“The Hypothalamic - Pituitary - Adrenal Axis in Depression: a focus on the hippocampus”

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences

2013

Catherine Sarah Symonds

School of Medicine
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<th>Description</th>
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<tbody>
<tr>
<td>5-HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<tr>
<td>ADD</td>
<td>Antiglucocorticoid augmentation of anti-Depressants in Depression</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AVP</td>
<td>Arginine Vasopressin</td>
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<tr>
<td>AVLT</td>
<td>Auditory Verbal Learning Test</td>
</tr>
<tr>
<td>BA</td>
<td>Brodmann Area</td>
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<tr>
<td>BDNF</td>
<td>Brain Derived Neutrotrophic Factor</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood oxygen level dependent</td>
</tr>
<tr>
<td>CA</td>
<td>Cornu Ammonis</td>
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<tr>
<td>CAR</td>
<td>Cortisol Awakening Response</td>
</tr>
<tr>
<td>CRF</td>
<td>Corticotrophin Releasing Factor</td>
</tr>
<tr>
<td>df</td>
<td>Degrees of Freedom</td>
</tr>
<tr>
<td>DHEA</td>
<td>Dehydroepiandrosterone</td>
</tr>
<tr>
<td>dlPFC</td>
<td>Dorsolateral Prefrontal Cortex</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>FDG-PET</td>
<td>Fludeoxyglucose (18F) - Positron emission tomography</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>FWE</td>
<td>Family Wise Error</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>GR</td>
<td>Glucocorticoid Receptor</td>
</tr>
<tr>
<td>HAMD / HDRS</td>
<td>Hamilton Depression Rating Scale</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic Pituitary Adrenal</td>
</tr>
<tr>
<td>HV</td>
<td>Healthy Volunteer</td>
</tr>
<tr>
<td>IAPS</td>
<td>International Affective Picture System</td>
</tr>
<tr>
<td>IQ</td>
<td>Intelligent Quotient</td>
</tr>
<tr>
<td>K</td>
<td>Cluster size</td>
</tr>
<tr>
<td>MADRS</td>
<td>Montgomery-Åsberg Depression Rating Scale</td>
</tr>
<tr>
<td>MDD</td>
<td>Major Depressive Disorder</td>
</tr>
<tr>
<td>MGH-TRD</td>
<td>Massachusetts General Hospital Treatment Resistant Depression staging</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
</tr>
<tr>
<td>MR</td>
<td>Mineralocorticoid Receptor</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger Ribonucleic Acid</td>
</tr>
<tr>
<td>n</td>
<td>number</td>
</tr>
<tr>
<td>NART</td>
<td>National Adult Reading Test</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>phMRI</td>
<td>pharmacological challenge Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>PTSD</td>
<td>Post Traumatic Stress Disorder</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular nucleus</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for DSM-IV</td>
</tr>
<tr>
<td>SMD</td>
<td>Standardized Mean Difference</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>SNP</td>
<td>Single nucleotide polymorphisms</td>
</tr>
<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
</tr>
<tr>
<td>TE</td>
<td>Echo Time</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition Time</td>
</tr>
<tr>
<td>TRD</td>
<td>Treatment Resistant Depression</td>
</tr>
<tr>
<td>Unc</td>
<td>uncorrected</td>
</tr>
<tr>
<td>VBM</td>
<td>Voxel Based Morphology</td>
</tr>
<tr>
<td>vlPFC</td>
<td>Ventrolateral Prefrontal Cortex</td>
</tr>
<tr>
<td>WAIS</td>
<td>Wechsler Adult Intelligence Scale</td>
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Abstract

“The Hypothalamic - Pituitary - Adrenal Axis In Depression: a focus on the hippocampus”: a thesis submitted to the University of Manchester for the degree of PhD in the Faculty of Medical and Human Sciences by Catherine Sarah Symonds (27th September 2013)

**Background:** The hypothalamic-pituitary-adrenal (HPA) axis has been implicated in the aetiopathology of depression, and the incidence of HPA dysfunction tends to increase with the severity of treatment resistance. In healthy volunteers (HV), both acute and chronic hypercortisolaemia causes cognitive impairment, including emotional memory. The exact mechanism of this remains unclear; however the action of cortisol on corticosteroid receptors in the hippocampus appears to be crucial and this may also be important in the aetiopathology of depression. The aim of this thesis was to investigate acute and chronic states of the HPA axis, and its role on neurocognition in HV and treatment resistant depression (TRD).

**Methods:** The acute action of cortisol in HV was examined through meta-analysis of the literature. In HV, the acute, non-genomic effects of hydrocortisone on the hippocampus were measured using pharmacological challenge functional magnetic resonance imaging (phMRI) and the effects on the working memory n-back task during functional magnetic resonance imaging (fMRI). Additionally, the neurocognitive effects in TRD patients, who are theorised to have chronically elevated corticosteroids, were compared to age and sex matched HV using the n-back task and a novel emotional encoding-retrieval task. Finally the acute effects of hydrocortisone on the whole brain were measured in TRD compared to HV using phMRI.

**Results:** Meta-analysis results demonstrated an adverse effect on performance in retrieval tasks, but not encoding, after an acute rise in cortisol in HV, with a trend towards sparing of emotional memories. Using phMRI, hydrocortisone caused a time dependent increase in signal in the hippocampus, as well as an increased signal in the ventrolateral prefrontal cortex and a decreased signal in the hippocampus during the n-back task. Patients with TRD, when compared with HV, had a decreased signal in the dorsolateral prefrontal cortex during the n-back task. Additionally, during the emotional encoding-retrieval task, regardless of the emotional content, the patients showed a decrease in signal in the posterior cingulate during encoding and an increase in the posterior insula during retrieval. During retrieval of positive versus neutral images, there was an increase in signal in the anterior cingulate. The earlier phMRI findings were not reproduced in either the TRD or age and sex matched controls.

**Conclusions:** This work developed and examined a new technique to explore the relationship between the HPA axis and depression, as well as exploring the neurocognitive difference between TRD and HV. A non-genomic, acute effect of cortisol on the hippocampus was demonstrated in HV, as well as differences in processing emotional memories both acutely in HV and also in TRD patients. Further work needs to be done to develop the phMRI technique further and explore the aetiopathological role of the HPA axis in depression, focussing on the hippocampus.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Acknowledgements

I would like to acknowledge the support of my three supervisors, especially to my main supervisor, Prof Ian Anderson, for having faith in me and his patience in teasing out of me the original idea for this thesis in a logical and practical form. His attention to detail and experience was very much appreciated. I would also like to thank Prof Bill Deakin, whose enthusiasm and inspiration was very much valued throughout.

I would also like to thank Dr Shane McKie, who taught a psychiatrist a bit of physics and mathematics with incredible patience until the penny finally dropped. No one else in the department was able to provide the same level of knowledge either about fMRI analysis or Dr Who. I also must thank the radiographers Neal, Barry, Amy and Lindsay (who sadly died in 2011) for being fantastic technical and moral support in the 1.5T scanner.

Recognition is also due to Bev Haggis, Rebecca Elliott (for fantastic advice on psychology and neurocognition) and everyone else in the NPU for making me so welcome and helping to solve many, many problems along the way.

I would like to thank Prof John McLaughlin, my advisor, who has been providing words of wisdom and support before I even moved to Manchester and remains an inspiration.

Most of all, I would like to thank my wife, Caroline, who has been at my side every step of the way, including through the darkest times. Without her love and devotion, this thesis would never have been written, and the punctuation would have been absolutely dreadful. I would like to dedicate this thesis to her.
**Format of Presentation**

This thesis is presented as alternative format as chapters 3, 4, 5 and 6 are all in different stages of publication with chapter 4 published in a peer reviewed journal. The author of this thesis is first named author and was responsible for designing the studies, recruitment, data gathering, analysis and writing of the papers with suitable support from the supervisory team.

The structure of this thesis is as follows: Chapter 1 is a general introduction and review of the literature; Chapter 2 contains an overall exploration of the methodology with additional methods sections associated with the papers in Chapters 3, 4, 5 and 6 with a general discussion section in Chapter 7. References are given at the end of each chapter, as well as an overall Reference section at the end.

Chapters 3, 5 and 6 are being prepared for submission to journals and they will be submitted in the next 6 months. Chapter 3 will be submitted to ‘Psychological Medicine’ and Chapter 5 and 6 will be submitted to European Psychopharmacology. Chapter 5 has been accepted as a poster for the Society of Biological Psychiatry’s 69th Annual Meeting, to be held in New York City, USA, May 8-10, 2014. Chapter 4 has been published as Symonds, C.S., McKie, S., Elliot, R., Deakin, J.F.W., Anderson, I.M. (2012). Detection of the acute effects of hydrocortisone in the hippocampus using pharmacological fMRI. European Neuropsychopharmacology 22: 867-874.
**Contribution**

This is to confirm that this thesis is the author’s own work. I wrote all the chapters of this thesis and the original concept of studying the acute effects of cortisol in depression was my own.

In Chapter 3, I performed the literature search, extracted all the data and performed the meta-analysis. I was taught how to use Rev-Man and received guidance on which statistical method to choose from Dr C Henson and the Cochrane Collaboration.

In Chapter 4, I designed the initial experiment with guidance on endocrinology from Prof David Rey. Dr Shane McKie advised on the scanning schedule and guided me through the fMRI/ phMRI analysis. I trialled the administration of drugs, I obtained the ethics and R&D approval, wrote the protocol, was responsible for liaising with the Wellcome clinical research facility, recruited, screened and consented all the participants. I also supervised all the scans and all the neurocognitive tests. I also designed a pilot neurocognitive scanner with Dr Montaldi, teaching myself to use e-Prime.

Chapters 5 and 6 were a mechanistic sub-study of the ADD (antiglucocorticoid augmentation of antidepressants in depression study), a randomised control trial. I designed the sub-study as presented in this thesis. I adapted a neurocognitive task and re-wrote it for e-Prime (see Chapter 2). I designed the scanning protocol and analysed this with minimal supervision. I was assisted in recruitment and screening of participants by Ms Williams, Research Assistant and was involved in determining eligibility for all the patients. I supervised all but 2 scans.
Additional Research and Training activities carried out during PhD studies

During my PhD, I also carried about 1-2 clinical sessions a week in psychotherapy and in the tertiary specialist service for affective disorders as part of my psychiatry training. I also completed the MRCPsych examinations. I wrote up the PhD whilst working full time in the NHS.

Additional research duties were being involved in research governance and R&D applications for the ADD study; liaising with pharmacy, secondary care and GPs for patients in the main ADD study; recruiting, screening and consenting patients for the main study; supervising additional neurocognitive tasks for the ADD study; performing domiciliary visits to perform monitoring visits; physical examinations and venepuncture on the main study patients; liaising with the University of Newcastle and writing e-Prime versions of neurocognitive tasks for the Newcastle sub-study. I was also involved in training research assistants in the use of the SCID and other matters.

During my PhD I wrote three review articles:


About the Author

Dr Cathy Symonds graduated from Newcastle University medical school in 2004, after gaining invaluable experience in research in psychiatry as an undergraduate doing an intercalated BMedSci. After working as a house-officer, she completed a two year medical senior house-officer rotation in Newcastle-upon-Tyne and obtained the MRCP (UK). After moving to Manchester, she was appointed as an academic clinical fellowship in psychiatry and took time out of programme during her core training (SHO) to study for a PhD, funded by a NIHR Biomedical Research Centre award. Over this time, she also gained the MRCPsych. She is currently working full-time as an ST4 in Old Age Psychiatry.
Chapter 1. Introduction & Literature Review
1.1 Introduction

Depression results in a huge burden, both economically and in terms of morbidity (WHO 2008). Approximately 10-30% of patients fail to respond to at least 2 treatments and are therefore classified as having treatment resistant depression (TRD) and about 10% of patients fail to respond to multiple treatments and have a chronic course (Anderson et al 2008a; Fava 2003). There is an urgent need for an improved mechanistic understanding of the aetiopathology of depression on which to base new therapeutic strategies.

Current pharmacotherapy is almost exclusively based on monoaminergic mechanisms however there is evidence that the hypothalamic-pituitary-adrenal (HPA) axis may have a pathophysiological role in depression. A key candidate for the site of action of cortisol is the hippocampus, possibly through glutamatergic mechanisms. A better understanding of the acute and chronic effects of corticosteroids is required to lead to advances in treatment.

The acute and chronic actions of the HPA axis have been demonstrated to affect cognition in healthy individuals. The presence of HPA axis abnormalities in depression has been considered a causative link with the cognitive changes and biases found in major depressive disorder (Pariante and Lightman 2008). It has been suggested that the persistent over-activity of the HPA axis leading to both cognitive deficits and depression may represent an underlying problem with corticosteroid receptor sensitivity during the acute phase of stress responses (Keeney et al 2006) and cognitive deficits may also predict poor response to treatment (McLennan and Mathias 2010). In this introduction, the relevant background literature is reviewed in terms of depression; the role of HPA axis in depression; evidence for the impact of HPA axis dysfunction on cognition; and the evidence for cognitive impairment in depression.
1.2 Depression

Depression is the leading cause of disability as measured by ‘years lived with a disability’ (WHO 2001). The cost of treating depression is greater than that of treating hypertension and diabetes combined (Department_of_Health 1996). Approximately 60% of people diagnosed with depression respond to existing antidepressants, with about 10-30% of patients being diagnosed with treatment resistant depression (Fava 2003; Nierenberg et al 2010). Given this high economic and morbidity burden, there is a clear need for a greater understanding of the aetiopathology of depression on which to base improved treatments.

The ‘biopsychosocial’ model is the most widely accepted model of the origins of depression (Engel 1977). Biological and psychological factors interact to precipitate and perpetuate depression, in combination with social and environmental factors such as childhood abuse as well as psychological factors, such as the personality trait of neuroticism (Charney and Manji 2004; Heim et al 2010).

The monoamine theory of depression explains the origins of depressive symptoms in terms of a relative or functional decrease in the neurotransmission of serotonin (5HT) and noradrenaline (Joca et al 2007). This abnormality can be reversed with the administration of drugs such as most conventional antidepressants including selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants. However, a major challenge to the monoamine theory has been the time-lag between when a monoamine antidepressant becomes pharmacologically active and when it begins to have a measurable effect on behavioural symptoms. This challenge has led to the development of the receptor sensitivity theory, which postulates that rather than an absolute deficit of monoamines, individuals with depression have reduced pre-synaptic sensitivity of 5HT autoreceptors leading to disrupted feedback control (Nutt 2002). Support for this theory comes from studies demonstrating de-sensitisation of 5-HT_{1A} autoreceptors using microdialysis in rats given fluoxetine (Hervas et al 2001) and reduced 5-HT_{1A} receptor binding in depressed human volunteers during positron emission tomography imaging (PET) (Drevets et al 1999).
However, there is a significant rate of non-response to conventional antidepressants and observational studies have noted the involvement of both the hypothalamic-pituitary-adrenal (HPA) axis and the glutamate systems in depression (Machado-Vieira et al 2009; Porter et al 2004). Drugs targeting the HPA axis have been identified as possible candidates either as antidepressants or agents to augment conventional antidepressant action. These have included metyrapone, mifepristone (RU-486), ketoconazole and dehydroepiandrosterone (DHEA) (Gallagher et al 2008).

The concept of classifying patients as having ‘treatment resistant depression’ remains a controversial one (Berlim and Turecki 2007). There has been a lack of consistency in the diagnostic criteria for TRD with some studies taking ‘resistance’ to mean anything from ‘a failure of complete resolution of symptoms’ to ‘a failure to reduce symptoms by 50% on the Hamilton Depression score’ to ‘remaining within the diagnosable domain of DSM-IV major depressive disorder (MDD)’ (Malhi et al 2005). There is also controversy regarding the definition of treatment adequacy, both in terms of adequacy and duration, and whether this definition should extend to non-pharmacological treatments (Fornaro and Giosuè 2010).

Beyond these nosological considerations, the question remains whether patients with TRD have a phenotypical difference compared to other patients with MDD. To date, there are no studies that compare directly the profile of cognitive deficits in TRD patients to other patients with MDD, however studies have shown that this group tend to show more severe cognitive deficits, especially verbal working memory (Gupta et al 2013), with treatment non-response predicted by executive dysfunction in older adults with MDD (Lenze et al 2012). However, whether this represents a specific characteristic of TRD as a separate sub-type of depression or merely reflective of the impact of being chronically unwell is unclear. It appears that TRD is characterised by more abnormalities of the HPA axis including reduced sensitivity of the glucocorticoid receptor (Bauer et al 2002; Bauer et al 2003) however no difference was found in the prednisolone suppression tests compared to controls was found despite the TRD group having higher baseline cortisol levels (Juruena et al 2009). One proposed mechanism for a poorer clinical course and impairment of
neurocognition is via reduced hippocampal volumes (see Section 1.5). Further exploration of the role phenotype of TRD in terms of cognition and neurochemistry may allow the development of new therapies.

1.3 The HPA axis and Depression

One of the most consistent findings in biological psychiatry has been the dysregulation of the HPA axis in depressed individuals (Porter et al 2004). A link between non-suppression of cortisol in the dexamethasone suppression test and relapse of depression has also been established, implying an underlying pathological link (Charles et al 1989). The HPA axis is regulated via a system of negative and positive feedback mechanisms. Through understanding the higher feedback mechanisms of the HPA axis, we can gain a further insight into the HPA axis’s role in regulating mood, cognition and this may provide a therapeutic opportunity.

1.3.1 The HPA Axis

The regulation of the HPA axis is illustrated in Figure 1-1 (de Kloet et al 2007). Corticotrophin Releasing Factor (CRF) and vasopressin (AVP) are released from the paraventricular nucleus (PVN) of the hypothalamus, causing adrenocorticotropic hormone (ACTH) to be released by the pituitary (Jacobson and Sapolsky 1991). In turn, cortisol is released in humans from the adrenal cortex. Increased levels of circulating cortisol are detected by glucocorticoid receptors in the pituitary, hypothalamus, hippocampus, amygdala and prefrontal cortex. These areas can trigger a negative feedback mechanism. In rats, a time-dependent BOLD signal in the hippocampus has corresponded with this rise in corticosteroids using pharmacological challenge functional magnetic resonance imaging (phMRI) (Ferris and Stolberg 2010). Corticosteroids regulate a variety of physiological mechanisms, including blood pressure, the regulation of fat, protein and carbohydrate metabolism; electrolyte balance; body water distribution; and immunosuppressant anti-inflammatory action (Sapolsky 2000). They are also involved in the regulation of neuronal apoptosis via Brain Derived Neutrotrophic Factor (BDNF) (Joca et al 2007; Smith et al 1995).
There are two types of corticosteroid receptor: mineralocorticoid receptor (MR or type I) and glucocorticoid receptors (GR or type II) (Lupien et al 2007). GRs have a low affinity for cortisol, whereas MRs have a higher affinity and are usually saturated at physiological levels. The hippocampus and limbic system have relatively high concentration of both GRs and MRs, whereas GRs are found more extensively in other areas including the dorsolateral prefrontal cortex, hypothalamus and pituitary (Kellner and Wiedemann 2008; Webster et al 2002). Neurones in area CA1 (Cornu Ammonis) in the hippocampus and dentate gyrus granule neurones express both GRs and MRs. Previously, it had been accepted that because of the near saturation of MRs at physiological levels that these receptors had little or no role in the feedback of the HPA axis but there is now evidence that this is not the case with the presence of ‘lower-affinity’ MRs in the limbic system (Karst et al 2005) and it has been argued from microdialysis work in rats that MRs are integral to rapid feedback following the bolus administration of a corticosteroid (Atkinson et al 2008; Pariante and
Lightman 2008), however, it could also be argued that their contribution to the feedback of the HPA axis is not significant overall.

Both MRs and GRs are classed as nuclear receptors effecting their action via nuclear DNA, although both are also present on the cell membrane (Joëls et al 2009). Cortisol is secreted in a pulsatile, diurnal rhythm (see Figure 1-2) under feedback regulated via GRs and MRs with additional dynamic changes in cortisol in response to demand (Weitzman et al 1971). The feedback occurs after binding of the corticosteroids with the intracellular receptor via alterations in nucleic DNA and subsequent protein transcription. This genomic action occurs four hours after the rise in cortisol (Joëls et al 2011). However, a behavioural change in neurocognitive function in response to the double-blinded administration of corticosteroids can be observed prior to this genomic effect occurring, strengthening the argument for a non-genomic action at the MRs and GRs (Het et al 2005).

Figure 1-2: The diurnal variation of cortisol value over a 24-hour period in man (Weitzman et al 1971)

Over the last ten years, it has been understood that membrane-bound MRs can produce rapid non-genomic responses to elevated stress hormones. Through the use of genetic knockout mice with forebrain-specific MR gene inactivation, in vitro work demonstrate a rise in intracellular recorded synaptic connection strength and glutamate secondary to corticosterone in hippocampal cells of control animals (Karst et al 2005). Another mechanism for the acute action of corticosterone on MRs is via the extracellular signal-
regulated kinase (ERK) 1/2 pathway, which controls synaptic plasticity and, when activated, also increases the chance of glutamate containing vesicles being released, both pre- and post-synaptically. This was demonstrated with the use of genetic knockout mice in vitro (Olijslagers et al 2008). As previously discussed, when corticosterone binds with mineralocorticoid receptors, it increases the probability of glutamate being released. It is also known that increased levels of GR activity can lead to an increase in NMDA Receptor expression in the hippocampus via increased genomic transcription (McEwen 2000). In rats, it has been demonstrated that an increase in corticosterone causes an increase of mRNA for kainate receptors (Hunter et al 2009). However, the applicability of this work to in vivo, human subjects has yet to be demonstrated.

As cortisol levels are both diurnal and reactive, in vivo testing in humans must take this into account by using a number of different methods to measure the HPA axis. The physiological stress effect of needles can induce an artefactual variation between subjects, leading to some researchers preferring to measure saliva and 24 hour urine collections. The latter method is less acceptable to subjects and only gives an aggregate measure, whereas the saliva can have methodological difficulties with reliability (Gallagher et al 2006). It has also been argued that cortisol measurements alone are not enough given the functional antagonism of cortisol by another ACTH controlled hormone, DHEA (Wolf et al 1997). However, random testing of the HPA axis, does not demonstrate its potential difference in reactivity between different patient groups and normal volunteers. Serial salivary measurements of cortisol on waking, or the cortisol awakening response (CAR) is one way of demonstrating this. A blunted response is seen in depressed individuals compared to controls (Pruessner et al 2003), however, this test is sensitive to extremes of early morning wakening and to other timing issues. The dexamethasone suppression test, the combined dexamethasone/CRF test and, more recently, the prednisolone suppression test, are dynamic measures of the HPA axis used in depressed subjects. Only GRs have a high affinity for dexamethasone, whereas both MRs and GRs have a high affinity for prednisolone (Juruena et al 2006). Therefore when dexamethasone suppression tests are used as dynamic tests of the HPA axis this only
measures the integrity of GRs not MRs. The dexamethasone test combined with CRF administration typically shows a blunted response of endogenous ACTH in depressed patients, although it fails to differentiate between remitted depressed and those with MDD (Van Den Eede et al. 2006). The prednisolone suppression test acts on both GRs and MRs (Pariante and Lightman 2008). Typically, patients with depression demonstrate a failure of negative feedback after a challenge with either prednisolone or dexamethasone (see section 1.3.2). These tests are relatively invasive and only give a measure of peripheral blood levels of cortisol/DHEA and do not indicate what is happening centrally, in the limbic system.

1.3.2 The Role of the HPA axis in depression

Over the past 30 years, abnormalities in the HPA axis have been one of the most consistent findings in biological psychiatry (Porter et al. 2004). Raised circulating cortisol levels and abnormalities of the HPA axis on dynamic testing have been demonstrated in several studies, a selection of which are summarised in Table 1-1. These have included both peripheral cortisol, ACTH and CRF in cerebrospinal fluid. The picture is not uniform and many areas remain controversial due difficulties in methodology, lack of specificity to depression and in interpreting results.

