimuli are encoded. This was used as converging evidence to show that older adults might have to recruit additional resources

to maintain information in WM through activation of the right AI/IFG. This may prevent irrelevant cues from being at the forefront of WM, so lessening the attention-capturing capability of the cues.

8.1.1.1. Effects of ageing on selection by visual saliency

In the first empirical chapter, the ability to select low saliency information, while ignoring high saliency distracting information, was examined in young and older individuals. Participants were asked to select local and global level of hierarchical stimuli that varied in perceptual saliency. The results demonstrated that, relative to young participants, older individuals were detrimentally impaired at inhibiting high saliency distractors and this was irrespective of the (global/local) level of processing. The data suggested that older adults might have pronounced deficits in non-spatial attentional selection of low-saliency stimuli. This further raised the question of investigating the effects of ageing on the neural mechanism involved in processing low-saliency information, while suppressing high saliency distractors.

8.1.1.2. Effects of neurocognitive ageing on WM-biased attentional selection

In respect to ageing, Chapter 5 showed that relative to young adults, older participants showed overall a greater drop in search performance under a condition where they had to hold an item in memory when compared to a baseline condition where the item was merely identified. Furthermore, older adults demonstrated a weaker guidance

effect on attentional selection from information held in the WM, when compared to young adults (Chapter 5). Taken together, it was proposed that older adults prioritize the maintenance of the task template, resulting in weaker interference of attention selection from irrelevant items in WM.

In a neural investigation of these effects (Chapter 6), the weaker behavioural validity effect in older participants was coupled with reduced activity in the ACC and the EVC, and in addition there was no validity modulation of the AI/IFG when compared to young adults. However, older adults also relied on the AI/IFG during the cue event to support encoding of the cue into WM. Correlational analysis revealed that the behavioural validity effect was associated with a strong coupling between the AI/IFG and the EVC in young but not in older participants. To reinforce these findings, PPI analyses showed a functional connectivity between the AI/IFG and the EVC, the ACC and the thalamus. This coupling was stronger for young relative to older adults. Similar findings were observed previously under high vs. low WM load conditions with young participants (Soto et al., 2012a; Jongen & Jonkman, 2011). For example, a functional connectivity analysis in Soto et al. (2012) revealed a strong coupling between the AI/IFG and the EVC related to the effects of cue validity under low but not high WM load conditions. Taken together fMRI findings suggest that the AI/IFG might play an important control role in WM. In particular, it is involved in biasing attention from WM to a matching stimulus in the search display provided the cognitive demands on WM are "moderate". However, if cognitive demands are high and there is insufficient WM

capacity to automate coding of the task template, then I suggest that the right AI/IFG will be recruited to support coding of the task template as well as the WM stimulus (Jongen & Jonkman, 2011). This increases overall activation in the right AI/IFG, whilst at the same time reducing its coupling with early visual areas. Ironically, the data indicate that having less capacity can be protective of search from extraneous interference.

There were other differences between the present imaging results and those previously reported on the WM-attention paradigm. For example, here only older participants showed an increased response to valid vs. invalid trials in the fronto-thalamic-occipital network. Before this effect was reported with young participants (Soto et al., 2007; Rotshtein et al., 2012). In addition here I observed increased responses to invalid compared with valid trials, while none of the previous studies have reported such effects (even at low thresholds) (Soto et al., 2007; Rotshtein et al., 2012; Grecucci et al., 2010; Soto et al., 2012). As hinted in Chapter 6, I speculate that these inconsistent results might relate to the different intervals between the cue event and the search event used across studies. For example, Soto et al. (2007) used a short interval of 250ms, whereas in the present fMRI experiment (Chapter 6) the interval was on average 3 seconds (2-4 seconds). In Chapter 4, I reported that at short intervals between the cue and the search display (< 1second) the validity effect was significantly stronger than at longer intervals (> 2 seconds). I suggested that this was due to the ongoing consolidation of the memory item that biased search with a short interval but was finished at the longer interval. Interestingly, in Chapter 5, I showed that the older adults may take longer than young