Loss of the normal diurnal variation has been an observed feature and relatively high levels of circulating cortisol have been reported repeatedly, as well as failure to suppress cortisol levels in the dexamethasone suppression test (Vreeburg et al. 2009). This was also found in bipolar depressed, treatment resistant depressed and psychotic depressed patients and also in many other psychiatric conditions, including dementia making a failure to suppress cortisol in the dexamethasone test not specific for depression (Hayes and Ettigi 1983). In addition, raised CRF has also been observed in depressed subject’s cerebrospinal fluid together with a blunted response to ACTH (Carpenter et al. 2003), which may imply an underlying problem with feedback control of the HPA axis in depressed patients, supporting the altered corticosteroid receptor hypothesis. Despite raised cortisol levels, Cushing-oid features are not routinely seen in depressed patients. The reason for this is
theorised to be reduced sensitivity and down-regulation of corticosteroid receptors (Rupprecht et al, 1991).

In contrast to the hypercortisolaemic picture described above, several studies have demonstrated lower cortisol levels in depressed subjects as compared to healthy controls and a blunted rise in morning cortisol levels. This apparent contradiction in results has raised several questions, including how seemingly opposite states can lead to the same clinical picture. It is known from animal studies that there is an inverted U shaped response to stress, with high and low levels leading to poor performance on cognitive tasks (Kovacs et al, 1976; Lupien and McEwen, 1997). It is possible that this also applies to mood: depression is seen both in Addison’s disease and Cushing’s Syndrome (Lishman, 1987). The exact mechanism for why this is the case is at present unclear, but may relate to the feedback mechanisms of the HPA axis at a higher level.
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Subjects</th>
<th>Method</th>
<th>Results</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strickland et al (2002)</strong> (Strickland et al 2002)</td>
<td>Unipolar depressed = 94 ‘vulnerable to depression’ = 166 HV=174</td>
<td>Morning and evening salivary cortisol</td>
<td>Morning cortisol reduced in depressed group</td>
<td>Vulnerability defined as an excess of life events and risk factors for depression, in the absence of depression itself</td>
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<tr>
<td><strong>Bhagwagar et al (2003)</strong> (Bhagwagar et al 2003)</td>
<td>Medication free, euthymic depressed = 31</td>
<td>Salivary cortisol on waking at 15 min intervals for next hour</td>
<td>Higher increase on waking in depressed group</td>
<td></td>
</tr>
<tr>
<td><strong>Young et al (2002)</strong> (Young et al 2002)</td>
<td>Unipolar drug free depressed = 39 HV= 41</td>
<td>Salivary DHEA and cortisol levels at 8am and 8pm</td>
<td>Raised cortisol: DHEA ratio in depressed, evening cortisol: DHEA ratio correlated with length of depressed episode</td>
<td></td>
</tr>
<tr>
<td><strong>Huber et al (2006)</strong> (Huber et al 2006)</td>
<td>72 psychotherapy inpatients (unipolar depressed = 57, anxiety = 21 OCD=9)</td>
<td>Salivary cortisol levels at 0, 15 and 30 mins post waking</td>
<td>Awakening cortisol reactivity blunted in depressed patients</td>
<td></td>
</tr>
<tr>
<td><strong>Vreeburg et al (2009)</strong> (Vreeburg et al 2009)</td>
<td>Remitted depressed = 579 Depressed = 701 HV = 308</td>
<td>Salivary cortisol in evening and 7 points post waking Dexamethasone suppression test</td>
<td>Higher waking cortisol in both remitted and depressed groups Higher evening cortisol in current depressed Both the MDD groups showed a failure to suppress cortisol</td>
<td>Largest study to date of unipolar depressed patients. No difference in dexamethasone suppression testing between remitted and currently depressed.</td>
</tr>
<tr>
<td>Author (year)</td>
<td>Subjects</td>
<td>Method</td>
<td>Results</td>
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<tr>
<td>Juruena et al (2006)</td>
<td>Treatment resistant depressed = 18 (15 with history of childhood sexual abuse) HV = 14</td>
<td>Baseline salivary cortisol, &amp; dexamethasone and prednisolone suppression tests.</td>
<td>Raised salivary cortisol Impaired cortisol suppression in the dexamethasone suppression test Normal prednisolone suppression</td>
<td>Implies an impairment of GRs not MRs in depression</td>
</tr>
<tr>
<td>Hennings et al (2009)</td>
<td>Unipolar recurrent depressed=457 Unipolar MDD = 276 Bipolar depressed = 109</td>
<td>Combined dex/CRH test</td>
<td>Remission was predicted by an early normalisation of previously dysregulated HPA Axis</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: A selection of studies illustrating the role of the HPA axis in depression, HV = healthy volunteer

Corticosteroid receptor polymorphisms have been explored as a possible explanation for both HPA abnormalities. Increased HPA axis activity has been found in non-affected family members of patients with affective disorders (Ising et al 2005) as well as GR and MR single nucleotide polymorphisms (SNP)s (Hashimoto et al 2006). However, this finding has not been replicated elsewhere and other studies have failed to find a link (DeRijk and de Kloet 2008). The mechanism and nature of HPA dysfunction in depression remains an area of controversy, but is most likely to be multifactorial and a combination of chronic desensitisation of GRs and MRs and maladaptive acute response.

Early life adversity has been demonstrated to be a non-specific risk factor for an increase in vulnerability for several psychiatric conditions, including depression (Heim et al 2010). In laboratory rats, early life trauma has been demonstrated to result in both HPA axis over-activity and behavioural problems. Women with a history of childhood abuse with and without depression showed a blunted response to a CRF challenge (Heim et al 2001). However, in a study comparing cerebrospinal fluid concentrations of CRF between depressed patients and healthy volunteers, depression was not a significant predictive factor, whereas a history of childhood abuse (in both the patients and controls) was
predictive (Carpenter et al 2003). This suggests that although abuse may contribute to a lifelong vulnerability to mood disorders, it is not specifically linked to HPA axis dysfunction and depression.

1.4 Evidence for the impact of HPA axis dysfunction impact on cognition

As discussed in section 1.2, above, cognitive impairment and bias are key features of depression, with concentration problems being part of the diagnostic criteria (American Psychiatric Association 1994), and cognitive biases forming a key basis of the theory of cognitive behavioural therapy (Wright and Beck 1983). Cognition is affected by corticosteroids and the effects of corticosteroids have been used as models of depression. The evidence for the impact of the HPA axis on cognition in healthy volunteers as well as non-depressed patient groups is presented in this section.

1.4.1 Healthy volunteers and cortisol

Both the acute and chronic elevation of cortisol can induce cognitive deficits in healthy volunteers in several different domains: declarative memory, executive function, and visuospatial memory. Declarative memory is subdivided into semantic memory (memory for facts) and episodic memory (memory for episodes experienced by an individual, also called autobiographical memory). The hippocampus is a key structure in declarative memory function, especially in encoding new material; the most compelling evidence for the importance of the hippocampus coming from hippocampectomised patients, such as H.M. (Schmolck et al 2002). Executive function encompasses working memory and controls and regulates cognitive processes (Baddeley 1996). A brief overview of the evidence of the impact of the HPA axis will be presented here.

1.4.2 Healthy volunteers, cortisol and declarative memory

The acute effect of corticosteroids has been shown to affect memory however the evidence to date is unclear, with studies reporting both improved and impaired
declarative memory. The timing of the rise in cortisol (prior to encoding or prior to retrieval); emotional aspects of the neurocognitive task and the method by which the cortisol rise was induced (exogenous drug administration or endogenous, stress-induced) also appears to affect participants ability to recall words. In Chapter 3, I present a systematic review with meta-analysis aimed to examine the acute effects of corticosteroids on declarative memory in 25 studies. This demonstrated that raised cortisol prior to retrieval, but not encoding, adversely affected performance in memory tasks. The effect was most consistently seen with exogenous cortisol and with the retrieval of neutral words. However, there were too few studies to draw firm conclusions regarding the effect of exogenous versus endogenous cortisol and the role of emotional material. The timing of the acute rise in cortisol related to encoding is important with a significant result only being found when there was a rise of cortisol post-encoding and pre-retrieval.

One recent study examined the effect of cortisol on the hippocampus during an emotional memory encoding-retrieval task (van Stegeren et al 2010). They found an enhanced encoding response and increased signal in the amygdala and hippocampus an hour post an infusion of hydrocortisone. They also examined the effects of noradrenaline by giving yohimbine, which blocks the effects of noradrenaline. This resulted in a decreased signal in the prefrontal cortex. They found no additive effect of these two stress hormones on hippocampal signal and no effect of the yohimbine on recognition memory. Although this was only a small study (n=12 in each group), it does imply that cortisol is a most important stress hormone in terms of laying down memories. The chronic effects of exogenous cortisol on declarative memory are more uniform and better studied with an adverse impact on both encoding and retrieving described (Roozendaal et al 2006; Sapolsky 2000). The reason behind this disparity between genomic and non-genomic steroid actions on declarative memory is not clear.
1.4.3 Healthy volunteers, cortisol and working memory

Non-declarative memory has been much less studied and conflicting effects of elevated levels of cortisol have been found in working memory, with visuospatial memory being studied the least. The acute administration of cortisol alters performance on working memory tasks, including the n-back task, with most effect being seen at higher levels of demand upon the working memory. It has been shown to both reduce (Al’Absi et al 2002; Lupien et al 2007; Schoofs et al 2008) and improve performance (Oei et al 2009).

1.4.4 The effect of exogenous steroids on cognition in non-depressed populations

Evidence in non-depressed populations for the role of corticosteroids in hippocampal apoptosis is less compelling. A study examining the effects of acute and chronic prednisolone in rheumatoid arthritis (Coluccia et al 2008) failed to demonstrate a change in hippocampal volume, although corticosteroids impaired hippocampal function as demonstrated by ability on a recall task.

1.5 Evidence for the role of depression on cognition

Patients with depression, in addition to low mood and vegetative symptoms, often display neurocognitive problems, with deficits in attention and concentration being amongst the core features and emotional memory biases being at the heart of the cognitive model of depression (American Psychiatric Association 1994; Wells et al 2012). Emotion-independent components in patients include attention-dependent working memory, encoding and recall (Coluccia et al 2008; de Quervain et al 2000) although these deficits are not consistent (Alhaj et al 2007). These deficits may persist beyond the remission of other symptoms, leading some researchers to liken this to a ‘scar’ left by the pathological process of depression (Hasler et al 2004). It is theorised that this could be driven by the volume reduction of key structures including the hippocampus and the prefrontal cortex (Koolschijn et al 2009) is caused by raised levels of corticosteroids causing apoptosis via BDNF. Dunham et al (Dunham et al 2009) demonstrated in a post mortem study
abnormalities in pro-BDNF in a patients with depression, suggesting a BDNF mediated apoptosis as an underlying mechanism.

In depression, memory for emotionally valenced material (or ‘emotional memory’) can be affected contributing to cognitive bias towards negative memories and material (Wells et al 2012). When neurocognitive tests which use emotionally valenced material have been given to depressed individuals, most aspects of memory have demonstrated selective deficits including encoding and retrieval, attentional-biases and working memory (Roiser and Sahakian 2013). In an emotional version of the go/no-go test of executive function, depressed individuals showed a decreased response time to negative stimuli (Murphy et al 1999). Patients with depression show a bias towards recalling negative autobiographical memories in greater detail, being over-general about positive events (Brittlebank et al 1993), as well as recalling fewer positive and more negative words (Liu et al 2012).

Additionally, there seems to be a bias against recognising positive material such as happy facial expressions in depression (Salvadore et al 2009), as well as a significant response in the frontal pole to positive pictures when compared to neutral images being shown to remitted depressed patients (Elliott et al 2012).

In addition to behavioural experiments, imaging studies have demonstrated cognitive deficits in patients with depression. FDG-PET imaging reveals abnormalities in patients with MDD in the resting state in regions linked to attention in healthy individuals: the dorsolateral prefrontal cortex (more frequently on the right), and anterior cingulate (Liotti and Mayberg 2001). Hippocampal volumes have been found to be reduced in depressed subjects which may be a state dependent phenomenon (Arnone et al 2012b). A meta-analysis with 2418 major depressed patients and 1974 controls demonstrated moderate volume reduction in the hippocampus, caudate and putamen, with larger volume reduction in the prefrontal cortex (Koolschijn et al 2009). A further meta-analysis with 4118 unipolar depressed patients and 3545 healthy controls echoed this with a decreased volumes demonstrated in the frontal cortex (including orbitofrontal cortex), hippocampus, striatum and cingulate, found in patients with unipolar depression (Arnone et al 2012a). Reduced hippocampal volume was associated with the duration of illness and with the
failure to respond to antidepressant treatment. In addition to these areas associated with emotional memory, patients also demonstrated an increased pituitary volume. This reduction in hippocampal volumes is associated with a failure to suppress cortisol in the dexamethasone suppression test in unipolar depression (Knoops et al 2010).

The severity of the cognitive impairment tends to correlate with the severity of the depression and psychotic symptoms are predictive of memory problems (Bulbena and Berrios 1993), but cognitive deficits independently predict social functioning (Roiser and Sahakian 2013). In addition, poor cognitive function predicts poor treatment response, at least in the later life population (McLennan and Mathias 2010; Potter et al 2004). It can therefore be surmised that in TRD, through studying cognition and elements that adversely affect it, we can learn more about possible future treatments.

1.6 Context of study

1.6.1 HPA Axis modulators as antidepressants

Although HPA axis abnormalities have been noted for the last 30 years in depression, this has not been widely exploited as a target for anti-depressant therapies. To date nine small trials have been carried out using agents including mifepristone (RU 486), metyrapone, ketonazole and DHEA. A recent Cochrane review of anti-glucocorticoid treatments for mood disorders (Gallagher et al 2008) concluded that although some small studies had produced promising results, especially with non-psychotic depressed patients, overall the review concluded that the agents trialled had no advantage over placebo based on currently available evidence. However, the evidence for metyrapone, appeared the strongest with a small study by Jahn et al (Jahn et al 2004) in TRD showing promise.

1.6.2 The ADD study

A Cochrane review suggests that antiglucocorticoid augmentation of antidepressants in TRD showed promise but overall evidence was inconclusive but they cited a promising pilot study of the cortisol synthesis inhibitor, metyrapone (Gallagher et al 2008). The
Antiglucocorticoid augmentation of anti-Depressants in Depression (The ADD Study) is a recently completed multicentre randomised placebo controlled trial of 500mg metyrapone twice daily for three weeks as an augmentation of serotonergic antidepressants in a large population of patients with TRD in the UK National Health Service (McAllister-Williams et al 2013), (theaddstudy.co.uk).

The overall aim of the ADD study was to evaluate efficacy as defined as an improvement in the Montgomery-Åsberg Depression Rating Scale (Carmody et al 2006) 2 weeks after medication had been discontinued. Secondary outcome measures included improved quality of life, an antidepressant effect persisting to 6 months, and tolerability measures. In addition, there were several sub-studies of the ADD study with the aim of further understanding about the underlying mechanisms of corticosteroids in TRD. Chapters 5 and 6 of this thesis form part of one sub-study of the ADD study. The author of this thesis designed and performed the research within the context of the wider study.

1.7 Rationale, Aims and Hypotheses of Thesis

1.7.1 Rationale of thesis

As outlined in the introduction and literature review (see Section 1.3.2), the HPA axis appears to play a crucial role in the aetiopathology of depression. Given that likelihood of incidence of abnormalities of the HPA axis varies with severity, this thesis will focus on patients with treatment resistant depression (TRD). Little work has been done regarding the acute action of the HPA axis on the hippocampus and the chronic role of the HPA axis on cognitive function, especially emotional memory, in this group of patients.

1.7.2 Aims and Hypotheses

1.7.2.1 Aims

I aimed to investigate the role of the HPA axis in depression, focussing on the cognitive and endocrine roles of the hippocampus. I aimed to examine the relative receptor sensitivities in relation to the HPA axis in acute versus chronic states to see whether there
was a difference in acute regulation as well as more chronic effects on cognition in treatment resistant depression.

Initially, I aimed to examine the role of the acute actions of cortisol on the hippocampus in healthy volunteers through meta-analysis of the existing literature and through functional magnetic resonance imaging (fMRI) experimentation. I also aimed to examine whether there was an acute effect that was measurable with pharmacological challenge fMRI (phMRI) of hydrocortisone the hippocampus in healthy volunteers in order to demonstrate the validity of this technique and the effects on working memory. Furthermore, I aimed to compare the presumed chronic effects of an abnormal HPA axis in TRD patients against normal volunteers, and compare the acute action of hydrocortisone on the hippocampus using phMRI to examine whether there is direct evidence for differences in MR and GR sensitivity between TRD and healthy volunteers.

1.7.2.2 Hypotheses

My hypotheses were:

1. That an acute rise in cortisol will have an adverse effect on cognition and cognitive processes, as measured by neurocognitive tests and fMRI. I hypothesized that this would represent a non-genomic action of cortisol.

2. That an acute rise in hydrocortisone will cause an increase in time-dependent BOLD signal in the hippocampus, and that this increase will be greater in healthy volunteers than for TRD patients, reflecting the hypothesised reduced sensitivity of corticosteroid receptors in the patient group.

3. That the chronic effects of HPA dysfunction in TRD patients will result in hippocampal under activity during fMRI cognitive emotional encoding-retrieval and working memory tasks. Additionally I hypothesize that the anterior cingulate, insula, and dorsolateral prefrontal cortex will show relative under-activity compared to healthy volunteers, in keeping with previous literature.
1.8 References


2 Chapter 2.

Methods
2.1 Introduction

As this is a thesis by alternative format, most of the methods used are contained within the papers in Chapter 3-6. However, additional detail on some the techniques are presented in this chapter. Methods are presented in the order they appear in subsequent chapters, and the chapter or chapters they relate to is indicated.

2.2 Meta-analysis and systematic review

Systematic review and meta-analysis are standard techniques and for the study outlined in Chapter 3, the principles of the Cochrane Collaboration were followed through-out (Higgins and Green 2011).

2.2.1 General principles of systematic review and meta-analysis

Systematic reviews aim to collate all the available evidence on an *a priori*, explicitly defined area, allowing reproducibility and transparency. The criteria for the inclusion and exclusion of studies should be clear and logical. Furthermore, the search criteria should maximize the chances of including all eligible data and be free of bias (Antman *et al* 1992).

Systematic reviews are frequently combined with meta-analyses, which use statistical methods to combine the results from several different studies and provide a more precise estimate of the effect size (Glass 1976). The studies must first be appraised for comparability in terms of study design and intervention. A summary statistic is then calculated and effect size of the intervention estimated.

The studies were analysed according to generic inverse variance. The DerSimonian and Laird method (DerSimonian and Kacker 2007) of generic inverse variance was used with random effects. A random effects model assumes that the pattern of distribution of the effects being estimated from the studies is a pattern that occurs as if by random. This method weights the effect more towards larger studies which produces a more precise estimate because the weighting is calculated as the inverse of the standard error,
incorporating the assumptions of random effects. It allows the combination of crossover with parallel designed studies. Further details on methods used are detailed in Chapter 3.

2.2.2 Protocol development and search strategy

The question to be addressed in this review was whether there was an effect of corticosteroid hormones on memory and to examine whether exogenous and endogenous rises caused an equivalent effect. Given the diversity of the literature in terms of subject, the method of inducing a rise in cortisol, the timing of this rise and the neurocognitive method used, this question was refined further and limited just the effects seen in healthy volunteers and the acute effects of cortisol on encoding and retrieval. I defined ‘acute’ as a stress induced or drug induced corticosteroid rise within 240 minutes of either the encoding or the retrieval task. This time frame was chosen to examine non-genomic effects of corticosteroids as beyond this time window genomic effects are likely to predominate (Losel et al 2003).

In order to develop a protocol that was transparent, reproducible, and answered the questions posed by the review in keeping with the Cochrane Collaboration methodology (Higgins et al 2011), I drew up an explicit search strategy designed to minimise bias and maximize the search yield. The Cochrane Collaboration primarily was designed to compare interventions assessed by randomised controlled trials in terms of predefined outcomes. I had to first consider whether this was applicable to my subject area. It was clear that the action that resulted in an acute rise in cortisol was an intervention and we were comparing two distinct types of intervention: acute drug induced and stress induced rises. As I wished to examine the acute effects of corticosteroids, I decided to exclude all trials that did not clearly demonstrate that this intervention had resulted in the effect I was interested in, namely a rise in cortisol. The most common outcome measure used was word list learning and as such this was chosen as our primary outcome. This resulted in a comparable outcome measure in terms of number of words recalled correctly. Meta-analysis is only a valid technique if the outcome measures and interventions are comparable across the studies, otherwise valid conclusions cannot be drawn. Given that
different neurocognitive tasks (e.g. memory for pictures, visuospatial memory, executive function) require different underlying neurocognitive processes (Migo et al 2012), these are not directly comparable.

Our final protocol (as outlined in Chapter 3) was to include all randomised controlled studies of encoding and retrieval memory in which stress was accompanied by a rise in cortisol or exogenously corticosteroid were administered. In addition, it was specified that there was an appropriate control (control task or placebo). We searched English language papers in humans in PubMed, Embase, Web of Knowledge, and Psychinfo, published before April 2013 using keywords “(cognitive or memory or cognition or neurocognition or neurocognitive or neuropsychological or executive) and (cortisol or corticosteroid or glucocorticoid or HPA)” as well as manually searching papers and reviews for further references.

Finally I restricted studies to those with healthy volunteer (HV) participants as the effects of stress cannot be assumed to be uniform across physical or psychiatric disorders. I wished to examine the evidence for the impact of an acute effect of a rise in cortisol on healthy volunteers’ ability to recall and recognise words to establish the baseline functioning prior to further experimental work with depressed patients given the conflicting results found in the literature (see Chapter 1, section 1.4) and my plans to explore this subject further experimentally.

2.3 Recruitment and Screening

2.3.1 Recruitment to study reported in Chapter 4

Healthy volunteers were recruited from advertisements placed in the University of Manchester. These ethics committee approved advertisements took the form of posters, advertisements on a volunteering page of the university’s website and emails. Over 500 replies were generated.
2.3.2 Inclusion and exclusion criteria for study reported in Chapter 4

A total of 14 people were recruited from staff and students of Manchester University after screening 18 people face to face. All subjects met the following inclusion criteria:

- Aged 18-45
- Right handed using the Briggs and Annett scale
- Free of substance abuse or psychiatric history as defined used the mini-SCID (miniature Structured Clinical Interview for DSM-IV) and SCID (First et al 2002a)
- All first degree relatives free of significant psychiatric illness
- Free of major physical illness (e.g. previous head injury, Neurological illness/abnormalities, endocrine abnormality, use of oral steroid medication, immunosuppression etc)
- Free of current infection
- Free of pregnancy or risk of pregnancy for females
- All females to be on long term hormonal contraception (e.g. oral contraceptive pill)
- Free of the presence of metal clips, orbital fragments or other exclusion to MRI scanning.
- IQ in normal range as measured by Ammon’s Quick Test (Ammons and Ammons 1962)

The initial screening included a medical and psychiatric history with a full physical examination performed by the investigator. The SCID, handedness questionnaires and IQ are discussed below (see section 2.3.5.1-2.3.5.3, below). All participants gave informed consent.

2.3.3 Recruitment to study reported in Chapter 5 and 6

Chapters 5 and 6 formed part of a sub-study of the ADD study. The author of this thesis designed and performed the research within the context of the wider study, the context of which was described in section 1.6.2, Chapter 1. Recruitment of the participants was defined as part of the wider study protocol (McAllister-Williams et al 2013), (theaddstudy.co.uk), and is further described in sections 2.3.3.1-2.3.4.
2.3.3.1 Recruitment of Healthy Volunteers

The recruitment strategy for healthy volunteers was similar for the study described in chapter 4. Healthy volunteers were recruited from advertisements placed in the University of Manchester. These ethics committee approved advertisements took the form of posters, advertisements on a volunteering page of the university’s website and emails. Over 200 replies were generated and 32 people were screened face to face resulting in 30 eligible people being recruited into the study.

2.3.3.2 Recruitment strategy for depressed patients

For patients in secondary care, the responsible clinician (RC) was approached and asked to consider referring potentially suitable patients who may benefit from the ADD study. If the RC identified such a patient, he or she then sought the consent of the patient to be contacted by the research team. Secondary care consultants in Manchester Mental Health NHS Trust, Greater Manchester West Mental Health NHS Foundation Trust and Pennine Care Mental Health NHS Foundation Trust were contacted via email and through a series of presentations. Information was also circulated to general practitioners (GPs) in Greater Manchester.

In addition, posters were displayed in patient waiting areas and the study website was available to be viewed by the public allowing patients to self-refer to the trial. If a patient self referred, consent was then sought from the RC. The RC and GPs were kept informed whether the patient had been included in the trial, the results of any screening tests, their progress and if there were any adverse incidents.

Sixty nine people were screened face to face for study, of which 27 people were eligible for the scanning sub-study reported as part this thesis. Three people subsequently withdrew their consent for the scanning study, two citing anxiety about the MRI scan and one citing anxiety about being part of a drug trial.
2.3.4 Inclusion and exclusion criteria for study reported in Chapter 5 & 6

The inclusion criteria of the healthy volunteers were the same as those for the study described in Chapter 4, however recruitment was targeted to match for sex and also at those aged over 30 to match for age.

The inclusion criteria for the patients included:

- Age 18-65 with DSM-IV Major Depressive Disorder (American Psychiatric Association 1994)
- Failure to respond to two or more antidepressants during their current episode of depression, with a Massachusetts General Hospital Treatment Resistant Depression (MGH-TRD) staging score of 2-10 (Fava 2003)
- Hamilton Depression Rating Scale -17 item (HDRS17 GRID-HAMD) score of ≥ 18 consistently for two weeks prior to starting the study drug (Williams et al 2008).
- On a serotonergic antidepressant at the current dose for at least 4 weeks at the point of randomisation

The exclusion criteria included:

- Other DSM-IV axis I diagnosis, other than an anxiety disorder considered to be secondary to a primary diagnosis of depression, confirmed using SCID (First et al 2002) or substance dependency.
- Contraindications to taking metyrapone including physical co-morbidities; lactation or pregnancy; co-prescription of an interacting medication.

2.3.5 Questionnaires used in Chapter 4-6

2.3.5.1 The SCID and mini-SCID

The SCID is a semi-structured interview which allows a standardised approach to making a diagnosis in keeping with DSM-IV criteria and increases inter-rater reliability (First et al 2002b). With training, non-clinicians can also use the SCID in a research context. The
mini screen for the SCID (see appendix 1) is an abbreviated, screening tool used in conjunction with the full SCID to allow a more focussed interview. The disadvantage of the SCID is that an over-strict or over-literal interpretation of the questions can lead inexperienced interviewers to rate the patient as having spurious diagnoses. However, SCID training emphasises the use of clinical acumen and experience alongside the interview to overcome this deficit.

2.3.5.2 IQ measures

At screening, all participants completed baseline measures of IQ, using estimates of full scale IQ, rather than use the time-consuming Wechsler Adult Intelligence Scale (WAIS). For the study described in Chapter 4, I administered Ammons’ Quick Test (Ammons and Ammons 1962) at screening. This is an estimate of full scale IQ consisting of a test of vocabulary using picture prompts. For the study described in Chapters 5 and 6, the National Adult Reading Test (NART) was used to measure IQ. The NART is a measure of pre-morbid intellectual ability and educational background (Nelson 1982). It consists of a page of phonetically irregular words which the subject must pronounce correctly. Errors are recorded and IQ estimated from the score. Both measures of IQ have the disadvantage of relying on verbal ability.

2.3.5.3 Handedness scales

Two versions of the Edinburgh handedness inventory were used. In Chapter 4, the Briggs and Nebbs modified Annett handedness scale was used to classify subject’s hand preference (Annett 1967; Briggs and Nebes 1975). Using this scale, anyone with a score between +24 (extreme right handedness) and +9 were included in this study, according to Briggs and Nebbs definition of right handedness. A modified version of this was used in the studies described in Chapters 5 and 6 (see appendix 2) which has been updated for modern activities and to take into account a greater degree of ambidexterity or individuals with a low hand preference (Gladue and Bailey 1995; Oldfield 1971). These scales are used for inferring hemisphere dominance. It is conventional in neurocognitive studies to include
just right handed individuals; however in the patient population the Edinburgh Handedness Scale does not provide a sensitive measure of brain laterality.

It has been known for half a century that individuals have a 73-96% likelihood of being left sided dominant for language processing (including left handed and ambidextrous individuals). This lead to the development of the Wada (sodium amytal) test prior to neurosurgery for epilepsy to determine accurately the lateralisation of an individual, especially as epileptic patients are more likely to have atypical or bilateral representations of language processing (Bauer et al 2013). Depressed patients, especially those with treatment resistance, also demonstrate abnormal laterality and asymmetry for language processing (Bruder et al 2012). This highlights the need for caution when interpreting hand preference as measured by the Edinburgh Handedness Inventory as a proxy for language laterality outside of the normal population.

Given the literature regarding laterality and hand preference combined with the relative difficulty to recruit and retain patients with TRD, I decided to take a pragmatic approach and recruit patients with preference for left handedness and perform a post-hoc sensitivity analysis. At the time of recruitment, the HVs were limited by the ethics committee approval to being right handed as they were also recruited into a wider neurocognitive study as part of the multicentre ADD trial, limiting the potential for matching the HVs and patients for handedness preference.

2.3.5.4 Massachusetts General Hospital Staging Method to Classify Treatment Resistant Depression

Treatment Resistance in the patient group studied in Chapters 4 and 5 was measured using the Massachusetts General Hospital (MGH) Staging Method to Classify Treatment Resistant Depression. Treatment resistant depression is commonly defined as a failure to respond to at least two adequate treatment trials, however, no standard consensus definition of TRD exists (Anderson et al 2008a; Fava 2003). This observer rated, structured staging tool was devised to allow a quantitative score to be calculated to aid diagnosis and help classify the severity of resistance. The MGH method measures number of treatment trials, duration of
each trial and attempts to optimise these trials by using high doses and augmenting agents. The scale is reproduced in Appendix 3. This scale was developed to supersede that of Thase and Rush (Thase and Rush 1997) and subsequent studies demonstrated that higher MGH scores predicted non-remission whereas Thase and Rush scores do not (Ruhé et al 2012).

The minimum score needed on the MGH method to be classified as TRD is 2. As likelihood of non-remission increases with MGH score, a maximum score of 10 was chosen for this study (Nierenberg and Amsterdam 1990). This range was also part of the inclusion criteria for the ADD study (McAllister-Williams et al 2013).

2.3.6 Mood Rating Scales

The end point of the ADD study was the Montgomery-Åsberg Depression Rating Scale (MADRS) score (Montgomery and Asberg 1979) and the grid version of the Hamilton Depression Rating Scale (GRID-HAMD; a version of the HDRS that separates out intensity and frequency to improve reliability) (Williams et al 2008)

2.3.6.1 Hamilton Depression Rating Scale - Grid Version

The seventeen item version of the Grid Hamilton Depression Rating Scale (Grid - HAMD) was used. This is an in interviewer-administered and rated questionnaire widely used in research (Williams et al 2008) and the standard version is used in clinical practise (Anderson et al 2008a). The grid element allows answers to be rated for both severity and duration to increase inter-rater reliability (Williams et al 2008).

2.4 Neurocognitive tasks

The n-back task was used in Chapters 4 and 5, and a novel picture based encoding retrieval task was used in Chapter 5. The development of these tasks is detailed in sections 2.4.1 and 2.4.2, below. A standardised verbal explanation of both tasks was given to the participants an hour before the test. Following pilot work, for all the in-scanner
neurocognitive tests the participants had a demonstration version of the task shown to them and they had to demonstrate they understood the task instructions actively.

2.4.1 The N-Back Task

The n-back task is a neurocognitive test of working memory. It has been well validated in several different patient groups (Kane et al. 2007). In fMRI experiments, this has been demonstrated to activate medial and lateral premotor cortex; medial and lateral posterior parietal cortex dorsal cingulate; dIPFC and ventrolateral prefrontal cortex (vIPFC); and the frontal poles (Owen et al. 2005).

The basic premise of this test is that participant is presented with a series of stimuli and instructed to remember the stimuli from n stimuli back and to indicate if they see a match with the current stimulus presented on the screen. There are different levels of complexity e.g. participants are asked to indicate if the stimulus shown is the same as the last one shown at the 0-back level; or if the stimulus shown is the same as the one before last at the 1-back level, etc. The stimuli can be numbers, letters or pictures (Jaeggi et al. 2003).

Three different versions of the n-back test were used in this thesis: a blocked version of Callicott et al. was used (Callicott et al. 2003) adapted for use in the fMRI scanner; an out of scanner version (Gevins and Cutillo 1993) and an adapted version of the task from Koychev et al. (Koychev et al. 2012). Both two former tasks have been described in detail elsewhere (Callicott et al. 2003; Gevins and Cutillo 1993) and are described further in chapter 4 (Symonds et al. 2012). However, the normal volunteers described the Callicott version as being particularly aversive as it presented a grid onto which the numbers were projected, introducing a spatial element which some participants reported as being particularly stressful and counter-intuitive. A simpler task was therefore introduced for the patient study to prevent a catastrophic reaction to the task (Elliott et al 1996). The Koychev et al. version (Koychev et al. 2012) of the task was adapted for the study detailed in Chapter 5 and is further described, below.
Koychev (Koychev et al 2012) used an event-related task and three levels of difficulty: zero-back, one-back and two-back. In order to maximise the number of comparisons at the most demanding level to the least demanding level without overly prolonging the task and tiring the depressed participants, the decision was made to only include blocks of zero-back and two-back. A block design was chosen given its increased statistical power and ease to administer, design and analyse (Chee et al 2003). The decision was also made to include rest periods, both to reduce fatigue and also to give the option of two control tasks. The rest task comprised of viewing a single cross in the centre of a screen for 20 seconds. This allowed two different contrasts between high intensity work load of working memory and a control condition: a contrast between the high intensity two-back and the low intensity zero-back; and a contrast between the two-back and rest. The former contrast provides a comparison between high and low demand on working memory with both conditions engaging similar processes and differing in only one variable, namely the intensity of the task. The latter provides a comparison between high demand working memory task and baseline, providing a greater magnitude contrast (Amaro Jr and Barker 2006).

The final version of the task had 4 blocks made up of a the zero-back task with 13 stimuli, each displayed for 2 seconds each (26 seconds in total per block), 4 blocks made up of a the two-back task with 13 stimuli, each displayed for 2 seconds each (26 seconds in total per block) and a rest block lasting 20 seconds (consisting of a black screen with a white fixation cross). In total the task lasted 7 minutes 12 seconds. The stimuli used in this task were letters.

2.4.2 Encoding and Retrieval Task

The emotional memory task used in Chapter 5 was adapted from Whalley et al (Whalley et al 2009) with permission. Initially, I developed a block-design, encoding and retrieval task in collaboration with Dr Daniela Montaldi. For this task, in the encoding block, participants are presented with a series of 12 sequential images of different objects to remember. Pictures were chosen as they are generally regarded as being easier to
remember than words, as suggested by the dual encoding theory (that pictures are encoded both as an image and verbally) (McBride and Dosher 2002) and that they are inherently superiorly encoded due to being semantically more distinct than words from one another (Mintzer and Snodgrass 1999). Experimental work has confirmed that pictures, as opposed to words, are recalled with greater accuracy. Event related potentials on EEG when recalling pictures are in-keeping with the pattern generated by both recollection (hence greater detail) and familiarity (recognition without detail) which supports the theory that pictures encode as a ‘richer’ memory (Curran and Doyle 2011).

Each picture was shown for 5 seconds in a block that lasted for 60 seconds. The pictures were of emotionally neutral scenes (e.g. a field of cattle, a picture of Reykjavik etc). Between blocks subjects completed a distraction task to prevent encoding. This comprised counting backwards in threes for 20 seconds to prevent rehearsal of the encoded images. The retrieval block was then presented for 45 seconds. In this block the subjects had to identify which of the objects had been previously shown in the encoding block using a four-button response box. They are presented with a series of scenes, including all the scenes they had previously seen, plus an additional 30% more scenes they had not viewed previously. The distracter scenes where of a similar theme but not closely matched to the target scenes. Subjects were asked to indicated with the button box if the scene was ‘old’ or ‘new’ to them, with a prompt visible on the screen as to which button to use. Such tasks have been shown to activate dorsolateral prefrontal cortex, hippocampus and parietal cortex (Rugg et al 2008).

There was no main effect of the task in any of the regions of interest (parahippocampus, hippocampus and dorsolateral prefrontal cortex). However, there was an effect of drug with hydrocortisone administration resulting in a reduced hippocampal BOLD signal during encoding, but not retrieval (a brief summary of results is presented in Appendix 4. Because of the methodological problem with the task it was not included in the paper presented in Chapter 4 (Symonds et al 2012). The pilot task demonstrated limitations in the design of the task. One interpretation is that the rest task was too demanding and therefore not an adequate control for the visual encoding/retrieval task. However,
without a simple rest task, no direct comparison could be made and no firm conclusion can
be drawn as to why this task failed. As no main effect was demonstrated, it implies that
the hippocampus was activated during the rest task. Given that there appeared to be
potential in a similar task, a review of the literature revealed the task by Whalley et al
(Whalley et al 2009) to have potential as it had a similar task but a much simpler rest task
(participants were asked to look at a fixation cross on a blank screen). They did not
demonstrate a problem with rehearsal and this task had the added advantage of an
emotional memory component as it used emotionally valenced and neutral pictures.

The original study used positive and neutral images from the International Affective
Picture System (IAPS), presented to patients with bipolar and schizophrenia for encoding
during an fMRI scan with recognition memory tested out of the scanner. Whalley et al
demonstrated an enhanced medial temporal lobe response to encoding emotional images,
more pronounced in bipolar patients. There is evidence that there is no difference
between the emotional response to words and pictures, but that people find visual
memory less effortful subjectively and show a greater limbic system activation of fMRI to
pictorial stimuli (Schlochtermeier et al 2013).

I retained the design of neutral and positive images based on evidence that people with
depression recall neutral images preferentially compared to positive (Elliott et al 2012),
with greater frontal polar activation. There were also methodological and ethical concerns
if negative images were to be used. Many of the IAPS negative images evoke shock,
revulsion or disgust which could potentially evoke distress in the depressed patients, many
of whom had significant trauma histories. If negative images were added to neutral and
positive ones this would increase the duration of the task, which was also a concern in
terms of potential fatigue.

The original event related task was adapted to a block design and scenes of a sexual
nature were removed as they were potentially culturally, sexuality or gender
inappropriate. There were 5 blocks made up of a block of 6 positive images lasting 24
seconds in total (4 second per image), a block of 6 neutral images lasting 24 seconds in
total (4 second per image) and a rest block lasting 24 seconds (consisting of a black screen with a white fixation cross). This is illustrated in Figure 2-1. In total the task lasted 6 minutes.

Subjects were asked to indicate whether the image was ‘not emotional’ or ‘emotional’ with a button box placed in their right hand. The subjects were instructed to give their opinions and that there was no right answer. They were also instructed to remember the images and told they would be tested on this shortly.

![Figure 2-1: Block design of the encoding task (adapted from Whalley et al 2009). Each block (neutral images, positive images or rest) lasted a total of 24 seconds, with a total task length of 6 mins.](image)

The original Whalley et al task was designed to test retrieval of images out of the scanner as one task with a mixture of neutral and positive images but without rest periods. It was intended to image the neurocorrelates of retrieval; therefore the task was adapted to a block design, similar to the encoding task. The original task had two parallel versions. In the retrieval section, participants were shown all the original images with a random order of old images interspersed with novel, distracter images. In the original version, some of the distracter images in the first version of the task were the same as the images to be encoded in the second version. This could introduce bias. Therefore additional images, matched for arousal and valency were chosen. Care was also chosen to match the number of neutral and positive images containing human subjects.

The retrieval component of the task took place 5 minutes after the end of the encoding task. In the interim, participants looked at a fixation cross and rested whilst a T1 structural scan was performed.
In the retrieval task, there were 6 blocks made up of a block of 8 positive images lasting 24 seconds in total (3 second per image), a block of 8 neutral images lasting 24 seconds in total (3 second per image) and a rest block lasting 24 seconds (again consisting of a black screen with a white fixation cross). This is illustrated in Figure 2-2. In total the task lasted 7 minutes 12 seconds. Subjects were asked to indicate whether the image had been previously ‘seen’ or ‘not seen’ with a button box placed in their right hand.

All subjects were given the opportunity to practice the task 2 hours prior to the scanning session to ensure they understood what was required of them. The much shorter, demonstration version of the task consisted of copyright free photographs not in the IAPS battery. Participants were able to ask questions to the investigators and the investigators checked the understanding of the participants.

![Figure 2-2: Block design of the retrieval task (adapted from Whalley et al 2009). Each block (neutral images, positive images or rest) lasted a total of 24 seconds, with a total task length of 7 minutes 12 secs.]

2.5 Functional MRI

Functional MRI (fMRI) techniques were used in the chapters 4, 5 and 6 and the principles of BOLD signal MRI, pharmacological challenge MRI as well as the analysis and scanning schedules is described in this section.
2.5.1 Principles of BOLD signal fMRI

Blood oxygen level dependent signal functional magnetic resonance imaging (BOLD signal fMRI) is a non-invasive imaging technique that measures changes in blood oxygenation as a proxy for neural activation. The measurement produced is quantitative and provides a non-quantified method for studying the regional effect of neuropsychological tasks. This contrast compares the paramagnetic properties of deoxyhaemoglobin (applied electromagnetic field is attracted into the material) with the diamagnetic properties of oxyhaemoglobin (applied electromagnetic field is repelled from the material). The image contrast is a measure of the extent to which an applied field interacts with the haemoglobin. Oxygenated haemoglobin increases in the areas of the brain being used to complete a task (e.g. a neurocognitive task). By utilising the differing magnetic properties of haemoglobin, brain activity can be inferred from the change in magnetic resonance signalling. Using the BOLD technique, rest or a control task MR data can be subtracted from data acquired during the task, and so attribute the BOLD signal in a brain area to the task. However, this is a non-quantified measurement, just a comparison and the coupling between BOLD signal and neural activity correlating with a higher cognitive function, emotion or other behavioural output is only indirectly attributed. Nevertheless, in addition to blood oxygenation, blood flow and volume contributes to BOLD signal and correlates with functional activity (Logothetis 2003). A time-lag, corresponding with the time it takes for blood to flow from arteries to capillaries, of approximately 2 seconds, occurs after a stimulus is presented. This is important when designing block and event related neurocognitive tasks for use during fMRI scans and for the interpretation of the results (Amaro Jr and Barker 2006). Chapters 4 and 5 contain further details regarding the analysis of BOLD fMRI.

2.5.2 Principle of pharmacological challenge fMRI

In standard fMRI, the stimulus condition being measured tends to be either a cognitive task or a sensory stimulus. In pharmacological challenge fMRI (phMRI or pharmacoMRI), the stimulus causing a change in BOLD signal is a drug or a hormone. Direct phMRI refers to
the ‘real-time’ effects caused by the ingestion of a drug during a scan, usually by a remotely operated intravenous syringe driver (used in chapters 4 and 6). Modulatory phMRI describes the technique where a participant receives a drug prior to the start of the scan in question (as used in chapter 4). (Anderson et al 2002; McKie et al 2005)

Direct phMRI is susceptible to the effects of motion and scanner drift, especially with longer scans, which can be overcome to a degree by having a placebo control group and correcting for motion by either co-varying individual movement parameters during analysis or using prospective motion correction (Churchill et al 2012). Using the p-block method of analysis, the ‘time bin’ (a block of 1-5 minutes) prior to the start of the infusion is used as the baseline to subsequent time bins using regression analysis (Del-Ben et al 2005; McKie et al 2005). If the baseline bin is atypical (e.g. because of movement artefact), this assumption of normality can distort the rest of the analysis. This is typically overcome by having a baseline scan prior to the infusion of several minutes.

2.5.3 Scanning protocol of study detailed in chapter 4

As described later in chapter 4, following a 6 minute T1-weighted structural scan a 25 minute challenge phMRI scan was performed. This consisted of a baseline period of 5 minutes then a slow bolus infusion over 90 seconds of 100mg hydrocortisone/saline followed by 18.5 minutes of continued scanning. A high resolution technique was used with a TR of 3 seconds, 500 volumes and a total of 26 slices angled obliquely to cover the hippocampus and a section of prefrontal cortex (see Figure 2-3). Plasma cortisol samples were taken at baseline, 25 minutes post hydrocortisone infusion and at 60 minutes at decannulation. These were sent for analysis at Oxford Brookes University Laboratories and analysed using a radioimmunoassay.
2.5.4 Scanning protocol of study detailed in chapter 5 and 6

Subjects were scanned using a parallel, between subjects design on a 1.5T scanner with prospective motion correction. The scanning session, which took place between 12noon and 3pm, consisted of a brief structural MRI scan, followed by three fMRI scans and a 6 minute structural MRI scan, followed by a 26 minute phMRI infusion scan (see Figure 2-4). Scanning was carried out on a Philips Intera 1.5 Tesla MRI scanner. Data for the fMRI scans were acquired with T2*-weighted, gradient echo, echo planar imaging (EPI). Full brain coverage was used with TR=2 sec, TE=40 ms, 3.5mm in-plane resolution, 4.5mm slice thickness with 0.5mm slice gap and 29 slices. For the phMRI scan, full brain coverage was used with a TR = 3 s, TE = 40 ms, isotropic 3 mm voxels with 29 slices and whole brain coverage. The decision was made to use a larger voxel size after experimentation with the analysis of the experiment detailed in Chapter 4 revealed that there was little loss of resolution with a voxel size of 3mm compared to 1.5mm, and this would allow whole brain coverage to be performed within the data storage limits of the 1.5T scanner. The advantage of whole brain coverage was that other limbic structures could be investigated and thus investigate the relationship between the acute effect of hydrocortisone on MRs (which are located mainly in the limbic area) and GRs which are located elsewhere (Berardelli et al 2013). The scan was 26 minutes in length and consisted of 520 volumes.

Figure 2-3: Scanning protocol for study detailed in chapter 4. VBM=T1 weighted structural scan, CORT=hydrocortisone, EMT= encoding-retrieval memory task WMT = working memory task
Cortisol samples: Plasma cortisol samples were taken at baseline and at 60 minutes at decannulation. These were sent for analysis at Central Manchester NHS Foundation Trust laboratories and analysed using a radioimmunoassay.

![fig](image)

**Figure 2-4:** Scanning protocol for study detailed in chapters 5 and 6. VBM=T1 weighted structural scan, CORT=hydrocortisone, EMT= encoding-retrieval memory task: EMT-E= encoding part, EMT-R= Retrieval part WMT = working memory task A= brief structural scan

2.5.5 Hydrocortisone administration

Subjects were randomised to receive either hydrocortisone (Pharmacia) 100mg first, or an equal volume of normal saline (36mls) in both the studies illustrated in chapter 4 and 6. The hydrocortisone/saline was given at 0.4ml/s over 90secs following the VBM Scan. This slow bolus was designed to reflect the effect of an acute stress response. Subjects were cannulated in the left antecubital fossa with a 20 gauge cannula one hour previously and normal saline delivered at 0.1ml/min to ensure patency of the line prior to the bolus. The methodology for this scanning procedure has been developed from experience within the research group of challenge phMRI using mCPP and citalopram (Del-Ben et al 2005; McKie et al 2011).

In normal individuals, studies of the pharmacokinetics of intravenous hydrocortisone have shown a plasma $t_{\text{max}}$ of approx 1 minute with a $t_{1/2}$ of 1.7 hours. Hydrocortisone crosses the blood-brain barrier rapidly (approximately 2 mins) by diffusion (Derendorf et al 1991;
Thomson et al 2007). The hydrocortisone dose of 100mg results in approximately 10-times the usual physiological levels and would be expected to saturate the GR and MR receptors in the brain (Derendorf et al 1991). This dose is well tolerated (Halonen et al 2007) and has not been associated with adverse effects in healthy subjects.

2.5.6 fMRI analysis

There is currently no consensus approach as to the optimum method to analyse fMRI data (Amaro Jr and Barker 2006). I used Statistical Parametric Mapping (SPM8) software (http://www.fil.ion.ucl.ac.uk/spm) due to this being standard within the research group and the advantage of the availability of atlas and other additional, compatible software.

A block design was chosen for fMRI analysis and a pseudo-block design chosen for reasons discussed in sections 2.4.1 and 2.5.4. The first step in data analysis is pre-processing which corrects for motion by realignment. Additionally, each set of scan data are normalised to a standardised map of the human brain, in this case the Talairach and Tournoux stereotactic space (Talairach and Tournoux 1998) using Montreal Neurological Institute (MNI) templates. These are then smoothed to compensate for addition anatomical variability.