participants to consolidate the cue in WM. Following these assumptions, it is likely that older adults (where consolidation might still be ongoing) are recruiting also the neural mechanism for consolidation through the thalamus. fMRI results supported these speculations, though activations were below threshold levels. Based on the findings in this thesis it can be proposed that the validity effect at short intervals might be driven by a neural mechanism (i.e. stimulus consolidation through thalamus) different to the neural mechanism at longer intervals (i.e. information held in WM, IFG/dlPFC which has direct link with the task salient network). In other words, there might be two mechanisms playing role in the modulation of attention through WM content: (a) a fronto-thalamic-occipital network during encoding of the WM cue (e.g. task effect (WM - MR) at short ISI in young adults (Soto et al., 2007, Chapters 4 and 5) and long ISI in older adults (Chapter 5) as an indication of ongoing consolidation) and (b) a second network implicated in conflict resolution post encoding of the WM cue (e.g. no task effect at long ISI in young adults (Chapters 4 and 5)).

8.1.1.3. Effect of ageing on GABA-BOLD

To my knowledge this thesis makes the first attempt to explicitly examine the effects of ageing on the relationship between resting GABA levels and BOLD signal change. The presented findings confirmed that resting levels of GABA in the right AI/IFG during encoding were inversely correlated with the BOLD response and with ageing. Due to the lack of direct correlations between GABA, the BOLD response and

behaviour it is not clear whether the observed effects are due to compensation (positive correlation with performance) or dedifferentiation (negative correlation with performance). How GABA levels might be related to existing findings and cognitive theories of ageing is discussed in the following section.

8.1.2. Conceptual contributions

Conceptually, the thesis aimed at providing converging functional and neurochemical measures in relation to existing theories of cognitive ageing. Particularly, I have proposed an expansion of two theories, the inhibitory deficit theory and the capacity deficit theory, which presented predictions about the neurofunctional and neurochemical results of ageing. In order to put the predictions to test, I have adopted two models: (i) the saliency model, based on the paradigm and findings of Mevorach etl al. (2006a; 2006b; 2009; 2010), testing the predictions of the inhibitory deficit theory, and (ii) the WM-biased attention model based on paradigm and findings of Soto et al. (2007; 2008; 2012) to test the predictions made about the capacity deficit model. Due to time limitations on the thesis, only the WM-biased attention model was tested on behavioural, functional and molecular levels.

In the Introduction I proposed that neuroimaging data in support of the capacity deficit model should present evidence of (i) older adults showing weaker activation in brain areas recruited by young adults, and (ii) overactivation of brain areas activated by older adults at low WM load, similarly to young adult at high WM load, but not low WM

load. The findings showed a similar pattern. Older adults under low WM load recruited the right AI/IFG relative to young adults under low WM load. Furthermore, the findings could be supplemented by Soto et al. (2012), where young adults showed a stronger recruitment of the same region (right AI/IFG) under conditions of high WM load (remember 3 items) relative to the low WM load condition (remember one item). Taken together, it is likely that the right AI/IFG involvement was related to capacity compensation during memory encoding. The nature of activation in the right AI/IFG during encoding in older adults (Chapter 6) and young adults under high WM load (Soto et al., 2012) could be either part of a task-specific neural mechanism (e.g. neural mechanism including regions explicitly involved in encoding) or a task non-selective neural mechanism (e.g. inhibition of the WM item from other information in WM, such as the task template). In my study there were no correlations of BOLD signal change during encoding with memory performance, whereas in Soto et al. (2012) this was not tested. Hence it is difficult to speculate what might be the nature of the right AI/IFG activation during encoding of the WM item. Either way, both studies suggest that the recruitment of the right AI/IFG during encoding occurs under conditions of reduced WM capacity, which further reduces the behavioural validity effect and the coupling with early visual cortex during search.