First level analysis was then performed creating contrasts between the neurocognitive task component and rest at an individual level. Second level analysis created contrasts that addressed the hypothesised questions at a group level. The details of these analyses for the individual experiments are detailed in sections 4.4.5, 5.4.1 and 6.3.6.
2.6 References


Chapter 3

The effect of acute stress and cortisol on encoding and retrieval: a systematic review of the literature with meta-analysis

Catherine S. Symonds*, Caroline Henson², John Francis William Deakin¹, Ian M. Anderson¹

*1. Neuroscience and Psychiatry Unit, G.907 Stopford Building, The University of Manchester and Manchester Academic Health Sciences Centre, Oxford Rd, Manchester M13 9PT. Tel 0044 161 275 1727 Fax: 0044 161 275 7429
email Catherine.Symonds@manchester.ac.uk

2. School of translational medicine, University of Manchester
3.1 Abstract

**Background:** An acute rise in corticosteroid has been shown to affect memory; however, the evidence to date is unclear, with studies reporting both improved and impaired declarative memory. The timing of the rise in cortisol (prior to encoding or prior to retrieval); emotional aspects of the neurocognitive task and the method by which the cortisol rise was induced (exogenous drug administration or endogenous, stress-induced) also appear to affect participants' ability to recall words. This systematic review with meta-analysis aimed to examine the acute effects of corticosteroids on declarative memory in 25 studies.

**Methods** Medical databases and citation lists of included studies and relevant reviews were searched to identify trials using a priori selection criteria, namely randomised controlled trials of healthy volunteers with an acute cortisol rise induced by exogenous drug administration or stress task as well as encoding or retrieval were assessed on quantified tests of word learning. Meta-analysis was performed using the generic inverse variance method displayed with heterogeneity to allow pooling of studies which measured outcomes on different scales. The effect of neutral, positive and negative stimuli material was also examined as subgroup analyses.

**Results:** Raised cortisol prior to retrieval, but not encoding, adversely affected performance in memory tasks. The effect was most consistently seen with exogenous cortisol and with the retrieval of neutral words. However, there were too few studies to draw firm conclusions regarding the effect of exogenous versus endogenous cortisol and the role of emotional material.

**Conclusions:** The timing of the acute rise in cortisol related to encoding is important with a significant result only being found when there was a rise of cortisol post-encoding and pre-retrieval. This is most likely to represent a non-genomic action of cortisol given the rapid onset of the effect. The impact of emotional content of memories in the context of acute stress needs to be studied further.
3.2 Background

Although hypercortisolaemia in depression is one of the most replicated findings in biological psychiatry (Porter et al 2004), reproducing its cognitive effects reliably in an experimental setting is challenging. Both depression (Abercrombie et al 2011) and post-traumatic stress disorder (PTSD) (Bremner et al 2004) results in altered hypothalamic-pituitary-adrenal (HPA) axis and selective memory deficits. The development of a reproducible model to mimic these cognitive impairments induced by disease states has been a matter of considerable debate. This is usually modelled using either a pharmacological induced rise in cortisol or a psychological stressor induced rise in cortisol. The latter model also affects neurotransmitter systems such as catecholamines (Abelson et al 2005). The former method yields a single hormone rise to mimic hypercortisolaemia however it can be argued that psychological stress gives rise to a more naturalistic model.

The acute action of cortisol and stress on the ability to encode and retrieve memories has been the subject of many different studies over the last four decades (Sauro et al 2003). Stress results in a rise in cortisol via the hypothalamic-pituitary axis with higher control of the HPA axis coming from the hippocampus, pre-frontal area and amygdala (de Kloet et al 2007). These areas of the brain are rich in mineralocorticoid and glucocorticoid receptors (Joëls et al 2011). On meta-analysis of encoding and retrieval functional magnetic resonance imaging (fMRI) tasks, the hippocampus and prefrontal cortex (as well as the secondary associative sensory cortices and posterior parietal cortex) were strongly implicated in recollection memory (Skinner and Fernandes 2007), with the hippocampus, parahippocampal cortex, entorhinal and perirhinal cortices implicated in recognition memory (Song et al 2011).

In a commonly used psychological model of memory, declarative memory is subdivided into semantic memory (memory for facts) and episodic memory (memory for episodes experienced by an individual, also called autobiographical memory). Recalled information can be semantic or episodic. In contrast, recognition memory relies in addition on implicit memory and familiarity (Migo et al 2012). It has been proposed that there are distinct neural regions for recollection and familiarity in addition to a common neural pathway...
(Montaldi et al 2006). These neural pathways may well be differentially vulnerable to the effects of cortisol.

In addition to the broad category of declarative memory, memory for different stimuli (e.g. word memory, categorical memory, learning faces etc.) and different modalities (e.g. auditory, visual etc) have different neural pathways. Pictures tend to be remembered with greater depth and this is theorised to be due to a parallel process of verbal and imaging coding (Ally 2012). These stimuli in turn can vary in terms of emotional valence and intensity. These variables can differentially affect both the neural processes involved and the accuracy of recall and recognition (Keil 2006).

There are many individual confounding factors affecting cognition and cortisol. Cortisol varies over the menstrual cycle (Symonds et al 2004) and gender has an independent effect on cognition (Kubzansky et al 2012). Additionally, as highlighted above, psychiatric illnesses such as depression and PTSD can affect both a person’s cortisol levels and their cognitive function, as can substance misuse (Tarter et al 2013) and several other medical conditions (Hawkins et al 2012).

Although previous reviews (Sauro et al 2003; Wolf 2009) have concentrated on chronically elevated cortisol levels, of increasing interest is the rapid, acute effect of cortisol on cognition. The neurophysiological effect of cortisol/corticosterone on the hippocampal formation is understood to occur in less than 10 minutes post administration with an increase in glutamate transmission (Karst et al 2005). This is well before genomic action of corticosteroids on the nucleus causing protein transcription occurs four hours after the rise in cortisol (Joëls et al 2011). The acute behavioural responses of humans to the early, non-genomic action of stress hormones on the brain represents an opportunity to further understand the functional impact of these hormones at a time when a steroidal, genomic action is unlikely. Whether these acute effects on cognition mimic the cognitive impairments seen chronic hypercortisolaemic states or long-term psychiatric conditions is unclear. It is argued that inappropriate reaction to stress can affect the functional ability of the glucocorticoid and mineralocorticoid-receptor rich prefrontal lobes, amygdala and hippocampus (de Kloet et al 2007). There have been many different paradigms used to
investigate this using a variety of different tasks to induce stress; several different drugs and doses; and a range of tasks to test neurocognition, both with and without emotional stimuli. In addition, a number of different timings for the administration of the drug or stressor have been used and there has been much debate as to how the timing of cortisol rises affects memory, particularly in its relationship to encoding and retrieval (Wolf 2009).

The aim of this review is to examine the effect of corticosteroid hormones on memory and to examine whether exogenous and endogenous, stress-related, rises are equivalent. Given that methodological variation between studies is likely to contribute to heterogeneity and conflicting findings, we chose to focus on the acute effect of cortisol on encoding and recalling words in normal volunteers.

3.3 Method

3.3.1 Literature Search

The Cochrane Collaboration methodology (Higgins et al 2011) was followed using an explicit a priori search strategy designed to minimise bias and maximize the search yield. A systematic analysis and pooling of data were subsequently performed (see below). We included all randomised controlled studies of memory encoding and retrieval in which stress accompanied by a rise in cortisol or exogenous corticosteroid were administered and where there was an appropriate control (control task or placebo corticosteroid). We searched English language papers in humans in PubMed, Embase, Web of Knowledge, and Psychinfo, published before April 2013 using keywords “(cognitive or memory or cognition or neurocognition or neurocognitive or neuropsychological or executive) and (cortisol or corticosteroid or glucocorticoid or HPA)” as well as manually searching papers and reviews for further references.

We selected studies in which encoding or retrieval were assessed on quantified tests of word learning in order to provide sufficient methodological similarity to allow the pooling of studies, as the mechanism for learning different types of subject matter has a different neural basis (Baddeley 1997). We defined ‘acute’ as a stress-induced or drug induced corticosteroid rise within 240 minutes of either the encoding or the retrieval task. This
time frame was chosen to examine non-genomic effects of corticosteroids as beyond this time window genomic effects are likely to predominate (Losel et al 2003). Studies that examined the effects of stress but either did not measure, or did not show a rise in cortisol, were excluded as it could not be assumed that a stress reaction had taken place. Finally we restricted studies to those with healthy volunteer participants as the effects of stress cannot be assumed to be uniform across physical or psychiatric disorders.

3.3.2 Analysis

The data were analysed using Review Manager (RevMan [Computer program] Version 5.17 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). Study eligibility, quality and risk of bias were determined by examining the following: allocation concealment; presence of a complete data set; selective outcome reporting and allocation concealment. The studies were grouped by intervention (stress versus control or drug versus placebo) and whether the intervention was before encoding or retrieval. Neutral, positive and negative stimuli were examined as planned subgroup analyses. Due to the different neural mechanisms and the discrepant results seen (Migo et al 2012), recall and recognition results were analysed separately. Papers that met the search criteria but could not be included in the meta-analysis were reported in narrative form.

Data was analysed using generic inverse variance (DerSimonian and Kacker 2007). The standardised mean difference and its standard error were calculated for each study to allow pooling of studies which measured outcomes on different scales. The methodology and type of study participants in each study was examined to assess clinical heterogeneity with quantitative pooling conducted using a random effects model (DerSimonian and Kacker 2007). Statistical heterogeneity was assessed using $\chi^2$ and quantified using the $I^2$ statistic (Higgins et al 2003). Studies with a cross-over design were only combined with parallel studies if there was no statistical effect of order or time on the outcome measures as recommended by the Cochrane Collaboration Handbook. (Higgins et al 2011)
3.4 Results

3.4.1 Results of Search

The literature search resulted in 5082 potentially relevant articles from which 280 full articles were identified for further evaluation from the title and abstract review. Eighty three articles reported an experimentally induced rise in cortisol associated with performing an encoding-retrieval task within four hours of this rise, and were included for closer critical appraisal. Twenty five articles met the criteria of involving a homogenous task (word learning) and a homogenous test population (healthy volunteers without significant co-morbidities) and were included in the meta-analysis. This selection process is illustrated in Figure 3-1.

**Figure 3-1: Flow chart illustrating the literature search**
3.4.2 Included Studies

The 25 included trials (Almela et al 2011; Beckner et al 2006; Boehringer et al 2010; Buchanan et al 2006; De Quervain et al 2003; de Quervain et al 2000; Domes et al 2002; Domes et al 2004; Domes et al 2005; Hoffman and al’Absi 2004; Jelici et al 2004; Kirschbaum et al 1996; Koessler et al 2009; Kuhlmann et al 2005a; Kuhlmann et al 2005b; Nater et al 2007; Rohleder et al 2009; Schoofs and Wolf 2009; Schwabe et al 2008; Schwabe and Wolf 2010b; Smeets et al 2006a; Tollenaar et al 2009b; Tops et al 2003; Wolf et al 2001a; Wolf et al 2001b) are described in Table 3-1, (describing the effects of an increased in cortisol prior to encoding) and Table 3-2, (describing the effects of an increase in cortisol after encoding and prior to retrieval). Six comparisons were made: 1) drug or stress-induced cortisol rise pre-encoding, 2) drug administration pre-encoding, 3) stress pre-encoding, 4) drug or stress-induced cortisol rise post-encoding and pre-retrieval, 5) drug administration post-encoding and pre-retrieval, and 6) psychological stress post-encoding and pre-retrieval.

3.4.3 Excluded studies

The characteristics of the rejected studies are detailed in supplementary Table A5-1 (Appendix 5).
<table>
<thead>
<tr>
<th>Trial</th>
<th>Participants</th>
<th>Intervention</th>
<th>Methods</th>
<th>Time pre-encode</th>
<th>Outcome</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug-induced rise in cortisol</td>
<td>De Quervain et al (2000)</td>
<td>Healthy volunteers (male &amp; female) age 18-30, n=18 (n=6 in three parallel groups receiving drug at 3 time points)</td>
<td>cortisone 25mg or placebo HC eqv** = 20mg</td>
<td>60 mins</td>
<td>No effect on memory of cortisone</td>
<td>Study duplicated in Table 3-2</td>
</tr>
<tr>
<td></td>
<td>Kirschbaum et al (1996)</td>
<td>Healthy volunteers (males) average age 24.7 n=40</td>
<td>hydrocortisone 10mg or placebo</td>
<td>60 mins</td>
<td>Placebo treated group recalled significantly more words than the hydrocortisone treated group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tops et al 2003</td>
<td>Healthy volunteers (male) age 18-27, n=22</td>
<td>hydrocortisone 10mg or placebo</td>
<td>60 mins</td>
<td>hydrocortisone impaired recall and recognition of neutral and pleasant words, but not unpleasant words</td>
<td>Recall 60mins after encoding task</td>
</tr>
</tbody>
</table>

Table 3-1: Characteristics of studies - studies which describe the effects of an increase in cortisol prior to encoding
<table>
<thead>
<tr>
<th>Trial</th>
<th>Participants</th>
<th>Intervention</th>
<th>Methods</th>
<th>Time pre-encode</th>
<th>Outcome</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stress-induced rise in cortisol</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Domes et al (2004)</td>
<td>Healthy volunteers (male) age 18-42 n=60</td>
<td>Trier stress test</td>
<td>Word list of 60 German words (20 neutral, 20 positive, 20 negative). Free recall &amp; recognition 24 hours after learning. Parallel design</td>
<td>5 mins</td>
<td>No effect of stress on recall. Recognition of positive words impaired in the group stressed before retrieval. Duplicated in Table 3-2 recognition not included as used sensitivity analysis.</td>
<td></td>
</tr>
<tr>
<td>Jelici et al 2004</td>
<td>Healthy volunteers (male &amp; female) mean age 20.1 n=20</td>
<td>Trier stress test</td>
<td>Emotional version Rey AVLT (15 neutral, 15 emotional words). Recall task 30 min after encoding. Parallel design</td>
<td>10 mins</td>
<td>Stress impaired recall of neutral words but enhanced memory of emotional words</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-1: Characteristics of studies - studies which describe the effects of an increase in cortisol prior to encoding
<table>
<thead>
<tr>
<th>Trial</th>
<th>Participants</th>
<th>Intervention</th>
<th>Methods</th>
<th>Time pre-encode</th>
<th>Outcome</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schwabe et al (2008)</td>
<td>Healthy volunteers (male &amp; female) age 19-36 n=85</td>
<td>Cold pressor</td>
<td>Word list of 10 positive, 10 negative and 16 neutral words. Recalled 1 hour after encoding (70 min post stress) and recognition test 24 hours later. Parallel design</td>
<td>10 mins</td>
<td>Stress enhanced neutral and positive word recall but not positive words</td>
<td>recognition not usable as not presented in format suitable for meta-analysis</td>
</tr>
<tr>
<td>Schwabe and Wolf (2010b)</td>
<td>Healthy volunteers (male &amp; female) age 19-39 n=50</td>
<td>Socially evaluated cold pressor</td>
<td>Word list of 8 negative, 8 positive and 8 contextual words. Recall and recognition 24 hours after encoding</td>
<td>1 min: during encoding</td>
<td>Stress decreased recall regardless of emotional valence</td>
<td>recognition not usable as not presented in format suitable for meta-analysis</td>
</tr>
<tr>
<td>Smeets et al (2006)</td>
<td>Healthy volunteers (male &amp; female) mean age 19.7, n=18</td>
<td>Trier stress test</td>
<td>30 word WVLT (10 positive, 10 negative and 10 neutral). Recalled 30 min after encoding. Parallel design</td>
<td>30 mins</td>
<td>Stress impaired recall in the stress group for neutral words but not for emotional words</td>
<td>recognition results did not include data that could be included in the meta-analysis</td>
</tr>
<tr>
<td>Wolf et al 2001b</td>
<td>Healthy volunteers mean age 24.9 (male and female) n= 58</td>
<td>Trier stress test</td>
<td>Word list of 25 German nouns (neutral) free recall 2 mins after encoding. Parallel design</td>
<td>10 mins</td>
<td>Stress did not impair recall</td>
<td></td>
</tr>
<tr>
<td>Nater et al (2007)</td>
<td>Healthy volunteers (male) mean age 23.7, n=20</td>
<td>Trier stress test</td>
<td>Rey AVL (30 neutral nouns). Recall task 30 min after encoding. Crossover design</td>
<td>15 mins</td>
<td>No effect of stress overall; higher cortisol responders showed enhanced recall</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-1: Characteristics of studies - studies which describe the effects of an increase in cortisol prior to encoding*Study duplicated in Table 3-2 (two parallel arms in examining effect of cortisol rise both before encoding and retrieval) ** HC eqv = hydrocortisone equivalence

Table 3-1: Characteristics of studies - studies which describe the effects of an increase in cortisol prior to encoding
<table>
<thead>
<tr>
<th>Trial</th>
<th>Participants</th>
<th>Intervention</th>
<th>Methods</th>
<th>Time pre-retrieve</th>
<th>Outcome</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug-induced rise in cortisol</td>
<td></td>
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<tr>
<td>De Quervain et al (2000)</td>
<td>Healthy volunteers</td>
<td>Cortisone 25mg or placebo</td>
<td>60 unrelated German nouns (all neutral). Recalled 24 hours after encoding; crossover study</td>
<td>60mins</td>
<td>Detrimental effect on memory of cortisone (p&lt;0.05)</td>
<td>Study duplicated in Table 3-1 Recalled 24 hours after encoding; crossover study with n=6 as tested at 3 time points. 1 time point not included</td>
</tr>
<tr>
<td></td>
<td>(male &amp; female) age 18-30, n=18</td>
<td>HC eqv** = 20mg</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>De Quervain et al (2003)</td>
<td>Healthy volunteers</td>
<td>Cortisone 20mg or placebo</td>
<td>20 unrelated German word pairs (all neutral). Recall and recognition 24 hours after encoding. Crossover study.</td>
<td>60 mins</td>
<td>Detrimental effect of cortisone on declarative memory task</td>
<td>Part of PET study</td>
</tr>
<tr>
<td></td>
<td>(male) age 21-27, n=14</td>
<td>HC eqv** = 16mg</td>
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<tr>
<td>Domes et al (2005)</td>
<td>Healthy volunteers</td>
<td>Hydrocortisone 25mg or placebo</td>
<td>Verbal subtest of Learning &amp; Memory Test (Baumler 1974) (20 paired Turkish and German neutral nouns). Recall 45 mins after encoding. Parallel design</td>
<td>45mins</td>
<td>Detrimental effect only seen in high cortisol responders</td>
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<tr>
<td></td>
<td>(male &amp; female) mean age 26.1, n=59</td>
<td></td>
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<tr>
<td>Kuhlman et al (2005a)</td>
<td>Healthy volunteers</td>
<td>Hydrocortisone 10mg or placebo</td>
<td>Word list of 15 negative &amp; 15 neutral words. Recall 30 mins after encoding. Crossover design</td>
<td>30mins</td>
<td>Hydrocortisone significantly impaired recall (p&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(female) n=16</td>
<td></td>
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<tr>
<td>Trial</td>
<td>Participants</td>
<td>Intervention</td>
<td>Methods</td>
<td>Time pre-retrieve</td>
<td>Outcome</td>
<td>Notes</td>
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<tr>
<td><strong>Drug-induced rise in cortisol</strong></td>
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<tr>
<td>Rohleder et al (2009)</td>
<td>Healthy volunteers (male &amp; female) age 21-35, n=19</td>
<td>Hydrocortisone 30mg or placebo</td>
<td>Word list of 15 negative and 15 neutral words. Recalled 3 hours after encoding. Crossover design</td>
<td>60mins</td>
<td>Trend towards hydrocortisone reducing recall of neutral and emotional words (p=0.07)</td>
<td></td>
</tr>
<tr>
<td>Tollenaar et al (2009)</td>
<td>Healthy volunteers (male) age 18-30, n=53</td>
<td>Hydrocortisone 35mg vs. placebo or propranolol 80mg vs. placebo</td>
<td>Word list of 30 emotional and 30 neutral words. Recalled 75 mins after encoding. Parallel design</td>
<td>75 mins</td>
<td>Memory retrieval of neutral and emotional information was impaired by hydrocortisone but not propranolol.</td>
<td></td>
</tr>
<tr>
<td>Wolf et al (2001a)</td>
<td>Healthy volunteers (male) mean age 24, n=9 and n=9mean age 64</td>
<td>Hydrocortisone 0.5mg/kg average dose = 35mg</td>
<td>10 neutral nouns (&quot;Shopping list&quot;). Recall 105 mins after encoding. Crossover design</td>
<td>60mins</td>
<td>Memory retrieval impaired in both young and elderly for word recall</td>
<td></td>
</tr>
<tr>
<td><strong>Psychological stress induced rise in cortisol</strong></td>
<td></td>
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<tr>
<td>Beckner et al (2006)</td>
<td>Healthy volunteers (males &amp; females), age 18.8 n=101</td>
<td>Public speaking</td>
<td>WAIS paragraph test B (neutral material). Free recall 48 hours after learning material. Parallel design</td>
<td>45 mins</td>
<td>Stress did not impair retrieval relative to controls.</td>
<td>Rejected after sensitivity analysis - task too dissimilar and accounted for 82% of variance in analysis</td>
</tr>
<tr>
<td>Trial</td>
<td>Participants</td>
<td>Intervention</td>
<td>Methods</td>
<td>Time pre-retrieve</td>
<td>Outcome</td>
<td>Notes</td>
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<tr>
<td>Psychological stress induced rise in cortisol</td>
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</tr>
<tr>
<td>Boehring er et al (2010)</td>
<td>Healthy volunteers (male) mean age 24.5 n=51</td>
<td>Trier stress test</td>
<td>Word list of 10 positive, 10 negative and 10 neutral German words. Stress task 60 mins after encoding. Free Recall 135 mins after stress. Parallel design.</td>
<td>135mins</td>
<td>No effect of stress on recall.</td>
<td>Subjects not warned they would be tested on word list.</td>
</tr>
<tr>
<td>Buchanan et al (2006)</td>
<td>Healthy volunteers (male &amp; female) mean age 18.6, n=14</td>
<td>Cold Pressor</td>
<td>Word list of 20 neutral, and 20 negative English words (10 high arousal, 10 low arousal) and free recalled 10 mins (1 hour before) stress. Delayed recall and recognition memory tested immediately after stressor. Parallel design</td>
<td>10 mins</td>
<td>Stress significantly impaired recall.</td>
<td>Immediate recall task completed before the stressor. Recognition results only quoted as an index of discrimination.</td>
</tr>
<tr>
<td>Domes et al (2004)</td>
<td>Healthy volunteers (male) age 18-42 n=60</td>
<td>Trier stress test</td>
<td>Word list of 60 German words (20 neutral, 20 positive, 20 negative). Free recall &amp; recognition 24 hours after learning. Parallel design</td>
<td>5 mins</td>
<td>No effect of stress on recall. Stress before recognition impaired recall of positive words</td>
<td>Retrieval results in Table 3-1</td>
</tr>
<tr>
<td>Kuhlman et al (2005b)</td>
<td>Healthy volunteers (male) age 19-40 n=48</td>
<td>Trier stress test</td>
<td>Word list of 10 positive, 10 negative and 10 neutral words. Recalled 10 mins after stress (30 mins after encoding). Crossover design</td>
<td>10 mins</td>
<td>Negative and positive recall was affected by stress but not neutral words</td>
<td></td>
</tr>
<tr>
<td>Schoofs et al (2009)</td>
<td>Healthy volunteers (female) mean age 24.7, n=36</td>
<td>Trier stress test</td>
<td>30 nouns (neutral) recalled 24 hours after encoding. Parallel design</td>
<td>10 mins</td>
<td>No effect of stress on memory</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-2: Characteristics of studies - studies which describe the effects of an increase in cortisol prior to retrieval. Study duplicated in Table 3-1 (two parallel arms in examining effect of cortisol rise both before encoding and retrieval) ** HC eqv = hydrocortisone equivalence
3.4.4 Study Characteristics

Thirteen studies (Almela et al 2011; de Quervain et al 2000; Domes et al 2002; Domes et al 2004; Hoffman and al’Absi 2004; Jelici et al 2004; Kirschbaum et al 1996; Koessler et al 2009; Nater et al 2007; Schwabe et al 2008; Schwabe and Wolf 2010b; Smeets et al 2006a; Tops et al 2003; Wolf et al 2001b) examined the effect of an acute rise in corticosteroids prior to encoding a declarative memory word list task and 14 studies (Beckner et al 2006; Boehringer et al 2010; Buchanan et al 2006; De Quervain et al 2003; de Quervain et al 2000; Domes et al 2004; Domes et al 2005; Kuhlmann et al 2005b; Kuhlmann and Wolf 2005; Rohleder et al 2009; Schoofs and Wolf 2009; Tollenaar et al 2009a; Wolf et al 2001a) examined the effect caused by an acute increase in corticosteroids after encoding had taken place and prior to retrieval. Of these, 2 studies (de Quervain et al 2000; Domes et al 2004) contributed to both categories as they examined the effects of exogenous corticosteroids both prior to encoding, and in a parallel group, prior to retrieval.

Of the studies examining the effects prior to encoding, 3 used exogenous corticosteroids (de Quervain et al 2000; Kirschbaum et al 1996; Tops et al 2003) and 11 used stress with an associated cortisol rise (Almela et al 2011; Domes et al 2002; Domes et al 2004; Hoffman and al’Absi 2004; Jelici et al 2004; Nater et al 2007; Schwabe et al 2008; Schwabe and Wolf 2010b; Smeets et al 2006a; Wolf et al 2001b). In the pharmacological challenge studies, the mean hydrocortisone equivalent dose was 13.3mg (range 10-20mg) and all 3 had 60mins between drug administration and learning the word lists. Two studies (Schwabe et al 2008; Schwabe and Wolf 2010b) used a cold pressor paradigm the remainder of the studies used the Trier Stress Test. The mean time between the stressor and learning the word lists was 14.1mins (range 0-30 mins). Of the studies focusing on the effect of corticosteroids prior to encoding, one of the 3 drug studies (Tops et al 2003) and 5 of the 11 stress studies (Domes et al 2004; Jelici et al 2004; Schwabe et al 2008; Schwabe and Wolf 2010a; Smeets et al 2006b) included emotional words in their memory tasks.
Seven studies examined the effect of exogenous corticosteroids given prior to retrieval (De Quervain et al 2003; de Quervain et al 2000; Domes et al 2005; Kuhlmann and Wolf 2005; Rohleder et al 2009; Tollenaar et al 2009b; Wolf et al 2001a) and 7 studied the effects of stress on retrieval (Beckner et al 2006; Boehringer et al 2010; Buchanan et al 2006; Domes et al 2004; Kuhlmann et al 2005b; Schoofs and Wolf 2009). In the pharmacological challenge studies, the mean hydrocortisone equivalent dose was 24.4mg (range 10-35mg) and the mean time between administration of the drug and the retrieval task was 55.7 mins (range 30-75mins). Two of the 4 stress studies used the cold pressor paradigm (Buchanan et al 2006), whilst the rest used the Trier Stress Test to induce an increase in cortisol. The mean time between stressor and retrieval of memories was 41.3 mins (range 2-135mins).

### 3.4.5 Bias Risk

We carried out a bias risk analysis (Higgins et al 2003) (Supplementary Figure 1) examining the following biases: randomisation, allocation, blinding of participants and personnel, blinding of outcome assessment, and selective reporting bias. Any characteristic not described adequately was listed as ‘unclear bias’. By the nature of the psychological stress tasks, blinding was limited as both the participant and personnel knew who was being stressed so we rated several between-subject studies as being of high risk of this bias. Drug intervention studies tended to be at low risk of performance or detection bias due to the use of placebo control.

### 3.4.6 The effect of cortisol on encoding

#### 3.4.6.1 Neutral words - All studies

Pooling the effect of all studies with an acute rise in cortisol prior to encoding neutral words, there was no significant effect on subsequent recall but a substantial degree of heterogeneity was present in this sample (14 studies, SMD = -0.08 [-0.33,0.16]; Z = 0.67, (P=0.50); $\chi^2 = 29.81$, df 13,$P= 0.005$, $I^2 = 56\%$), (See Figure 3-2).
3.4.6.2 Neutral words - Drug studies

There was a trend towards impaired recall with exogenous administration of cortisol prior to encoding, with a small to moderate effect size and low heterogeneity between studies (3 studies, SMD = -0.40(-0.80, 0.01); Z= 1.93, P=0.05; $\chi^2 = 1.44$, df= 2, $P=0.49$ $I^2 = 0\%$), (see Figure 3-3).

3.4.6.3 Neutral words - Stress studies

There was no effect of stress prior to encoding on recall of neutral words with a substantial degree of heterogeneity between studies (11 studies, SMD = -0.03(-0.37, 0.30); Z= 0.18, P=0.86; $\chi^2 = 35.42$, df =10, $P<0.001$ $I^2 = 72\%$), (See Figure 3-4).

3.4.6.4 Positive words - All studies

Pooling the effect of all studies with an acute rise in cortisol prior to encoding positive words, there was no significant effect on subsequent recall with a moderate degree of heterogeneity present in this sample (6 studies, SMD =-0.14 [-0.44,0.17]; Z = 0.87, ($P=0.38$); $\chi^2 = 8.14$, df 5,$P=0.15$, $I^2 = 39\%$) (See Figure 3-5).
Figure 3-2: Forest plot showing the intervention of all studies (exogenous and endogenous rise in cortisol) vs. placebo with increase prior to encoding for neutral words only.

Figure 3-3: Forest plot showing the effect of exogenous, drug-induced cortisol rise vs. placebo with increase prior to encoding for neutral words

Figure 3-4: Forest plot showing the effect of endogenous, stress-induced cortisol rise vs. placebo with increase prior to encoding for neutral words
3.4.6.5 Positive words - Drug studies

Only one study, Top et al. (2003) (Tops et al. 2003), examined the effect of drugs on positive word encoding. This study showed a significant but small, detrimental effect of cortisol on the encoding of positive words (F(1, 20) = 5.72, p = 0.013).

3.4.6.6 Positive words - Stress studies

There was no effect of stress prior to encoding on recall of positive words with a small to moderate degree of heterogeneity between studies (5 studies, SMD = -0.07 [-0.36, 0.22]; Z = 0.47, p = 0.64; \( \chi^2 = 5.77, df = 4, (p=0.22) \) \( I^2 = 31\% \)).

3.4.6.7 Negative words - All studies

Pooling the effect of all studies with an acute rise in cortisol prior to encoding negative words, there was no significant effect on subsequent recall with a small degree of heterogeneity present in this sample (6 studies, SMD = -0.17 [-0.45, 0.11]; Z = 1.19, (p=0.23); \( \chi^2 = 6.86, df 5, (p=0.23) \) \( I^2 = 27\% \). (see Figure 3-6)

3.4.6.8 Negative words - Drug studies

Only one study, Top et al. (2003) (Tops et al. 2003) examined the effect of drugs on negative word encoding. This study showed a no effect of cortisol on word learning.

---

Figure 3-5: Forest plot showing the intervention of all studies (exogenous and endogenous rise in cortisol) vs. placebo with increase prior to encoding for positive words.
3.4.6.9 Negative words - Stress studies

There was no significant effect of stress prior to encoding on recall of negative words with a moderate degree of heterogeneity between studies (5 studies, SMD = -0.18(-0.50, 0.13); \( Z = 1.13, P=0.26 \); \( \chi^2 = 6.68, df = 4, (P=0.15) \) \( I^2 = 40\% \)).

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Std. Mean Difference</th>
<th>SE</th>
<th>Weight</th>
<th>IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smeets 2006</td>
<td>-0.4835</td>
<td>0.2622</td>
<td>20.0%</td>
<td>-0.48 [-1.00, 0.03]</td>
</tr>
<tr>
<td>Domes 2004</td>
<td>-0.3208</td>
<td>0.3185</td>
<td>15.1%</td>
<td>-0.32 [-0.95, 0.30]</td>
</tr>
<tr>
<td>Schwabe 2010</td>
<td>-0.4163</td>
<td>0.2921</td>
<td>17.1%</td>
<td>-0.42 [-0.99, 0.16]</td>
</tr>
<tr>
<td>Jelici 2004</td>
<td>0.4959</td>
<td>0.3216</td>
<td>14.9%</td>
<td>0.50 [-0.13, 1.13]</td>
</tr>
<tr>
<td>Schwabe 2008</td>
<td>-0.1137</td>
<td>0.229</td>
<td>23.8%</td>
<td>-0.11 [-0.56, 0.34]</td>
</tr>
<tr>
<td>Tops 2003</td>
<td>0</td>
<td>0.4336</td>
<td>9.2%</td>
<td>0.00 [-0.85, 0.85]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>100.0%</td>
<td></td>
<td></td>
<td>-0.17 [-0.45, 0.11]</td>
</tr>
</tbody>
</table>

Heterogeneity: \( \text{Tau}^2 = 0.03; \chi^2 = 6.86, df = 5 (P = 0.23); I^2 = 27\% \)

Test for overall effect: \( Z = 1.19 (P = 0.23) \)

Figure 3-6: Forest plot showing the intervention of all studies (exogenous and endogenous rise in cortisol) vs. placebo with increase prior to encoding for positive words.

3.4.7 The effect of cortisol on retrieval

3.4.7.1 Neutral words - All studies

Pooling the effect of all studies with an acute rise in cortisol prior to recalling neutral words, there was no significant effect with a substantial degree of heterogeneity present in this sample (12 studies, SMD = -0.10[-0.54,-0.33]; \( Z = 0.47, (P=0.64); \chi^2 = 61.79, df 11,P<0.000001, I^2 = 82\% \)) (See Figure 3-7)

A sensitivity analysis was performed excluding the outlier study Beckner et al (Beckner et al 2006). The task used in this study was different to the others in the analysis with subjects learning a paragraph by hearing it read out loud. Pooling now showed a significant small effect on subsequent recall with little heterogeneity present in this sample (11 studies, SMD = -0.21 [-0.40,-0.01]; \( Z = 2.10, (P=0.04); \chi^2 = 7.65, df 10, P=0.66, I^2 = 0\% \)).

A further sensitivity analysis on the other outlier study (Schwabe and Wolf 2010b) was performed but found no significant effect of this study on the effect size or heterogeneity in this analysis or subsequent sub-analyses.
3.4.7.2 Neutral words - Drug studies

There was a significant detrimental effect of exogenous administration of cortisol prior to retrieval on subsequent recall with a small effect size and low heterogeneity between studies (7 studies, SMD = -0.28 (-0.55, -0.02); Z = 2.08, P = 0.04; $\chi^2 = 4.37$, df = 6, $P=0.63$ $I^2 = 0\%$). (See Figure 3-8).

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Std. Mean Difference</th>
<th>SE</th>
<th>Cortisol Total</th>
<th>Control Total</th>
<th>Weight</th>
<th>Std. Mean Difference</th>
<th>IV, Random, 95% CI</th>
<th>Std. Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckner 2006</td>
<td>1.6439 0.2314</td>
<td>50</td>
<td>51</td>
<td>9.3%</td>
<td>1.84 [1.19, 2.10]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boehringer 2010</td>
<td>-0.3254 0.3133</td>
<td>33</td>
<td>18</td>
<td>8.6%</td>
<td>-0.33 [-0.94, 0.29]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>de Quervain 2000</td>
<td>-0.637 0.3928</td>
<td>6</td>
<td>6</td>
<td>7.8%</td>
<td>-0.64 [-1.41, 0.13]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>de Quervain 2003</td>
<td>-0.5365 0.3858</td>
<td>14</td>
<td>14</td>
<td>7.9%</td>
<td>-0.54 [-1.29, 0.22]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domes 2004</td>
<td>-0.4696 0.321</td>
<td>20</td>
<td>20</td>
<td>8.5%</td>
<td>-0.47 [-1.10, 0.16]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domes 2005</td>
<td>0.0399 0.2607</td>
<td>28</td>
<td>31</td>
<td>9.1%</td>
<td>0.04 [-0.47, 0.55]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuhlmann 2005a</td>
<td>-0.2585 0.3552</td>
<td>16</td>
<td>16</td>
<td>8.2%</td>
<td>-0.26 [-0.95, 0.44]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuhlmann 2005b</td>
<td>0.031 0.3245</td>
<td>19</td>
<td>19</td>
<td>8.5%</td>
<td>0.03 [-0.61, 0.67]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rohleder 2009</td>
<td>-0.0391 0.3345</td>
<td>19</td>
<td>19</td>
<td>8.4%</td>
<td>-0.04 [-0.69, 0.62]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schools 2009</td>
<td>0.0933 0.2358</td>
<td>36</td>
<td>36</td>
<td>9.3%</td>
<td>0.09 [0.37, 0.56]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tollenaar 2009</td>
<td>-0.6126 0.4454</td>
<td>16</td>
<td>17</td>
<td>7.3%</td>
<td>-0.61 [-1.49, 0.26]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wolf (a) 2001</td>
<td>-0.6285 0.4861</td>
<td>9</td>
<td>9</td>
<td>6.9%</td>
<td>-0.63 [-1.58, 0.32]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>266</td>
<td>256</td>
<td>100.0%</td>
<td>-0.10 [-0.54, 0.33]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\text{Tau}^2 = 0.47$; $\text{Chi}^2 = 61.79$, df = 11 ($P < 0.000001$); $I^2 = 82\%$

Test for overall effect: $Z = 0.47$ ($P = 0.64$)

Figure 3-7: Forest plot showing the intervention of all studies (exogenous and endogenous rise in cortisol) vs. placebo with increase prior to retrieval for neutral words only

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Std. Mean Difference</th>
<th>SE</th>
<th>Cortisol Total</th>
<th>Control Total</th>
<th>Weight</th>
<th>Std. Mean Difference</th>
<th>IV, Random, 95% CI</th>
<th>Std. Mean Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Quervain 2000</td>
<td>-0.637 0.3928</td>
<td>6</td>
<td>6</td>
<td>12.0%</td>
<td>-0.64 [-1.41, 0.13]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>de Quervain 2003</td>
<td>-0.5365 0.3858</td>
<td>14</td>
<td>14</td>
<td>12.4%</td>
<td>-0.54 [-1.29, 0.22]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domes 2005</td>
<td>0.0399 0.2607</td>
<td>28</td>
<td>31</td>
<td>27.2%</td>
<td>0.04 [-0.47, 0.55]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuhlmann 2005a</td>
<td>-0.2585 0.3552</td>
<td>16</td>
<td>16</td>
<td>14.7%</td>
<td>-0.26 [-0.95, 0.44]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rohleder 2009</td>
<td>-0.0391 0.3345</td>
<td>19</td>
<td>19</td>
<td>16.5%</td>
<td>-0.04 [-0.69, 0.62]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tollenaar 2009</td>
<td>-0.6126 0.4454</td>
<td>16</td>
<td>17</td>
<td>9.3%</td>
<td>-0.61 [-1.49, 0.26]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wolf (a) 2001</td>
<td>-0.6285 0.4861</td>
<td>9</td>
<td>9</td>
<td>7.8%</td>
<td>-0.63 [-1.58, 0.32]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>108</td>
<td>112</td>
<td>100.0%</td>
<td>-0.28 [-0.55, -0.02]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\text{Tau}^2 = 0.00$; $\text{Chi}^2 = 44.54$, df = 6 ($P = 0.63$); $I^2 = 0\%$

Test for overall effect: $Z = 2.08$ ($P = 0.04$)

Figure 3-8: Forest plot showing the intervention of the effect of exogenous, drug-induced cortisol rise vs. placebo with increase prior to retrieval for neutral words only

3.4.7.3 Neutral words - Stress studies

There was no significant effect of stress prior to retrieval on recall of neutral words with a substantial degree of heterogeneity between studies (5 studies, SMD = 0.21 (-0.61, 1.03); Z = 0.50, $P=0.62$; $\chi^2 = 44.54$, df =11, $P<0.000001$ $I^2 = 77\%$). The results were unchanged after
removal of the outlier study, Beckner et al (Beckner et al 2006), (4 studies, SMD = 0.05(-0.30, 0.40); Z= 0.29, P=0.77; \( x^2 = 47.73, df =11, P<0.000001 \; I^2 = 77\% \), (see Figure 3-9).

3.4.7.4 Positive words - All studies

There were no studies examining the effect of exogenous cortisol on the retrieval of words so the analysis looked at the effect of psychological stress on retrieval. There was a trend towards a detrimental effect on subsequent recall with a low degree of heterogeneity present in this sample (4 studies, SMD = -0.25 [-0.53, 0.04]; Z = 1.68, (P=0.09); \( x^2 = 1.73, df 5,P=0.63, I^2 = 0\% \), (see Figure 3-10).

3.4.7.5 Negative words - All studies

Pooling the effect of all studies with an acute rise in cortisol prior to retrieving negative words, there was a significant effect on subsequent recall with a moderate degree of heterogeneity present in this sample (6 studies, SMD = -0.31 [-0.62,0.00]; Z = 1.95, (P=0.05); \( x^2 = 7.86, df 5,(P=0.16), I^2 = 36\% \), (see Figure 3-11).

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Std. Mean Difference</th>
<th>SE</th>
<th>Total</th>
<th>Control Total</th>
<th>Weight IV, Random, 95% CI</th>
<th>Std. Mean Difference IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckner 2006</td>
<td>1.6439</td>
<td>0.2314</td>
<td>50</td>
<td>51</td>
<td>20.7%</td>
<td>1.64 [1.19, 2.10]</td>
</tr>
<tr>
<td>Boehringer 2010</td>
<td>-0.3254</td>
<td>0.3133</td>
<td>33</td>
<td>18</td>
<td>19.6%</td>
<td>-0.33 [-0.94, 0.29]</td>
</tr>
<tr>
<td>Domes 2004</td>
<td>-0.4696</td>
<td>0.321</td>
<td>20</td>
<td>20</td>
<td>19.5%</td>
<td>-0.47 [-1.10, 0.18]</td>
</tr>
<tr>
<td>Kuhlmann 2005b</td>
<td>0.031</td>
<td>0.3245</td>
<td>19</td>
<td>19</td>
<td>19.5%</td>
<td>0.03 [-0.61, 0.67]</td>
</tr>
<tr>
<td>Schoofs 2009</td>
<td>0.0933</td>
<td>0.2358</td>
<td>36</td>
<td>36</td>
<td>20.6%</td>
<td>0.09 [-0.37, 0.56]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td></td>
<td>158</td>
<td>144</td>
<td>100.0%</td>
<td>0.21 [-0.61, 1.03]</td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.79; Ch² = 44.54, df = 4 (P < 0.00001); I² = 91%
Test for overall effect: Z = 0.50 (P = 0.62)

Figure 3-9: Forest plot showing the intervention of endogenous, stress-induced rise in cortisol vs. placebo with increase prior to retrieval for neutral words only

---

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Std. Mean Difference</th>
<th>SE</th>
<th>Total</th>
<th>Control Total</th>
<th>Weight IV, Random, 95% CI</th>
<th>Std. Mean Difference IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boehringer 2010</td>
<td>-0.4815</td>
<td>0.3156</td>
<td>33</td>
<td>15</td>
<td>21.3%</td>
<td>-0.48 [-1.10, 0.14]</td>
</tr>
<tr>
<td>Kuhlmann 2005b</td>
<td>-0.4574</td>
<td>0.3291</td>
<td>19</td>
<td>19</td>
<td>19.5%</td>
<td>-0.46 [-1.10, 0.19]</td>
</tr>
<tr>
<td>Schoofs 2009</td>
<td>-0.1293</td>
<td>0.236</td>
<td>36</td>
<td>36</td>
<td>38.0%</td>
<td>-0.13 [-0.59, 0.33]</td>
</tr>
<tr>
<td>Domes 2004</td>
<td>-0.0197</td>
<td>0.3162</td>
<td>20</td>
<td>20</td>
<td>21.2%</td>
<td>-0.02 [0.64, 0.00]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td></td>
<td>108</td>
<td>90</td>
<td>100.0%</td>
<td>-0.25 [-0.53, 0.04]</td>
</tr>
</tbody>
</table>

Heterogeneity: Tau² = 0.00; Ch² = 1.73, df = 3 (P = 0.63); I² = 0%
Test for overall effect: Z = 1.68 (P = 0.09)

Figure 3-10: Forest plot showing the intervention of endogenous, stress-induced rise in cortisol vs. placebo with increase prior to retrieval. Encoding positive words only
3.4.7.6 Negative words - Drug studies

Only two studies (Kuhlmann et al 2005a; Rohleder et al 2009), examined the effect of cortisol on the retrieval of words. Kuhlman et al (2005) (Kuhlmann et al 2005a) reported a significant detrimental effect of cortisol on retrieval of negative words (p<0.01) and Rohleder et al (Rohleder et al 2009) reported no effect. When there were combined there was overall no effect on subsequent recall with a low degree of heterogeneity present in this sample (2 studies, SMD = -0.24 [-0.71, 0.23]; Z = 1.00, (P=0.32); χ² = 0.00, df 1, (P=0.95), I² = 0%) This analysis should be interpreted with caution given it only had two studies.

3.4.7.7 Negative Words - Stress studies

There was no significant effect of stress prior to retrieval on recall of negative words with a substantial degree of heterogeneity between studies (4 studies, SMD = -0.36(-0.84, 0.11); Z= 1.50, P=0.13; χ² = 7.81, df =3, (P=0.05) I² = 62%). (Figure 3-12)

Figure 3-11: Forest plot showing the intervention of all studies (exogenous and endogenous rise in cortisol) vs. placebo with increase prior to retrieval, negative words

Figure 3-12: Forest plot showing the intervention of endogenous, stress-induced rise in cortisol vs. placebo with increase prior to retrieval. Encoding negative words only
### 3.4.8 Recall pre and post encoding

The key results of the meta-analysis (sections 3.4.6-3.4.7) are summarised in Table 3-3.

<table>
<thead>
<tr>
<th>Drug + Stress</th>
<th>Effect of Intervention Prior to Encoding</th>
<th>Effect of Intervention prior to Recall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral</td>
<td>↔</td>
<td>↓*</td>
</tr>
<tr>
<td>Positive</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Negative</td>
<td>↔</td>
<td>↓</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Neutral</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>(↓)</td>
<td>↓</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stress</th>
<th>Neutral</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
</tbody>
</table>

Table 3-3: Summarised findings of the meta-analysis. ↑ = beneficial effect of cortisol, ↓ = detrimental effect of cortisol, ↔ = no effect, ( ) denotes a trend, [ ] indicates single study only, n/a = no studies, * excluding outlier.

### 3.4.9 The effect of cortisol on recognition memory

We found 8 studies that examined recognition memory; however we did not undertake quantitative analysis due to methodological differences in their analysis, and so present their results narratively here for comparison.

Only two studies examined the effect of hydrocortisone prior to encoding on neutral, positive and negative words. Tops et al (2003) (Tops et al 2003) examined the effect, recorded as ‘hit rate minus false alarm rate’. In line with their results with a similar recall task (included in our analysis), they found a significant detrimental effect of cortisol on neutral words (F(1,20)56.91, p = 0.008,) but not on positive or negative words. One study, de Quervain et al (2003) (De Quervain et al 2003) examined the effect of drug induced cortisol rise prior to recognising a previously learnt word list. They displayed their results
as a percentage of the participant’s immediate free recall score and it showed no effect of cortisol on recognition memory.

Four studies (Domes et al 2004; Schwabe et al 2008; Schwabe and Wolf 2010b; Smeets et al 2006a) examined the effect of stress pre-encoding on recognition memory of neutral, positive and negative words. Both studies by Schwabe et al (Schwabe et al 2008; Schwabe and Wolf 2010b) gave their results as a sensitivity index. In the first study (Schwabe et al 2008) they found no effect of stress on recognition but in the later study (Schwabe and Wolf 2010b) they found a significant negative effect of cortisol ($F (1, 44) = 4.1, p < 0.05$). There was no effect of word valence in either study. Smeets et al (Smeets et al 2006a) gave their results as a proportion of recalled words to recognised words and found a significant, detrimental effect of cortisol on neutral words ($p<0.01$) but did not demonstrate an effect on positive and negative words.

Domes et al (2004) had two parallel groups and examined the effect of stress both pre-encoding and pre-retrieval. Results were recorded as ‘hit rate minus false alarm rate’. They examined the effect of stress on learning of neutral, positive and negative words and found that stress pre-retrieval significantly detrimentally effected the recognition of positive words only ($p=0.025$).