In respect to the MRS findings in Chapter 7, it is tempting to speculate on the role of GABA with respect to the neurocognitive models of ageing. One possibility is that reduced resting GABA levels with age might be linked to the dedifferentiation

hypothesis. This proposes that older adults show a less distinct pattern of activation during task performance (i.e. through the reduced specificity and selectivity of neural mechanisms). This becomes evident from neuroimaging findings reporting that relative to young adults, older adults recruit task-specific regions to a lesser extent, coupled with an over-recruitment of non-selective brain regions (Logan et al., 2002; Morcom et al., 2007; Garrett et al., 2011). For example, Logan et al. (2002) showed that, after matching performance across age groups, older participants showed under-activation in task specific regions coupled with less efficient inhibition of task-irrelevant brain regions. Similarly, reduced GABA levels in animals result in lower performance associated with higher spontaneous activity and lower signal-to-noise ratio (Hua et al., 2006; Wang et al., 2006). Thus, less efficient GABA inhibition, related to ageing, might result in less efficient modulation of task-specific networks coupled with non-selective recruitment of irrelevant or competing brain regions (Fujiwara et al., 2011). Furthermore, it has been proposed that non-selective recruitment is likely to be a result of an age-associated breakdown of inhibitory connections (Hasher & Zacks, 1988; Head et al., 2004). It is possible that high GABA levels are required for the efficient processing of a signal within a task-specific network. Thus, age-related decline of GABA levels might result in a less efficient modulation of neural mechanisms with the signal "spilling over" to other non-selective regions.

In line with the dedifferentiation hypothesis, an account in terms of reduced GABAergic inhibition could be further extended to explain findings related to decreased

variability of neural systems with ageing (Garrett et al., 2011; Garrett et al., 2012). Garrett et al. (2012) showed that in young adults there was an increased variability of neural response during a task condition relative to a fixation, while in older adults were observed fewer changes in brain variability within and across experimental conditions. Similar effects were found when comparing fast and slow performing adults. The authors proposed that the age-related decreases in neural variability across and within conditions represent a more rigid and uniform neural system. Thus switching between different neural states is less efficient. The impact of this may be shown behaviourally through evidence that there is a reduced ability to process efficiently varying and unexpected external stimuli (Garrett et al., 2012). Intuitively, neural variability might be related to overall resting GABA levels, where high GABA levels can provide an environment for a complex neural system, while low GABA levels will lose the ability to flexibly switch between "inhibition" and "activation" in response to different neural states. Taken together, dedifferentiation in ageing might be a result of a natural process related to loss of GABA resulting in a less efficient modulation task-specific mechanism and wider recruitment of non-selective brain areas.

The proposal on how GABA might fit to the dedifferentiation hypothesis might account for neuroimaging studies observing overactivations in brain regions correlated with negative performance. There is another set of neuroimaging studies, however, related to the compensatory mechanism, where older adults show overactivation of task-specific brain regions correlated positively to performance. In the view of the STAC

theory, it is likely that the brain regions showing compensatory functions in the older adults are related to multi-purpose 'scaffolding areas'. It is suggested that as the task-specific region of a network loses its specificity due to neural atrophy, that 'scaffolding areas' might take-over the role of processing a signal from the task-specific regions. It might be hypothesized that efficient modulation of the scaffolding areas may also depend on the resting levels of GABA – individuals with higher GABA levels have better chances of modulating the scaffolding areas to compensate for neural atrophy. If that is the case, there should be a positive correlation between GABA within the scaffolding areas and performance only in older adults, but not young adults.

Taken together, I suggest that resting GABA levels might play an important role in the capacity deficit theory, with the depletion of GABA with age posing challenges on the neural system to recruit task-specific regions for the efficient processing of a particular signal.