One further study examined the role of pre-retrieval stress on recognition memory. Buchanan et al (2006) (Buchanan et al 2006) reported their results of neutral words using sensitivity analysis and found no significant effect of stress on recognition sensitivity.

### 3.5 Discussion

This meta-analysis found that raised cortisol prior to retrieval, but not encoding, adversely affected performance in memory tasks. The effect was most consistently seen with exogenous cortisol and with the retrieval of neutral words. The effects of emotional words largely followed the same pattern but there were too few studies to be able to confidently differentiate effects of drug and stress.
These findings are in keeping with a previous meta-analysis on the subject (Het et al 2005). They included fewer studies and also combined the analysis of recall with recognition memory, and encoding of words and pictures. However the different neural mechanisms thought to underlie these different psychological processes (Migo et al 2012) suggests that the pooling of these studies and the subsequent result should be interpreted with caution.

Similarly Wolf (2009) (Wolf 2009) concluded in his narrative review that retrieval rather than encoding is the process most vulnerable to corticosteroid. However, he stated that the emotional arousing material was most vulnerable to the effects of stress, a finding we were not able to confirm. Our results also differ from the conclusions drawn by Putman & Roelofs (2011) (Putman and Roelofs 2011) who, in their selective review of the literature, concluded that stress acted to enhance learning.

In addition to these findings in human volunteers, there is a large animal literature.

Animal studies use several different paradigms to those seen in human studies. Recognition memory is usually measured in rats by exploiting the animals’ curiosity for novel items placed in an open space, whereas spatial memory is usually measured via maze models, with both paradigms being approximations for human cognition rather than exact parallels (Lupien and McEwen 1997). Studies using both stress (usually either electric shock or predator scent exposure) and corticosterone have been performed both pre-encoding and pre-retrieval. Using a stress paradigm, Baker and Kim (Baker and Kim 2002) demonstrated acute corticosterone rise pre-encoding caused impaired performance on a non-spatial recognition memory task. Several other studies also demonstrated both tail shock and drug induced rise in corticosterone levels caused a poorer functional performance both at the encoding and retrieval stage. (Birrell and Brown 2000; de Quervain et al 1998; Diamond et al 1999), however Conrad et al (Conrad et al 2004) showed female rats showed enhanced performance in the water maze. The chronic effects of raised corticosterone (either exogenous or endogenous) in animals results in dendritic remodelling, especially in the CA3 area of the hippocampus, and a decrease in the number of synapses overall in the hippocampus, associated with a decline in functional performance in memory tasks (Rothman and Mattson 2010).
Animal work has shown that membrane-bound mineralocorticoid and glucocorticoid receptors can produce rapid non-genomic responses to elevated stress hormones and this can lead to an increase in NMDA receptor expression in the hippocampus (Joëls 2008; McEwen 2000; Olijslagers et al 2008). Presynaptically these receptors, via the ERK1/2 pathway, increase the chance of glutamate containing vesicles being released. As a hypothesized mechanism for cognitive impairment caused by acute glucocorticoids in humans, this non-genomic action would explain the rapid effect of stress hormones on hippocampal mediated learning.

Pharmacological challenge functional MRI studies have demonstrated a rapid effect of hydrocortisone on the hippocampus (Symonds et al 2012), prefrontal area and amygdala (Henckens et al 2009; Henckens et al 2010). Many studies excluded ‘cortisol non-responders’ from their results to study the effect of stress-induced hypercortisolaemia on cognition. However, this may well be introducing a source of bias into the literature. One fMRI study demonstrated that those subjects who failed to produce elevated levels of cortisol in response to a stressor showed a lower level of hippocampal activity during encoding at baseline (Khalili-Mahani et al 2010). There was a marked degree of variability in the psychological stress intervention results and some of this could be due to selecting for the subset of the population which produce high levels of cortisol in response to a social stress task.

This meta-analysis does not demonstrate a major impact of the emotional valence of words on the effects of cortisol on their encoding or retrieval although it is not possible to exclude a modulating effect. In animal work, it has previously been argued that the amygdala acted as a ‘neural gateway’ for the action of corticosteroids on the hippocampus (Roozendaal et al 2006), with a mutual enhancement facilitating accurate recall of emotionally charged, and therefore evolutionarily important, pieces of information.

As discussed above, we specifically examined cortisol increases occurring less than 4 hours before retrieval in order to explore non-genomic effects on behaviour occurring around the time of stressful events. Given the effects were found after administration of drug alone, the deleterious effect of corticosteroids/acute stress on retrieval can be
hypothesised to be mediated by mineralocorticoid and glucocorticoid receptors located in frontal lobes, amygdala and hippocampus. An alternative explanation could be that these results represent an interruption to consolidation of memories, however given the wide range of times of administration of cortisol after encoding (30mins - 24 hours) this is unlikely. Following a stressful event, a number of catecholamines including noradrenaline and adrenaline, as well as corticosteroids, are released. There is an immediate action of noradrenaline, measurable on the hippocampus and amygdala (Joëls et al 2011). Some researchers have argued that this is the key mechanism underlying any immediate stress effect on memory given the temporal relation between the fast acting non-genomic catecholamines and the later genomic action of cortisol. However, the rapid action of corticosteroids via mineralocorticoid and glucocorticoid receptors (Karst et al 2005) demonstrates an equally immediate, non-genomic action of cortisol on the hippocampus. The effect of a stress induced rise of cortisol on encoding and retrieval seems far more variable and also gave a less consistent effect on memory. This could represent a greater variability in the effectiveness in these tests in causing a neurochemical effect. Indeed all studies reported that there were a proportion of participants who failed to show a rise in cortisol, pulse or bioelectrical impedance, although these participants were discarded for the purpose of our analyses. The effect of other catecholamines (e.g. noradrenaline) may enhance performance and ameliorate the detrimental effects of cortisol. Additionally, other psychological effects of the non-pharmacological stressor may have effects, both detrimental and enhancing, on memory. This greater variability of a stress task may explain the greater heterogeneity seen in the findings of this current review.

This meta-analysis is the largest to date but the overall number of studies and participants was still relatively small. We attempted to include only well-defined groups to try and reduce heterogeneity and to increase validity; however considerable heterogeneity was still present suggesting unaccounted variation between studies. In addition studies were excluded if they did not meet our strict criteria which may have altered the overall conclusions. The sub-group analyses allowed for a useful exploration of the effect of emotion, however these analyses were small and therefore should be interpreted with caution. We only examined verbal memory using list-learning tasks and our results cannot
be generalised to memory for non-verbal material. Another limitation of this study was that only studies published in English were included which may result in publication bias however a funnel plot analysis did not reveal an absence of small, negative studies.

3.6 Conclusions

This meta-analysis showed that there was a significant, detrimental effect of a drug-induced rise in cortisol after encoding on the recall of neutral words, with no clear effect on emotional words. Furthermore, more robust conclusions can be drawn from pharmacological intervention studies, given the lack of heterogeneity compared to the higher level of heterogeneity seen in the psychological stress tasks. The timing of the interventions related to encoding is important with a significant result only being found when there was a rise of cortisol post encoding and pre-retrieval.

There was insufficient evidence to on which to draw firm conclusions regarding whether the acute effects of cortisol differentially influenced retrieval of emotional, compared with non-emotional, words. Given the potential importance of this for understanding cognitive processes in stress-related disorders more research using emotional stimuli is required.

3.7 References


4 Chapter 4

Detection of the acute effects of hydrocortisone in the hippocampus using pharmacological fMRI

Catherine S. Symonds*, Shane McKie, Rebecca Elliott, John Francis William Deakin, Ian M. Anderson

Neuroscience and Psychiatry Unit, G.907 Stopford Building, The University of Manchester and Manchester Academic Health Sciences Centre, Oxford Rd, Manchester M13 9PT. Tel 0044 161 275 1727 Fax: 0044 161 275 7429 email Catherine.Symonds@manchester.ac.uk

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4.1 Abstract

Impaired hippocampal function is believed to be important in the pathogenesis of depression. The hippocampus contains a high concentration of both mineralocorticoid (MR) and glucocorticoid receptors (GR), and the experimental administration of corticosteroids has been reported to mimic memory impairments seen in depression. Using pharmacological functional magnetic resonance imaging (phMRI) we investigated whether hippocampal function is altered after acute administration of hydrocortisone. Changes in BOLD signal following infusion of 100mg hydrocortisone given as a rapid intravenous bolus were measured in 14 healthy volunteers in a within-subject placebo-controlled crossover design. Subsequently, subjects completed an n-back task during an fMRI scan. Hydrocortisone infusion caused a significant, time-dependent increase in fMRI BOLD signal in hippocampus reaching a maximal effect at 11-19 minutes. The n-back task increased BOLD signal in prefrontal and parietal cortical areas and decreased it in the hippocampus. After hydrocortisone the left hippocampal decrease in BOLD signal was attenuated with the magnitude of attenuation correlating with the increase seen after hydrocortisone infusion. No difference in behavioural task performance was observed. The results suggest acute hydrocortisone has rapid direct and modulatory influences on hippocampal function, probably acting through non-genomic GR or MR signalling. Hydrocortisone infusion phMRI may be a useful tool to investigate hippocampal corticosteroid receptor function in depression.

Keywords: hippocampus, working memory, fMRI, corticosteroids

4.2 Introduction

Abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis have been reported in a number of different psychiatric conditions, including depression and anxiety (de Kloet et al 1998). This dysregulation is characterised by elevated levels of cortisol (Porter et al 2004) as well as altered acute HPA axis responses (Kudielka and Wust 2010). Corticosteroids, through their action on corticosteroid receptors, modulate neuronal networks underlying the control of mood and memory processes (Lupien et al 2007).
Altered function of central corticosteroid receptors may play a key role in changes in function of the HPA axis in psychiatric disorders (Porter et al 2004).

Previous work has focused on HPA axis control at the level of the hypothalamus and pituitary. However in the last decade there has been increasing emphasis on the importance of higher centres concentrating on the hippocampus, along with the prefrontal cortex and amygdala (de Kloet et al 1998; Herman et al 2005). It has been recognised that the hippocampus exerts a mainly inhibitory feedback of the HPA axis, with studies on hippocampectomised rats demonstrating higher basal ACTH levels (Feldman and Weidenfeld 1993; Wilson et al 1980). In addition, a recent study demonstrated that the human hippocampus is necessary to mount a cortisol response to psychological stress (Buchanan et al 2009).

Homoeostasis of the HPA axis is achieved via the action of cortisol on glucocorticoid (GR) and mineralocorticoid receptors (MR). These receptors are found in high concentrations in the prefrontal cortex, amygdala, and hypothalamus. Particularly high concentrations of both MR and GR are expressed in the hippocampus, with evidence that MRs are located mainly in the ventral hippocampus in rats (van Eekelen et al 1991). Previous research had emphasised that MR are occupied at 90% levels at basal levels of cortisol and it has been believed that occupation by its major ligand, aldosterone, contributes little to the feedback control of cortisol (Spencer et al 1993). However it has recently been shown that the MR agonist fludrocortisone acutely decreases afternoon/evening cortisol concentrations in humans showing that there is remaining functional capacity and involvement in feedback control (Buckley et al 2007). GR and MR predominantly reside in the cytoplasm; upon binding corticosterone/cortisol they are quickly translocated into the nucleus where they regulate the expression of target genes (Nishi and Kawata 2007). In addition to these genomic effects, corticosteroids also have rapid non-genomic effects that influence glutamate release and affect neuronal signalling. These acute actions are mediated by GR receptors (Löwenberg et al 2008), but research also suggests this rapid action is mediated at least in part by membrane bound MR (Joëls et al 2009; Olijslagers et al 2008). Hippocampal electrophysiological responses have been observed within minutes
of corticoid infusion corresponding to reports of brain penetration within 2 minutes of infusion (Makara and Haller 2001; Schwartz et al 1972; Venero and Borrell 1999).

Tests of working memory, the ability to keep information active for a short time, are associated with enhanced brain response in a network of regions critically involving frontal and parietal cortical regions including the dorsolateral prefrontal cortex (dIPFC) and ventrolateral prefrontal cortex (vIPFC) (Owen et al 2005). They also lead to deactivation of other brain areas, including the anterior hippocampus (Esposito et al 2006). The hippocampus forms part of what has been termed the ‘default network’, areas which show greater activity in the resting state, or internally focused tasks compared to those with an external focus during which a deactivation is seen (Passamonti et al 2011). However, following psychological stress this hippocampal deactivation during working memory tasks is attenuated (Qin et al 2009; Weerda et al 2010). In addition to the functional neuroimaging effects, the acute administration of cortisol also alters performance on working memory tasks, including the n-back task, but this is situation dependent and has been shown to both reduce (Al’Absi et al 2002; Lupien et al 2007) and improve performance (Oei et al 2009) depending on the task demand. These studies suggest that corticosteroid modulates performance on working memory tasks and that this is associated with frontal and hippocampal function.

Our aim in this study was to use functional MRI combined with drug challenge (pharmacoMRI, phMRI) to image the effects of acute hydrocortisone infusion on human hippocampal function. Based on the recent animal literature that the non-genomic action of GR and membrane bound MR increase the probability of glutamate release in hippocampus (Di et al 2009; Olijslagers et al 2008), we hypothesised that acute hydrocortisone would lead to a rapid increase in bold oxygen level dependent (BOLD) signal in the hippocampus. In addition, we hypothesised that acute hydrocortisone would impair working memory and attenuate hippocampal deactivation during an n-back task.
4.3 Experimental Procedures

4.3.1 Participants

Fifteen right-handed, healthy volunteers (7 females) were recruited from staff and students of the University of Manchester (mean age 23.9 ± 3.8, range 19-30). One male subject was later excluded due to a head-coil malfunction after his first infusion scan; therefore results are reported for 14 subjects. All subjects were screened for substance misuse and psychiatric disorder using the SCID (First et al 2002a) and were free from major physical and psychiatric illness; and from psychiatric illness in first degree relatives. All the female subjects were on long term hormonal contraception to control for the effects of menstrual cycle on endogenous cortisol levels to allow reliable within subject comparison (Symonds et al 2004) but subjects were otherwise medication-free, drank less than 21 units of alcohol/week and were caffeine free on the scan days. All subjects were right-handed (Briggs and Nebes 1975), and had no contraindications to MRI scanning. Their IQ was measured using Ammons’ quick test (mean 110 ±S.D. 9.3) (Ammons and Ammons 1962). Local research ethical approval was obtained and all participants gave written, informed consent.

4.3.2 Experimental design

Subjects were scanned on two occasions separated by 1 week in a randomized, single blind, balanced order, cross-over design in which they receiving an intravenous bolus of hydrocortisone 100mg on one occasion and saline on the other. One hour before the scanning session subjects were cannulated with the intravenous line kept patent using normal saline. The scanning session, which took place between 12noon and 3pm, consisted of a structural MRI scan, a challenge phMRI scan, during which they received hydrocortisone/saline infusion, followed by two modulatory phMRI scans in which participants undertook a memory task and an n-back task, with only the latter reported here. Blood samples were taken for measurement of serum cortisol through the cannula at baseline and at 25 min and 60 min post infusion.
Immediately, following the scanning session participants undertook a series of computerised neuropsychological tasks including an out-of-scanner n-back. All computerised tasks were run on a PC in E-Prime 2.0 (Psychology Software Tools Inc.). Only the n-back tasks (in scanner and out of scanner versions) are reported here.

4.4 Hydrocortisone challenge phMRI scan

Hydrocortisone sodium succinate (Pharmacia) 100mg diluted in normal saline, or an equal volume of normal saline (36 mls), were administered by infusion pump over 90 seconds starting 5 min into a 25 minute high resolution fMRI scan (scanning parameters detailed below). A supra-physiological dose was used to overcome inter-subject variability and ensure saturation of corticosteroid receptors (Buttgereit et al 2002). During the scan, participants performed computerised subjective self rating at 2 min. intervals.

4.4.1 N-Back scan

A blocked version of the n-back task adapted from the event-related task of Callicott et al was used (Callicott et al 2003), and back projected onto a screen visible to the participant via two mirrors attached to the head coil. The task started approximately 35 minutes after the infusion. The task responses were acquired from the participant using a fibre-optic button box held in the right hand as illustrated in Figure 4-1.
4.4.2 Out of scanner n-back task

Numbers were displayed sequentially for 500ms each with the participant prompted to state the number displayed on the current screen (zero back) or 1, 2 or 3 screens back. The levels were presented in increasing order of difficulty with 30 numbers per level 6 prompts per level at variable intervals. The task duration was 7.3 mins and commenced 10 mins after the end of the infusion scan.

4.4.3 Scanning protocol and data acquisition

Scanning was carried out on a Philips Intera 1.5 Tesla MRI scanner. Data were acquired with T2*-weighted, gradient echo, echo planar imaging (EPI).

For the hydrocortisone challenge phMRI scan a high resolution technique was used with a TR = 3 s, TE = 40 ms, isotropic 1.5 mm voxels, and a total of 26 slices angled obliquely centred on the hippocampi but also covering the orbital frontal and anterior cingulate.
cortices and the top of the cerebellum. The scan was 25 minutes in length and consisted of 500 volumes.

For the n-back task, full brain coverage was used with TR=2 sec, TE=40 ms, 3.5mm in-plane resolution, 4.5mm slice thickness with 0.5mm slice gap and 29 slices.

### 4.4.4 Cortisol assays

These were analyzed at Oxford Brookes University Laboratories. Cortisol was measured by a double-antibody radioimmunoassay (RIA) with the inter- and intra-assay coefficients of variation (CVs) 5.7% and 4.4% respectively over the range of the standard curve and the limit of detection was 0.5ug/100ml respectively.

### 4.4.5 Data Analysis

Behavioural, hormonal and demographic data were analyzed using Statistical Package for Social Science version 15.0 (SPSS Inc, 2006). Behavioural data from the n-back tasks and hormonal data were analyzed using repeated measure ANOVA with two within subject factors, condition (hydrocortisone and saline) and difficulty (0-back, 1-back, etc) or time, followed by post-hoc paired t-tests. The outcome measures used for the n-back task were speed of response, number omitted and number correct. Results are presented as mean ± SD.

fMRI and phMRI data were processed and analysed using Statistical Parametric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk/spm). The pharmacological imaging data were analyzed using a pseudo-block pharmacological MRI analysis technique (McKie et al 2005) using a drug x time repeated measure random effects ANOVA.

The data from both the fMRI and phMRI scans were spatially pre-processed using standard protocols. They were realigned, using the first scan as a reference, normalised into the Talairach and Tournoux stereotactic space (Talairach and Tournoux 1998) using MNI templates then spatially smoothed using a 8 mm Gaussian kernel.
For the phMRI, first level analysis was performed with each scan split into 10 time-bins of 2 minute duration starting 1 minute after the start of scanning with each time-bin compared with a baseline time-bin leading up to the start of the infusion (-2 to 0 min). Each individual’s realignment parameters were also added as covariates of no interest. Scans were controlled for the effects of drift by subtracting the time-bin effects for each subject’s saline scan at the individual subject level. The statistical parametric maps from each individual data set were then entered into second-level, random effects analyses to account for both scan-to-scan and subject-to-subject variability. A fully-factorial analysis of variance was then performed with subsequent hydrocortisone-saline and saline-hydrocortisone contrasts carried out on the data following baseline to examine the effect of hydrocortisone infusion. The a priori region of interest (ROI) was the hippocampus, which expresses high concentrations of GR and MR (Young et al 1998). In particular, given the association with novelty and the action of corticosteroids, a mask of the anterior hippocampus was used with a 7mm sphere bilaterally at ±30, -16, -18 using dimensions and co-ordinates chosen from the Talairach Atlas (Talairach and Tournoux 1998). We report significant responses at a small volume corrected threshold of p(Family Wise Error; FWE)<0.05 for the anterior hippocampus and at a restricted coverage corrected threshold of p(FWE)<0.05 for non-hypothesised areas. The cerebellar vermis was chosen as a reference region to control for non-specific effects (McEwen et al 1986).

For the n-back task, both levels of n-back were contrasted to 0-back and drug vs. saline comparisons made at the first level for each subject. Second level processing was performed using one-sample t-tests to explore 1) the main effect of task at the highest working memory load under saline (2-back minus 0-back and 0-back minus 2-back) and 2) effect of drug (hydrocortisone minus saline and saline minus hydrocortisone) during the task (2-back minus 0-back and vice versa). A priori regions of interest taken from the literature (Owen et al 2005) were identified using the WFU Pick Atlas tool (Maldjian et al 2003). These consisted of lateral premotor cortex (BA6), dorsal anterior cingulate cortex (BA32), dLPFC (BA9, 46) vLPFC (BA44, 45, 47), medial posterior parietal and inferior parietal lobule (BA 7, 40) and medial cerebellum. In addition we included the anterior hippocampal region of interest from the challenge phMRI. An effect of hydrocortisone was
expected to be seen in the hippocampus, vPFC and dLPFC. Within these areas we report as significant areas Family Wise Error (FWE) corrected p(FWE)<0.05 for the region of interest but not corrected for multiple comparisons. Other areas surviving p(FWE)<0.05 at a whole brain level are also reported for interest but not further interpreted.

We were also interested in examining whether the degree of hippocampal activation after hydrocortisone challenge was related to its effects on task-mediated neuronal activation. We therefore correlated Area under the Curve (AUC) BOLD signal difference between cortisol and saline for challenge phMRI and percentage BOLD signal change for n-back task after cortisol-saline subtraction.

4.5 Results

4.5.1 Serum cortisol levels

The hydrocortisone infusion was well tolerated with no participants experiencing any subjective effects (data not reported). There was a significant effect of hydrocortisone administration on serum cortisol concentration (drug x time: F (2, 24) =74.650, p<0.001). After saline, cortisol concentrations fell slightly (baseline 8.6 ±5.7 µg/100ml, +25min 6.4 ±5.2 µg/100ml, +60min 6.1 ±6.4 µg/100ml) whereas after hydrocortisone robust increases were seen (baseline 12.8 ±4.7 µg/100ml, +25min 27.3 ±11.4 µg/100ml, +60min 22.8 ±10.5 µg/100ml). There was no significant drug x sex interaction (F(1,22)=0.114, p=0.742) neither was there a significant drug x time x sex interaction observed (F(2,22)= 0.362, p=0.700).

4.5.2 Hydrocortisone challenge phMRI

Following hydrocortisone infusion, compared with saline, there was a significant increase in BOLD signal in the right hippocampus (x=30, y= -18, z= -19, Z=3.39, p (FWE) =0.039, p_{unc}=0.00035) with a trend towards a significant increase in the left anterior hippocampus (x=-26, y= -12, z= -14, Z=3.14, p(FWE)=0.091, p_{unc}=0.00085). No activation was seen in the control regions (right and left cerebellar vermis). The change in BOLD signal over time
(hydrocortisone-saline subtraction) is illustrated for both hippocampi and the right
cerebellar vermis in Figure 4-2.

The reverse subtraction (saline-hydrocortisone) showed no significant activation.

Figure 4-2: This illustrates the increase in BOLD signal following a subtraction of the
hydrocortisone minus saline phMRI scans, with error bars indicating standard error
(SE). Clockwise from top left: a) an overlay shown with a threshold of 0.05 for
illustrative with ROI highlighted b) left hippocampus b) right hippocampus and c) in the
control region of the vermis during the phMRI infusion, cortisol-saline subtraction.
Ordinate on bar chart shows degree of BOLD signal increase across time post-infusion
in the subtraction

4.6 N-Back imaging data

Under the saline condition the 2-back compared to 0-back subtraction showed increased
BOLD signal in bilateral medial posterior parietal and lateral premotor cortices, and dlPFC
and vlPFC in keeping with the areas previously identified (Owen et al 2005). A decrease in
BOLD signal during the task was seen in a number of frontal areas and the left
hippocampus (see Supplementary Table S1 for results, Appendix 6).

In the hydrocortisone-saline there was significant increased signal in the left hippocampus,
right vlPFC (pFWE = 0.035) and in the non-pre hypothesised area of the left precentral
gyrus at pFWE (whole brain corrected) p<0.05 (Table 4-1). The result in the hippocampus reflected a reversal of the decreased signal seen under saline (Figure 4-3). There were no significant results in the saline-hydrocortisone subtraction.

<table>
<thead>
<tr>
<th>Area</th>
<th>Co-ordinates (MNI)</th>
<th>Cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BA  Side  x  y  z  K   Z   pFWE</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>28  L  -33  -14  -15  14   3.29  0.007</td>
<td></td>
</tr>
<tr>
<td>vlPFC</td>
<td>47  R  31  25  -10  19   3.72  0.035</td>
<td></td>
</tr>
<tr>
<td>Precentral Gyrus</td>
<td>4   L  -40  -11  40  66   4.82  0.049*</td>
<td></td>
</tr>
</tbody>
</table>

Table 4-1: Shows results of cortisol minus saline 2-0 back subtraction by area for the n-back task. *whole brain corrected (FWE=Family wise error, BA = Brodmann Area, K=cluster size at punc<0.005).
4.6.1 N-Back behavioural data

In the out of scanner n-back task, there was a significant effect of task difficulty ($F(3, 39) = 5.129$, $p=0.004$) but not of drug condition ($F(1, 39) = 2.804$, $p=0.12$) or of task difficulty by drug interaction ($F(3, 39) = 1.190$, $p=0.33$). However participants had a lower number correct in the hardest 3-back condition following hydrocortisone compared with saline with a trend to significance using a paired $t$-test ($t=1.790$ df= 13; $p=0.097$). No significant effects were demonstrated in reaction time. Due to technical problems with the fibre-optic button box, behavioural data could only be analyzed for 11 subjects. In the in-scanner version there was a significant effect of task difficulty ($F(2, 20) = 23.25$, $p=0.001$) but no significant effect of condition ($F(1.20) = 0.615$; $p=0.451$) or task difficulty by drug interaction ($F(2.20) = 45.28$, $p=0.615$).
4.6.2 Correlations between challenge phMRI and n-back phMRI responses

As illustrated in Figure 4-4, there were significant, positive correlations between the BOLD signal during the phMRI and the n-back tasks. During the phMRI scan, the increases in BOLD signal after hydrocortisone in the left and right hippocampi were highly correlated ($r=0.909$, $p<0.001$). Furthermore, the hydrocortisone-induced BOLD signal change in the left hippocampus during challenge phMRI correlated with the BOLD signal in the left hippocampus (i.e. attenuation of the decrease during the task) following the 2-back vs. 0-back subtraction ($r=0.784$, $p=0.002$). These correlations remained significant when re-analyzed without the two most extreme values ($r=0.650$, $p=0.030$ and $r=0.597$, $p=0.041$, respectively). There were no significant correlation between hippocampal changes and cortical changes in n-back.

![Graphs showing correlations between BOLD signal changes in hippocampus and phMRI responses](image)

Figure 4-4: Illustration of the correlations between Area Under the Curve (AUC) BOLD signal cortisol-saline differences for challenge phMRI and with percentage signal change for n-back task after cortisol-saline subtraction.
4.7 Discussion

In this study we were able to demonstrate acute effects of hydrocortisone in the brain using both challenge and task modulatory phMRI (Anderson et al 2008b). The hydrocortisone challenge resulted in increased hippocampal BOLD signal compared with saline and in a subsequent n-back task it increased right vlPFC signal and reversed the decrease found in the left hippocampus under saline. We found a high correlation between hydrocortisone-induced BOLD signal increase to challenge and failure to suppress hippocampal activity under hydrocortisone during the n-back task.

The use of functional magnetic resonance imaging (fMRI) to investigate the effects of drugs on neuropsychological tasks (modulatory phMRI or phMRI) is well established (Anderson et al 2008b). We, and others, have shown that it is also possible to examine the acute effects of a drug given intravenously using BOLD signal over time as a measure of functional neuronal activation or deactivation by that drug (Anderson et al 2008b). This is a technique that is being increasing used in both animal (Ceolin et al 2007; Ferris and Stolberg 2010) and human studies (Rose et al 2006b) and we have previously demonstrated acute brain activation by the serotonergic drugs mCPP (Anderson et al 2002; McKie et al 2011) and citalopram (McKie et al 2005).

Our challenge phMRI results suggest that hydrocortisone has a rapid effect on hippocampal function with the maximal effect between 11-19 minutes post infusion consistent with an action through non-genomic signalling. Although significant activation was only found in the right hippocampus there was a trend to significance in the left hippocampus (Table 4-1) and a high correlation between the increases in BOLD signal the two sides in (Figure4- 4) supporting a probable bilateral effect as would be expected from a drug action. The percentage increase in BOLD signal demonstrated here using phMRI is in keeping with signal change demonstrated by others using the technique, both in humans and in animals (Ferris and Stolberg 2010; McKie et al 2005). Hydrocortisone rapidly enters the brain after an intravenous bolus with maximal concentrations seen at 2 mins in mice using microdialysis (Schwartz et al 1972). In non-sedated rats using a challenge phMRI paradigm (Ferris and Stolberg 2010) a significant, dose-related response in the hippocampus to
corticosterone after 1 minute was demonstrated. This rapid effect is consistent with in vitro studies which indicate that this may be a non-genomic mechanism acting via membrane bound MR, suggesting corticosteroid modulation of glutamate neurotransmission (Karst et al 2005). The only other published challenge phMRI study using hydrocortisone in humans, in a small parallel group study, recently reported reductions in BOLD signal in hippocampus and amygdala after a bolus of 10mg hydrocortisone, maximal at 30-35min after injection (Lovallo et al 2009). However the findings were not robust and indeed statistically questionable since they only report effects at a significance level of one standard deviation difference between hydrocortisone and saline conditions. In addition the methodological differences between studies, particularly in the dose and the time course studied, make direct comparisons difficult.

As discussed in the introduction, acute stress and corticosteroids affect cognitive function more rapidly than can be explained by genomic effects. Our study administered the n-back task 35 min after a large intravenous bolus of hydrocortisone and we found that hydrocortisone increased right vIPFC activation, an area which is part of the working memory network. In addition it attenuated the decreased hippocampal BOLD signal found under the saline condition. This can be interpreted as deactivation of the default network as a part of focusing on external stimuli or goal related behaviour (Esposito et al 2006). One interpretation of our results is therefore that acute hydrocortisone impairs the function of the working memory network related to decreased ability to switch attention to external stimuli or goals. Whether this is an adaptive (i.e. protective) or a non-adaptive response to acute stress is likely to depend on the circumstances. We did not find that hydrocortisone significantly affected performance on the n-back task in our subjects making the functional significance of our imaging findings uncertain. However we tested high functioning subjects and the study numbers were small making it likely that we had insufficient power to detect any effect. Previous studies have reported impaired working memory performance after acute hydrocortisone administration (Lupien et al 1999; Oei et al 2006; Qin et al 2009) and it could contribute to deficits in learning under stress that have been shown in both health and in disease states, such as depression and post traumatic stress disorder (Hinkelmann et al 2009; Kaymak et al 2010; LaGarde et al 2010).
Limitations include the relatively small number of participants included and the single blind nature of the study. In addition our use of high resolution fMRI for the challenge phMRI scans led to limited brain coverage. However there were no detectable subjective effects from hydrocortisone making it less likely those participants were aware of the randomization, and the cross-over, within-subject design increased the power of the study. Challenge phMRI is potentially susceptible to scanner drift when measuring BOLD signal change overtime (Anderson et al 2008b) but we have controlled for this by using the saline control and the balanced order of scans. Previous research suggests that a rise in systemic blood pressure following hydrocortisone can be first detected after 140 minutes post infusion (Dodt et al 2000) making blood pressure-induced changes in cerebral blood flow during our study unlikely to account for the results. In addition we found no effect in our a control area, the cerebellum, an area with a low concentration of GR and MR but which would be susceptible to any global change in blood flow. This argues against a global effect on blood flow explaining our findings. The dose of hydrocortisone we used was supra-physiological and therefore may not reflect physiological effects of cortisol acting on the hippocampus, for example during response to a stressful stimulus. However, the dose administered in this study was designed to provide proof of concept of activation of corticosteroid receptors by a ‘pharmacological’ challenge designed to saturate MR and GR. Our results suggest that high dose challenge may be useful as a test of glucocorticoid receptor sensitivity in areas involved the higher control of the HPA axis, such as the hippocampus. It will however be important in the future to examine the effects of hydrocortisone challenge in the physiological range to understand the actions of cortisol on the brain during stress.

In summary, we have been able to show acute effects of hydrocortisone both directly on hippocampal function and on neural activation during an n-back task using phMRI. This effect is likely to be mediated by non-genomic effects of hydrocortisone on MR and/or GR and may be a mechanism by which stress exerts rapid effects on brain function. The use of hydrocortisone-infusion phMRI may therefore be a useful tool to investigate hippocampal corticosteroid receptor function in psychiatric conditions such as depression where abnormalities of HPA axis function are believed to play an important role in aetiology.
This work was supported by a clinical fellowship from NIHR Manchester Biomedical Research Centre and a grant from the University of Manchester Magnetic Resonance Imaging Facility.

4.8 Role of the funding source

This work was supported by a clinical fellowship from NIHR Manchester Biomedical Research Centre and a grant from the Magnetic Resonance Imaging Facility, Manchester Wellcome Trust Clinical Research Facility; the funders had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

4.8.1 Acknowledgements:

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4.9 References


Chapter 5

Emotional and working memory in treatment resistant depression: an fMRI study

Catherine S. Symonds*, Shane McKie†, Rebecca Elliott†, R. Hamish McAllister-Williams‡, I. Nicol Ferrier‡, John Francis William Deakin†, Ian M. Anderson†

*1. Neuroscience and Psychiatry Unit, G.907 Stopford Building, The University of Manchester and Manchester Academic Health Sciences Centre, Oxford Rd, Manchester M13 9PT. Tel 0044 161 275 1727 Fax: 0044 161 275 7429

email Catherine.Symonds@manchester.ac.uk

2. Academic Psychiatry, Wolfson Research Centre, Campus for Ageing and Vitality, Newcastle upon Tyne, NE4 5PL

* Corresponding author
5.1 Abstract

Neurocognitive deficits and mood-congruent biases have been reported in depression, although there are inconsistencies which may be related to the population studied. We investigated the neural basis of working and episodic memory, including the encoding and retrieval of emotional memories in patients with treatment resistant depression (TRD) on the basis that this population may be more homogeneous and likely to display neurocognitive deficits than an undifferentiated depressed group. Using functional magnetic resonance imaging (fMRI) we compared the behavioural and BOLD signal response of 30 healthy volunteers and 27 medicated TRD patients using the working memory n-back task and an emotional encoding and retrieval pictures task. There was no difference in behavioural performance on the memory tasks. During the n-back task the TRD patients showed a reduced activation of the dorsolateral prefrontal cortex compared to the controls. In the emotional memory task, the TRD group had less activation of the posterior cingulate cortex whilst encoding both positive and neutral images, and reduced anterior cingulate cortex activation whilst retrieving positive images compared to neutral. Whilst retrieving images irrespective of valence, the TRD group demonstrated an increased activation of the posterior insula compared with controls. The decreased activation of the prefrontal cortex during the n-back task was demonstrated here in a treatment resistant population. This study also suggests there is an alteration in the functioning of the cingulate and insular cortex in the encoding and retrieval of positive emotional memories in this group of patients with TRD. Further study is needed to determine whether there is alteration of negative emotional memory processing and what relationship this has to the degree of depression or a failure to respond to treatment.

Keywords: emotional memory, working memory, fMRI, treatment resistant depression, cingulate, insula.
5.2 Introduction

Mood-congruent cognitive biases and neurocognitive deficits are considered to be central to the diagnosis of depression (American Psychiatric Association 1994; Castaneda et al 2008). In particular, impairments in attention and concentration; working memory; and declarative memory have been demonstrated (Chamberlain and Sahakian 2006). These neurocognitive impairments have been shown to correlate with under activity of the amygdala, anterior cingulate and hippocampus using functional Magnetic Resonance Imaging (fMRI) (Cooney et al 2010; Davidson et al 2003; Harmer et al 2011).

Several studies of patients with depression have demonstrated deficits in executive function (Castaneda et al 2008) with failures of attention and concentration being most commonly reported (Basso and Bornstein 1999; Chamberlain and Sahakian 2006; Egeland et al 2003). Working memory appears to be commonly affected in depression with problems with memory load demonstrated by the digit span task (especially backward span) and other tests of verbal and non-verbal working memory (Castaneda et al 2008); as well as problems with set-shifting (Lange et al 2012). However, other studies have not found working memory deficits in depressed patients (Purcell et al 1997). This disparity may be accounted for by differences in the study populations as these deficits appear to be more pronounced in both more severe illness and in older patients (Austin et al 2001). More subtle deficits are also evident using fMRI with reduced BOLD signal in the dorsolateral prefrontal cortex and anterior cingulate during higher levels of the n-back working memory task (Lange et al 2012; Rose et al 2006a). In a version of the n-back task using emotional faces as the stimuli, similar deficits have been demonstrated with reduced activity of the ventrolateral prefrontal cortex as well as the dIPFC in response to positive distracters (Kerestes et al 2012).

Depression also affects memory during encoding and retrieval tasks with patients performing less well on tests of word learning, such as the Rey Auditory Verbal Learning Test (Neu et al 2005) and of pictures (Ally 2012). The differences between people with depression and controls are more pronounced when an emotional component is introduced.
to the task. It has been argued that problems with emotional memory are at the heart of depressive cognitions and biases towards negative material (Scher et al 2005). Patients with depression show a bias towards recalling negative autobiographical memories (Brittlebank et al 1993), and words (Liu et al 2012). They also show a bias against recognising positive material such as happy facial expressions (Anderson et al 2011b; Salvadore et al 2009). A bias is also evident in patients in remission from depression, with a significant response in the frontal pole to positive pictures when compared to neutral images (Elliott et al 2012). The anterior cingulate deactivation has also been demonstrated in response to positive stimuli in a depression (Zhang et al 2013). However, this is not a consistent finding and several studies have failed to find a difference in emotional memory compared to controls (Anderson et al 2011a).

Amongst depressed individuals, approximately 30% of patients fail to respond to ≥2 treatments (treatment resistant depression, TRD) and about 10% of patients fail to respond to multiple treatments and have a chronic course (Anderson et al 2008a). To date, BOLD signal fMRI during emotional memory tasks in the TRD population has been under-researched. The aim of this study was therefore to compare healthy volunteers and patients with TRD using fMRI during an emotional encoding and retrieval task, as well as a working memory task. We adapted a task previously used by Whalley et al (Whalley et al 2009), as it had been shown to demonstrate a difference in emotional memory processing between patients with bipolar disorder and schizophrenia, as well as using pictures which are thought to be easier to encode (Curran and Doyle 2011).

We hypothesised that the TRD group would show a reduced BOLD signal response in our regions of interest (ROI) taken from the literature when encoding and retrieving the emotional images (Migo et al 2012; Skinner and Fernandes 2007; Spaniol et al 2009). For the encoding task, these consisted of amygdala, hippocampus, parahippocampal gyrus, dorsolateral prefrontal cortex (dLPFC), and inferior temporal area (Brodman Areas (BA)s 9, 20, 45, and 46). For the retrieval task, the a priori regions of interest were amygdala, hippocampus parahippocampal gyrus, anterior cingulate cortex (BA 32), insula, dLPFC and anterior prefrontal cortex (BAs 9 and 10). We also hypothesised that they would also show reduced BOLD signal when comparing positive to neutral images in our ROIs. In addition,
we hypothesised that the TRD patients would have reduced BOLD signal activation in the
dLPFC during higher working memory in the n-back task. This was in keeping with our
previous study of healthy volunteers following a hydrocortisone challenge and from the
literature of depressed individuals (Harvey et al 2005).

5.3 Method

Normal volunteers and patients with DSM-IV depression with treatment resistance were
recruited as part of the ADD study (Anti-glucocorticoid augmentation of antidepressants in
Depression) (McAllister-Williams et al 2013) (www.theaddstudy.co.uk), a placebo
controlled, randomised controlled trial investigating the efficacy of metyrapone as an
augmentation agent for conventional antidepressants. This current study formed part of a
mechanistic sub-study and took place before the participants had taken the study
medication. Local research ethical approval was obtained and all participants gave
written, informed consent. All subjects had no contraindications to MRI scanning.

5.3.1 Patients

Sixty nine patients were recruited from outpatient clinics and general practitioners in the
Greater Manchester area. Of these, 27 were eligible for randomisation into this study, one
of whom subsequently withdrew consent. Twenty six patients participated in the final
study (13 females). All participants (including healthy volunteers) had their IQ measured
using the NART (National Average Reading Test) (Nelson 1982) and handedness was
measured using the Edinburgh handedness Inventory (Annett 1967; Oldfield 1971).

Inclusion criteria comprised having TRD (defined as being moderate to severely depressed
on the SCID (First et al 2002b) despite being on a treatment dose of an antidepressant for
at least 6 weeks and having failed to recover on two other antidepressants), Grid Hamilton
Depression Rating Scale (grid-HAMD; a semi-structured interview version of the HAMD that
separates out intensity and frequency to improve reliability) (Williams et al 2008) score
≥18, age 18-65, no other primary DSM-IV (American Psychiatric Association 1994) axis I
diagnoses, no neurological diagnoses or unstable medical conditions and no recent or
current use of corticosteroids.
Twenty patients were right handed, 6 ambidextrous and 1 left handed. Given the literature regarding laterality (Bruder et al 2012) and hand preference combined with the relative difficulty to recruit and retain patients with TRD, it was decided to take a pragmatic approach and recruit patients with preference for left handedness and perform a post-hoc sensitivity analysis. One scan from the patient group was excluded from analysis due to movement artefact and another subject was withdrawn from the study due to the discovery of a structural abnormality, therefore data are presented for 25 patients.

5.3.2 Healthy volunteers

Thirty two right-handed, healthy volunteers (HV) were recruited from by advertisements in the University of Manchester. Thirty were eligible (15 females). All subjects were screened for substance misuse and psychiatric disorder using the SCID (First et al 2002a). They were free from major physical illness and had no psychiatric illness themselves or in first degree relatives. All the female subjects were on long term hormonal contraception to control for the effects of menstrual cycle on endogenous cortisol levels to allow reliable within subject comparison (Symonds et al 2004) but subjects were otherwise medication-free, drank less than 21 units of alcohol/week and were caffeine free on the scan days.

One male subject was later excluded due to a head-coil malfunction; therefore data are reported from 29 subjects.

5.3.3 Experimental design

Subjects were scanned using a parallel, between subjects design on a 1.5T scanner. The scanning session, which took place between 12noon and 3pm, consisted of a brief localisation MRI scan, followed by two fMRI protocols in which participants undertook the n-back task followed by the encoding emotional pictures memory task. After a 6 minute structural MRI scan, subjects completed the retrieval emotional pictures memory task.
5.3.4 fMRI Tasks

All computerised tasks were run on a PC in E-Prime 2.0 (Psychology Software Tools Inc.) and back projected onto a screen visible to the participant via two mirrors attached to the head coil. The task responses were acquired from the participant using a fibre-optic button box held in the right hand. All participants received standardised training on how to perform the tasks prior to the scan.

5.3.5 Emotional Pictures

The task used by Whalley et al (Whalley et al 2009) was adapted to create an in-scanner recognition memory task. Additional images from the IAPS (International Affective Pictures System) battery were used to lengthen the task.

For the encoding task, a total of 60 images were shown in 5 blocks of 6 positive and 6 neutral images (each block lasting 48 seconds). These were followed by a block of rest, consisting of a fixation cross, which also lasted 24 seconds each. There were 5 blocks of each condition shown making the task length 360 seconds in total. Participants were asked to indicate using the button box whether they felt the picture was ‘emotional’ or not, and instructed to remember the images.

During the recognition segment of the task, a total of 96 images were shown in 6 blocks of 8 positive images and 6 blocks of 8 neutral images (each block lasting 48 seconds). These were followed by a blocks of rest, consisting of a fixation cross, which also lasted 24 seconds each. There were 6 blocks of each condition shown making the task length 432 seconds in total. All images shown in the encoding section were re-shown. Participants were asked to indicate using the button box whether they recognised the image from the encoding task or not.

5.3.6 N-Back scan

A blocked version of the n-back task was adapted from the task of Koychev et al (2012)(Koychev et al 2012). It consisted of four blocks of zero-back and four blocks of
two-back, each lasting 32 seconds. These required 3 correct responses from 13 stimuli per block using the button box. These blocks were interspersed with 4 blocks of rest consisting of a fixation cross, lasting 20 seconds each.

5.4 Scanning protocol and data acquisition

Scanning was carried out on a Philips Intera 1.5 Tesla MRI scanner with prospective motion correction. Data were acquired with T2*-weighted, gradient echo, echo planar imaging (EPI). Full brain coverage was used with TR=2 sec, TE=40 ms, 3.5mm in-plane resolution, 4.5mm slice thickness with 0.5mm slice gap and 29 slices.

5.4.1 Data Analysis

Behavioural and demographic data were analyzed using Statistical Package for Social Science version 20.0 (SPSS Inc, 2012). Behavioural data from both tasks were analyzed using repeated measure ANOVA with one within subject factor condition (difficulty: e.g. emotional valency: positive, neutral or 0-back, 2-back) and one between subject factor (participant group), as well as independent t-tests. The outcome measures used for the tasks were speed of response, number omitted, number of false positives and number correct. Results are presented as mean ± SD.

fMRI data were processed and analysed using Statistical Parametric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk/spm). Scans were spatially pre-processed using standard protocols. They were realigned, using the first scan as a reference, normalised into the Talairach and Tournoux stereotactic space (Talairach and Tournoux 1998) using MNI templates then spatially smoothed using a 8 mm Gaussian kernel. A mask was created which was a sum of the ROIs for each task and used as single comparison in the analysis.

For both the emotional picture tasks (encoding and retrieval), positive and neutral conditions were contrasted to rest. Second level processing was performed using one-sample t-tests to explore 1) the main effect of task on positive pictures minus rest, neutral pictures minus rest, all pictures minus rest and vice versa and 2) the effect of depression on each level of the task (healthy volunteers minus patients and vice versa). For the
encoding task, our ROIs were the amygdala, hippocampus and parahippocampal gyrus, dorsolateral prefrontal cortex (dIPFC), and inferior temporal area (BAs 9, 20, 45, and 45).

For the retrieval task the *a priori* regions of interest were amygdala, hippocampus, parahippocampal gyrus, anterior cingulate cortex (BA32), insula, dIPFC and fronto-polar areas (BAs 9 and 10).

For the n-back task, both levels of n-back were contrasted to rest. Second level processing was performed using one-sample t-tests to explore 1) the main effect of task at the highest working memory load (2-back minus 0-back) and 2) effect of depression on each level of the task (healthy volunteers minus patients and vice versa). Our ROIs were lateral premotor cortex (BA 6), dorsal anterior cingulate cortex (BA32), dIPFC (BA9, 46) ventrolateral prefrontal cortex (vIPFC) (BA44, 45, 47), medial posterior parietal and inferior parietal lobule (BA 7, 40) and medial cerebellum, as per Symonds et al (Symonds et al 2012).

Within these areas we report as significant areas Family Wise Error (FWE) corrected peak level p(FWE)<0.05 for the region of interest. Other areas surviving p(FWE)<0.05 at a whole brain level are also reported for interest.

### 5.5 Results

#### 5.5.1 Participant demographics

The mean age of patients was 44.1 ± 7.7 (range 26-57) versus mean age 36.47 ± 5.94 (range 31-55) for the healthy volunteers. The mean NART IQ score for the patient group was 109.7 ± 10.0 and for the healthy volunteers 113.3 ± 10.4. There was no significant difference between the patient and healthy volunteer groups in either age or IQ (p=0.22 and p=0.15, respectively).

The baseline grid HAMD for the patient group was 23.74 ± 3.7 (versus 0.28±0.5 for the healthy volunteers).

There was a significant difference in handedness between the healthy volunteer group and the patients, with one left handed individual and 5 ambidextrous individuals in the TRD
group; however the results were not substantially altered by the removal of this individual in post hoc sensitivity analysis.

Patients were one at least one serotonergic antidepressant with 5 patients on monotherapy. The rest were on 1-5 additional agents (mean number of drugs 2.28±2.5 ). Antidepressants (in order of frequency) were mirtazepine, venlafaxine, duloxetine, sertraline, citalopram, escitalopram, trazadone, paroxetine, agomelatine, and reboxetine. Mood stabilizers used were lithium and lamotrigine. Atypical antipsychotics were quetiapine, aripiprazole and risperidone. Additionally, some patients were also on other psychotropic agents (pregabalin, diazepam, promazine, zopiclone and liothyronine).