8.1.3. Methodological contributions

From a methodological point of view the thesis as a whole can be regarded as a multimodal protocol for the investigation of a specific age-associated cognitive deficit on behavioural, functional and neurochemical levels of analysis, in three stages. The protocol requires no *a priori* assumptions about the neural mechanism of interest. In the first stage, I adopted a well-established paradigm and identified cognitive deficits related to the interaction between WM and visual attention driven by WM capacity deficits. In

the second stage, based on behavioural findings, the paradigm was adopted to be "fMRI-friendly" for identifying the neural circuits specific to the cognitive task. In the third stage, GABA-edited MRS measurement was implemented in the regions of interest, based on the fMRI findings. The protocol can be implemented for testing different cognitive functions and disorders without the need of pre-existing knowledge about the underlying neural mechanism.

A potential drawback of the protocol might be the time interval between fMRI and MRS measurement required for the identification of the ROIs from the fMRI data analysis. In the case of this thesis the average timing between 2nd and 3rd stage was about six months, which might introduce confounding due to transient or temporal factors having an effect on neurochemical levels. However, if the researchers have previous experience with the protocol, and if the protocol is already implemented and tested within the research facility, the timescale for execution of the protocol should be relatively short.

The protocol might be found particularly useful with potential advancements of MEGA-edited MRS to reduce the voxel size. It is known that structure and function are not uniformly linked, and there are intersubject differences in functional areas i.e. the task-selective region in response to one condition in one subject is close to, but not exactly the same brain structure. A possible solution to this would be to use functional localizers within subjects based on fMRI data as guidance for the localization of the MRS voxel.

8.1. Future directions

8.1.1. Neuroscientific viewpoint

The saliency model proposed in this thesis was tested only on a behavioural level. It would be interesting to investigate the mechanism related to inhibition of high saliency distractors on the functional and neurochemical levels too. One advantage of this model is that the *a priori* knowledge about placement of the MRS voxel can be based on previous fMRI, TMS and neuropsychological findings. Therefore, both fMRI and MRS measurements can be conducted in a single one-hour session.

One more particularly interesting and novel point would be to investigate the change of GABA levels from rest to task-specific modulation in response to inhibition of high saliency distractors in young and older individuals. Taken together the neural mechanism related to inhibition of highly saliency irrelevant information is well described in young adults and the findings in Chapter 3 suggest it might be affected by ageing. Therefore, it would be interesting to investigate how the mechanism might be changed on a molecular level using GABA-edited MRS.

8.1.2. Methodological viewpoint

One important issue that was discussed in **Chapter 7** was with regard to accurate correction for partial tissue volume effects within the voxel of interest. Although most image analysis packages for neuroimaging data provide tools for tissue segmentation,

there is no available tool for registration of the MRS voxel with the anatomical structure and the procedure is not straightforward. In this thesis, I have developed a semi-automatic procedure using a number of tools from different software packages for the re-creation of the VOI and the calculation of the partial tissue volume of the VOI. The procedure could be developed further into a fully-automatic procedure in the environment of MATLAB for use by other researchers.

Accounting for partial tissue volume is another point that needs addressing. It is suggested that grey matter contains two times higher GABA concentration to white matter (Jensen, deB Frederick, & Renshaw, 2005a; Petroff, Ogino, & Alger, 1988; Petroff, Spencer, Alger, & Prichard, 1989). In addition, the relaxibility of GABA in different tissues might be different and might change with age differently. Change in T2-relaxation times might affect quantification of the MRS measurement, so that age-related reduction in metabolite signal intensity does not reflect a decline in concentration, but rather a change of T2-relaxation times. For example, a recent study by Kirov et al (Kirov et al., 2008) demonstrated an age-related shortening of the T2-relaxation times of NAA, Cr and Cho, which might partially account for observed age-related decrease of concentrations in these metabolites. These findings suggest that potential age-related changes in the GABA T2-relaxation times might account for some of the findings in the literature. Additional research in this area requires attention to rule out or adjust for possible age-related changes of GABA's T2-relxation times.

8.2. Conclusion

Combinations of fMRI and GABA-edited MRS provide non-invasive means of probing neurocognitive changes in ageing. The present approach allowed, for the first time, an examination of the role of GABA in age-related functional and behavioural differences. The data demonstrated that age-associated changes in cognitive control of visual selection from WM might be linked to neural scaffolding (compensation) processes as a result of reduced resting GABA levels. The findings extend our understanding that the age-related deficits in attentional selection are affected by changes in both inhibitory and capacity mechanisms. The cause of the change might be a common decrease of resting levels of GABA with age. The results however should be interpreted with caution until future studies combining negative BOLD response, GABA measures and more direct measures of neural activity (EEG/MEG) are conducted to further support interpretations of the findings.

Peer-reviewed Publications

- **Tsvetanov KA**, Mevorach C, Allen H, Humphreys GW. "*The Effects of Aging on Selection by Visual Saliency*". Attention, Perception, & Psychophysics (accepted)
- **Tsvetanov KA**, Arvanitis TN, Humphreys GW. "*Effects of stimulus identity and load in working memory on visual search: Eliminating the effect of load but not identity by lengthening encoding time*". QJEP 2012
- **Tsvetanov KA**, Rotshtein P, Arvanitis TN, Humphreys GW. *"Attentional Guidance from WM: Age Dissociates Effects of Overall Load and Attentional Guidance",* Journal of Experimental Psychology (submitted)
- **Tsvetanov KA**, Rotshtein P, Arvanitis TN, Humphreys GW. "*Reduced memory guidance of selection in ageing: Increased responses in anterior insula and reduced frontal-occipital coupling.".* Cerebral Cortex (submitted)
- **Tsvetanov KA**, Rotshtein P, Edden RA, Arvanitis TN, Wilson M, Humphreys GW. *"Resting GABA modulates the effects of ageing on memory-dependent BOLD signals in the right Anterior Insula / Inferior Frontal Gyrus"*. (in preparation)
- Mayhew SD, Li S, Storrar JK, **Tsvetanov KA**, Kourtzi Z. "*Learning shapes the representation of visual categories in the aging human brain*". J Cogn Neurosci., December 2010
- Hartaigh B, Jiang CQ, Thomas GN, **Tsvetanov KA**, Bosch JA, Cheng KK, Lam TH. "*Usefulness of physical fitness and the metabolic syndrome to predict vascular disease risk in older Chinese (from the Guangzhou Biobank Cohort Study-Cardiovascular Disease Subcoh*". Am J Cardiol., September 2011
- Chirimuuta M, **Tsvetanov KA**, Humphreys GW, *"Visual Effects of Working Memory Load and Timins During Visual Search*" (in preparation)

Peer-reviewed Presentations

- **Tsvetanov KA**, Rotshtein P, Arvanitis T, Humphreys GW, "*Dissociating load and memory in the fronto-parietal network: an aging study*". Poster presentation at the Organisation for Human Brain Mapping Annual Meeting, Beijing, 10-14 June 2012
- **Tsvetanov KA**, Rotshtein P, Arvanitis T, Humphreys GW, "*Effects of stimulus identity and load in working memory on visual search: Eliminating the effect of load but not identity by lengthening encoding time*". Poster accepted for presentation at the Vision Sciences Society, Naples, 11- 16 May 2012
- **Tsvetanov KA**, Mevorach C, Allen H, Humphreys GW, "*The Effects of Aging on Selection by Visual Saliency*". Talk presentation at the Experimental Psychology Society, London, 5-6 January 2012
- **Tsvetanov KA**, Chechlacz M, Humphreys GW, "*Regional Brain Alterations in the Healthy Senescensing Population using VBM and TBSS*". Poster presentation at the British Society for Research on Ageing Annual Scientific Meeting, Manchester, 2-3 July 2009

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