5.5.2 Behavioural results

5.5.2.1 Emotional pictures encoding task

There was no significant difference in the number of responses made between participant groups \( (F(1,51)=0.02, \ p=0.88) \) or reaction time \( (F(1,51)=0.84, \ p=0.36) \). There was a main effect for group for subjective emotionality \( (F(1, 51)=5.14, \ p=0.03) \) with patients classifying fewer positive images as ‘emotional’, however there was no significant participant group x valence effect \( (F(1,51)=1.19, \ p=0.28) \).

5.5.2.2 Emotional pictures retrieval task

There was no significant effect of the emotional valence (positive or neutral image) in the number of words omitted in the retrieval tasks \( (F(1,50)=2.40, \ p=0.13) \) and no effect of group (TRD or HV) by emotional valence on number omitted \( (F(1, 50) = 2.03, \ p=0.16) \). There was also no effect of emotion on reaction time \( (F(1,50) = 0.91, \ p=0.35) \) and no effect of group by emotion on reaction time \( (F(1,50) = 0.12, \ p=0.73) \). There was also no effect of emotion on number of words correctly recalled \( (F(1,50)=0.01, \ p=0.94) \) and no effect of emotion by group on correct recognition \( (F(1,50)=10.51, \ p = 0.25) \).
5.5.2.3 N-back task

There was no significant main effect of group on number correct (F(1,53)=0.09, p=0.76), omitted (F(1,53)=1.19, p=0.28), or reaction time (F(1,53)=1.12, p=0.29). There was also no effect of level of difficulty or level by group on any of these measures.

5.5.3 fMRI results

5.5.3.1 Emotional pictures encoding task

The main effect of task (all participants, all encoding images minus rest) showed increased BOLD signal in bilateral parahippocampal, bilateral inferior frontal gyrus (BA 47, 9), left temporal gyrus (BA 20) and right hippocampus in keeping with findings of meta-analyses of encoding tasks literature (Skinner and Fernandes 2007; Spaniol et al 2009) (see Supplementary Table A7-1 for full results and Supplementary Figure A7-1 for an array illustrating the significant areas, Appendix 7).

When the healthy volunteers were compared to the patients, patients with TRD showed a significant decreased BOLD signal at whole brain level in the posterior cingulate when compared to the healthy volunteers during the encoding task (healthy volunteers minus patients, all encoding images minus rest contrast), (BA 31; x =9.5, y= -35, z= 35, Z=3.47 pFWE(whole brain corrected)= 0.043). There were no significant findings in the pre-hypothesised ROIs. (See Table 5-1 and Figure 5-1). There were no significant results in the opposite subtraction.
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Table 5-1: Shows results of the three fMRI tasks. <sup>a</sup> = whole brain corrected pFWE<0.05, <sup>b</sup> = pFWE<0.05 following <i>a priori</i> region of interest analysis (ROI) (FWE=Family wise error, BA = Brodmann Area, K=cluster size at punc<0.005). MNI= Montreal Neurological Institute Space.
Figure 5-1: Significant BOLD decrease in the patients compared to the controls in the posterior cingulate (9.5, -35, 35), pFWE whole brain = 0.043 during the encoding task (Controls minus Patients, all encoding images minus rest, error bars indicated SE)
5.5.4 Emotional pictures retrieval task

The main effect of task (all participants, all retrieval tasks minus rest) showed increased BOLD signal in the parahippocampus bilaterally, the left inferior temporal lobe (BA36), the left lingual gyrus (BA 18) and left BA 9 and 6. There was no significant change in BOLD signal in the hippocampus (see supplementary table A7-2 for full results, Appendix 7 and Figure A7-2, for an array illustrating the significant areas Appendix 7).

In the a priori region of interest of the insula, the BOLD signal was increased in the patients compared to the controls whilst completing the retrieval task, as compared to the rest condition (patients minus controls, retrieval images minus rest contrast), (posterior insula, BA 40; x =-46.5, y=-21, z= 20, Z=3.85 pFWE(ROI analysis) = 0.046) (see Table 5-1 and Figure 5-2). In the anterior cingulate, another a priori region of interest, the BOLD signal was reduced in the patients compared to the controls whilst retrieving positive images as compared to neutral images (controls minus patients, positive images minus neutral images contrast), (anterior cingulate, BA 33; x =-4.5, y=17.5, z= 25, Z=3.95 pFWE(ROI analysis) = 0.033), (see Table 5-1and Figure 5-3).
Figure 5-2: Significant BOLD increase in the patients compared to the controls in the posterior insula (-46.5, -21, 20), ROI pFWE=0.046 during the retrieval task (Patients minus controls, all retrieval images minus rest, error bars indicated SE)
Figure 5-3: Significant BOLD increase in the patients compared to the controls in the anterior cingulate (-4.5, 17.5, 25) ROI pFWE = 0.033 during the retrieval task (controls minus patients, positive minus neutral, error bars indicated SE)

5.5.5 N-Back task

The main effect of the task (using all participant scans), the 2-back compared to 0-back subtraction showed increased BOLD signal bilaterally in the dIPFC, medial posterior parietal and lateral premotor cortices, in keeping with the areas reported in a previous meta-analysis (Owen et al 2005). In the controls minus patients, 2-back minus 0-back
contrast, the patients showed a decrease in BOLD signal compared to the controls in the dlPFC (BA 6; x = -25.5, y=7, z= 25, Z=50 pFWE(whole brain corrected) = <0.001 (see Table 5-1 and Figure 5-4), However, there was no difference seen in our ROI areas.

Figure 5-4: Significant BOLD increase in the controls compared to patients in the dlPFC (-25.5, 7, 50) whole brain pFWE<0.001 in the 2-back minus 0-back contrast (error bars indicated SE)
5.6 Discussion

We demonstrated that in treatment resistant depression, the patients showed a relative under-activity of the posterior cingulate whilst encoding both positive and neutral images, as well as a reduced BOLD signal in the anterior cingulate whilst retrieving positive images. In addition, the patients demonstrated an increased activation of the insula during the retrieval- rest contrast and reduced dIPFC activation during the n-back task compared to the controls. However, we did not find a significant difference between the patients with TRD and the controls in the accuracy or reaction speed during any of the tasks.

To our knowledge, brain activation during retrieval has not been previously examined using this particular emotional encoding-retrieval task (Whalley et al 2009), nor encoding in patients with treatment resistant unipolar depression. Few studies to date have focussed on emotional memory in TRD, and this study adds to that literature. Our results demonstrate that during encoding, people with depression show a greater degree of negative BOLD signal in the posterior cingulate compared to control. There is no clear consensus about the function of the posterior cingulate (Leech and Sharp 2013). It has been associated in the past with motor intention, attention and spatial awareness (Mann et al 2011), however it has more recently been linked to pre-attentive processing (Stoitsis et al 2008). The ventral posterior cingulate is connected to the hippocampus and parahippocampal gyri (Kobayashi and Amaral 2007), together with BA 25 and BA 31, it is the most connected area of the human brain (Mann et al 2011). The posterior cingulate is part of the default mode network and is known to deactivate during tasks requiring external attention (Gusnard et al 2001). The failure of this mechanism during an emotional memory task may suggest a possible mechanism for attention and emotional-bias based deficits seen in depression.

During the retrieval task, a decreased activity in the anterior cingulate bilaterally during positive stimuli was identified, however no difference between positive and neutral was found during the encoding task. In our study, the decrease in BOLD signal was found in BA 33, an area associated the anterior cingulate affect, along with BA 24 and 25 (Devinsky et
al 1995). As previously demonstrated in a meta-analysis of 16 previous studies in major depressive disorder by Zhang et al (Zhang et al 2013), the anterior cingulate shows decreased BOLD signal activation in response to positive stimuli. Glucose hypermetabolism in the anterior cingulate (specifically BA25) predicted non response to treatment in one PET study (Konarski et al 2009), but not in BA 33. Previously an over-activity of the anterior cingulate has been associated with a greater subsequent response to antidepressants (Davidson et al 2003) and with the effects of tryptophan depletion in remitted depression (Neumeister et al 2004). The anterior cingulate has been identified as a target for neuro-stimulation in treatment resistant depression (Neimat et al 2008).

Non-response to antidepressant treatment has been predicted by hypometabolism of glucose in a PET study in the anterior cingulate (Mayberg et al 1997). The under-activity of the anterior cingulate in response to positive stimuli demonstrated in our current study may go some way to explain the cognitive biases against the accurate recall of positive memories seen in depression, and also be a marker treatment resistance (Kennedy et al 2007).

We demonstrated an increase in activity in the posterior insula of the TRD group during retrieval of both positive and neutral images, compared to rest. The posterior insular cortex has connections to the hippocampus, anterior cingulate, temporal lobes and the somatosensory cortex (Chang et al 2012). It has been shown to be activated in disgust and somatosensory stimuli. However in subjects with anxiety disorder, a relative increase in insular activity has been reported (Stein et al 2007) and in remitted depression an increase in posterior insular BOLD signal whilst viewing happy faces has been reported (Anderson et al 2011a). It has been theorised that activation of the posterior insula in response to emotion could be due to a greater degree of empathy shown by depressed individuals (De Vignemont and Singer 2006).

In keeping with the literature, this study demonstrated depressed patients’ failure to activate the dLPFC during higher working memory loads (Kerestes et al 2012), however this is the first time it has been demonstrated in treatment resistant depressed individuals to our knowledge. Performance in the n-back task has been found to correlate with several severity markers of depression and in addition, an increase in BOLD signal activation of the
dlPFC in depressed individuals has been noted in other fMRI studies (Fitzgerald et al 2008; Harvey et al 2005). Previous work by these authors demonstrated an increased BOLD signal in dlPFC following hydrocortisone infusion in healthy volunteers (Symonds et al 2012) and this provides some indirect evidence for the theory that the cognitive deficits seen in depression mimic hypercortisolaemia (Porter et al 2004).

The limitations of this study are the small numbers, the ethnically homogenous population reducing the generalisability of the study, and the fact that that all of the depressed individuals were on medication. Although they were all on at least one serotonergic agent, they were also on a variety of other agents, including mood stabilizers and antipsychotics. These results, therefore, could be attributed to the medication and not to the illness. The design of the encoding-retrieval task also introduced limitations as it did not contain negative images. The decision was made not to include negative images partly because of the positive results in depression regarding positive versus neutral stimuli and also because of the ethical issues of showing patients with depression negative images from the IAPS battery, which can cause significant distress. However, this meant that no direct conclusions about a bias towards negative material in depression could be made.

This study reinforces the role of the anterior cingulate and insular cortex in cognitive processing in depression. However, future research needs to focus on the contrast between treatment resistant depression and treatment responsive depression, especially in relation to emotional memory, to allow firmer conclusions to be drawn. In particular, this study sheds some light on the unclear role of the posterior cingulate in the cognitive biases and deficits seen in depression. The role of this area, and in particular its neural connections, in treatment resistant depression could further our understanding of the emotional biases at the heart of depressive cognitions.

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5.7 Role of the funding source

This work was supported by a clinical fellowship from NIHR Manchester Biomedical Research Centre and a grant from the Magnetic Resonance Imaging Facility, Manchester Wellcome Trust Clinical Research Facility; the funders had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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5.8 References


6 Chapter 6

The acute effects of hydrocortisone in treatment resistant depression using pharmacological fMRI

Catherine S. Symonds¹, Shane McKie¹, R. Hamish McAllister-Williams², I. Nicol Ferrier²,
John Francis William Deakin¹, Ian M. Anderson¹

1. Neuroscience and Psychiatry Unit, G.907 Stopford Building, The University of Manchester and Manchester Academic Health Sciences Centre, Oxford Rd, Manchester M13 9PT. Tel 0044 161 275 1727 Fax: 0044 161 275 7429
   email Catherine.Symonds@manchester.ac.uk

2. Academic Psychiatry, Wolfson Research Centre, Campus for Ageing and Vitality, Newcastle upon Tyne, NE4 5PL
6.1 Abstract

Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is associated with depression. Previously, we demonstrated the acute, time dependent effects of hydrocortisone on the hippocampus in a normal volunteer population. Impaired hippocampal function caused by HPA axis dysfunction may account for some of the neurocognitive deficits and cognitive biases seen in depression. We aimed to examine the effect of an acute bolus of hydrocortisone on treatment resistant depression (TRD) patients in comparison with age and sex matched healthy volunteers. Using pharmacological functional magnetic resonance imaging (phMRI) we investigated whether BOLD signal is altered after acute administration of 100mg of intravenous hydrocortisone in a between-subject placebo-controlled parallel designed study. The final sample consisted of 20 patients with TRD and 27 healthy volunteers. Our a priori regions of interest were the hippocampus, hypothalamus, amygdala and dorsolateral and ventrolateral prefrontal cortex (all areas rich in corticosteroid receptors). We failed to demonstrate with a difference between the TRD and healthy volunteers groups and also a main effect of hydrocortisone. We discuss reasons that may have accounted for this result in comparison to our previous research, including lack of power and artefact.

**Keywords**: hippocampus, fMRI, treatment resistant depression, corticosteroids.
6.2 Introduction

Elevated levels of cortisol have been demonstrated in patients with depression (Porter et al 2004) as well as altered acute hypothalamic-pituitary-adrenal (HPA) axis responses, with a blunted response to stress and to wakening being typical findings (Kudielka and Wust 2010). A causative link between the cognitive deficits seen in depression and elevated levels of cortisol has been proposed (Pariante and Lightman 2008). Additionally, diurnal variation of symptoms and disruption of sleep has been causally linked to the presence of dysregulation of the HPA axis in depressed patients (Linkowski 2003). However, we have much to understand about the mechanisms underlying these processes.

Using peripheral measures, patients with depression have demonstrated abnormalities of the HPA axis. In addition to the blunting of salivary cortisol response to stress, these have included a failure of dexamethasone to suppress cortisol levels on dynamic testing, a trait that persists after remission in some studies (Vreeburg et al 2009), but not others (Aubry et al 2010). There is also evidence that remission can be predicted by an early normalisation of the HPA axis as tested by the combined dexamethasone/CRH test (Hennings et al 2009). Therefore inducing a normalisation of the HPA axis in patients with treatment resistant depression (TRD) using metyrapone as an augmenting agent has been a recent target for therapeutic intervention in studies of depression (Gallagher et al 2008; Jahn et al 2004). However, the underlying mechanism for metyrapone’s actions are not clear (Sigalas et al 2012) and traditional methods of measuring HPA axis abnormalities rely on peripheral blood and saliva samples, which may not reflection the action of cortisol on the limbic system.

One possible site of the dysregulation of the HPA axis in depression is the hippocampus. The regulation of the HPA axis occurs not just at the level of the hypothalamus and pituitary, but also in higher centres including the hippocampus, amygdala and prefrontal cortex (Herman et al 2005). These areas are particularly rich in mineralocorticoid and glucocorticoid receptors (MRs and GRs) (de Kloet et al 1998). Animal studies have demonstrated higher basal ACTH levels and a failure to regulate the HPA axis in
Patients with unipolar depression have a reduced hippocampal volume, as well as reduced cingulate, frontal and orbitofrontal cortices, as demonstrated with a meta-analysis of data from 4118 patients (Arnone et al 2012a). Reduced hippocampal volume was associated with the duration of illness and with the failure to respond to antidepressant treatment. In addition to these areas associated with emotional memory, patients also demonstrated an increased pituitary volume. This reduction in hippocampal volumes is associated with failure of dexamethasone to suppress cortisol levels in unipolar depression (Knoops et al 2010).

Both MRs and GRs are classed as nuclear receptors exerting their effects through action on nuclear DNA, although both are also present on the membrane in addition (Joëls et al 2009). However, a behavioural change in neurocognitive function in response to the double-blind administration of corticosteroids can be observed prior to this genomic effect occurring, strengthening the argument for a non-genomic action at the MRs and GRs (Het et al 2005). Over the last ten years, it has been understood that membrane-bound MRs can produce rapid non-genomic responses to elevated stress hormones. Through the use of genetic knockout mice with forebrain-specific MR gene inactivation, in vitro work demonstrates a rise in intracellular recorded synaptic connection strength and glutamate secondary to corticosterone in hippocampal cells of control animals (Karst et al 2005).

Another mechanism for the acute action of corticosterone on MRs is via the extracellular signal-regulated kinase (ERK) 1/2 pathway, which controls synaptic plasticity and, when activated, also increases the chance of glutamate containing vesicles being released, both pre- and post-synaptically (Olijslagers et al 2008).

Damage to the human hippocampus has been shown to cause an inability to mount an appropriate cortisol response to a psychological stressor (Buchanan et al 2009). The acute response to psychological stress, a combination of catecholamines and corticosteroids, not only impaired hippocampal dependent recall in depressed patients (Abercrombie et al 2008), but in healthy volunteers as well (Het et al 2005). Depressed individuals with
anxiety demonstrate an increased ACTH response compared to healthy controls (Young et al 2004). Pharmacological studies with corticosteroids have demonstrated an acute effect on hippocampus function in healthy volunteers (Het et al 2005). Additionally, depressed individuals demonstrated altered hippocampal activations following the administration of hydrocortisone during an fMRI encoding and retrieval task (Abercrombie et al 2011). The direct action of cortisol has not yet been studied using direct pharmacological challenge fMRI (phMRI) to our knowledge.

phMRI has the advantage of providing a measure of the acute effects of drugs over time. Hydrocortisone challenge phMRI is a promising technique which has been shown to cause an increase in hippocampal BOLD signal in humans and rats (Ferris and Stolberg 2010; Symonds et al 2012). Given that there appears to be a difference in the acute effect of cortisol and the higher regulation of cortisol in depressed individuals, hydrocortisone phMRI may help us understand the mechanism underlying HPA axis dysfunction. The TRD population are of particular interest given the potential therapeutic gain in targeting the HPA axis, as illustrated by promising small scale trials into the antidepressant action of antiglucocorticoids (Gallagher et al 2008; Jahn et al 2004).

Our aim in this study was to use functional MRI combined with drug challenge (pharmacomRI, phMRI) to image the effects of acute hydrocortisone infusion on human hippocampal function in patients with TRD and normal, healthy age and sex matched controls. Based on our pilot study (Symonds et al 2012), the recent animal literature that the non-genomic action of GR and membrane bound MR increase the probability of glutamate release in hippocampus (Di et al 2009; Olijslagers et al 2008), we hypothesised that acute hydrocortisone would lead to a rapid increase in bold oxygen level dependent (BOLD) signal in the hippocampus, hypothalamus, amygdala and dorsolateral and ventrolateral prefrontal cortex. We further hypothesised that given the failure of dexamethasone to suppress cortisol levels demonstrated by depressed patients; we would expect a reduced response in the hypothesised regions of interest (ROI) in patients compared to controls.
6.3 Methods

Normal volunteers and patients with DSM-IV depression with treatment resistance were recruited as part of the ADD study (Anti-glucocorticoid augmentation of antidepressants in Depression) a double-blind randomised, placebo controlled trial investigating the efficacy of metyrapone as augmentation agent for conventional antidepressants (McAllister-Williams et al 2013) (www.theaddstudy.co.uk). This current study formed part of a mechanistic sub-study and took place before the participants had taken the study medication. Part of this study has already been reported (see Chapter 5). Local research ethical approval was obtained and all participants gave written, informed consent. All subjects had no contraindications to MRI scanning.

6.3.1 Patients

Sixty nine patients were recruited from outpatient clinics and general practitioners in the Greater Manchester area. Of these 27 were eligible for randomisation into this study, two of whom subsequently withdrew consent. Twenty five patients participated in the final study (13 females). Inclusion criteria included having treatment resistant depression (TRD). This was defined as being moderate to severely depressed despite being on a treatment dose of an antidepressant for at least 6 weeks and having failed to recover on two other antidepressants. Diagnosis was confirmed with the SCID (First et al 2002b); a Massachusetts General Hospital Treatment Resistant Depression (MGH-TRD) staging score of 2-10 (Fava 2003); and Grid Hamilton Depression Rating Scale score ≥18 (grid-HAMD; a semi-structured interview version of the HAMD that separates out intensity and frequency to improve reliability) (Williams et al 2008). They were aged 18-65, had no other primary DSM-IV (American Psychiatric Association 1994) axis I diagnoses, no neurological diagnoses or unstable medical conditions and no recent or current use of corticosteroids.

All participants’ IQ was measured using the NART (National Average Reading Test) (Nelson 1982) and handedness was measured by the Edinburgh handedness Inventory (Annett 1967; Oldfield 1971). Twenty patients were right handed, 6 ambidextrous and 1 left handed.

Given that depressed patients are more likely to have atypical language laterality (Bruder
et al 2012) and hand preference combined with the relative difficulty to recruit and retain patients with TRD, it was decided to take a pragmatic approach and recruit patients with preference for left handedness and perform a post-hoc sensitivity analysis.

Twenty-five patients began the scanning study. One scan from the patient group was excluded from analysis due to movement artefact, three people could not tolerate the whole scan and another subject was withdrawn from the study due to the discovery of a structural abnormality, therefore data are presented for 20 patients.

6.3.2 Healthy volunteers

Thirty two right-handed, healthy volunteers (HV) were recruited from by advertisements in the University of Manchester. Thirty were eligible (15 females). All subjects were screened for substance misuse and psychiatric disorder using the SCID (First et al 2002a) and were free from major physical and psychiatric illness; and from psychiatric illness in first degree relatives. All the female subjects were on long term hormonal contraception to control for the effects of menstrual cycle on endogenous cortisol levels to allow reliable within subject comparison (Symonds et al 2004) but subjects were otherwise medication-free, drank less than 21 units of alcohol/week and were caffeine free on the scan days.

One male subject was later excluded due to a head-coil malfunction, another male for terminating the scan early due to accidently pushing the emergency alarm and a third due to movement; therefore results are reported for 27 subjects.

6.3.3 Drug allocations

Participants were allocated to the drug groups using double-blind randomization. The final group allocations were as follows: saline HV n=14, hydrocortisone HV n=13, saline TRD n=12, hydrocortisone TRD n=8.

6.3.4 Experimental design

The scanning session, which took place between 12 noon and 3pm, consisted of a brief structural MRI scan, followed by three fMRI scans and a 6 minute structural MRI scan, and
finally, a 26 minute phMRI infusion scan. Only the latter two scans are reported here with 
the former scans being reported elsewhere (See Chapter 5).

6.3.5 Scanning protocol and data acquisition

Scanning was carried out on a Philips Intera 1.5 Tesla MRI scanner with prospective motion 
correction. Data were acquired with T2*-weighted, gradient echo, echo planar imaging 
(EPI) and prospective movement correction (PMC) (see Discussion, section 4, below for 
further discussion). Full brain coverage was used with a TR = 3 s, TE = 40 ms, isotropic 3 
mm voxels with 29 slices and whole brain coverage. The scan was 26 minutes in length 
and consisted of 520 volumes.

6.3.6 Data Analysis

Behavioural and demographic data were analyzed using Statistical Package for Social 
Science version 20.0 (SPSS Inc., 2012). Results are presented as mean ± SD.

fMRI data were processed and analysed using Statistical Parametric Mapping (SPM8) 
(http://www.fil.ion.ucl.ac.uk/spm). Scans were spatially pre-processed using standard 
protocols. They were realigned, using the first scan as a reference, normalised into the 
Talairach and Tournoux stereotactic space (Talairach and Tournoux 1998) using MNI 
templates then spatially smoothed using an 8 mm Gaussian kernel.

The pharmacological imaging data were analyzed using a pseudo-block pharmacological 
MRI analysis technique (McKie et al 2005) using a drug x time repeated measure random 
effects ANOVA.

The data from both of the phMRI scans were spatially pre-processed using standard 
protocols. They were realigned, using the first scan as a reference, normalised into the 
Talairach and Tournoux stereotactic space (Talairach and Tournoux 1998) using MNI 
templates then spatially smoothed using an 8 mm Gaussian kernel.

For the phMRI, first level analysis was performed with each scan split into 8 time-bins of 4 
minute duration starting 1 minute after the start of scanning with each time-bin compared
with a baseline time-bin leading up to the start of the infusion (-2 to 0 min). The statistical parametric maps from each individual data set were then entered into second-level, random effects analyses to account for both scan-to-scan and subject-to-subject variability. Scans were controlled for the effects of drift by subtracting the time-bin effects for the saline scans. A fully-factorial analysis of variance was then performed with subsequent hydrocortisone-saline and saline-hydrocortisone contrasts carried out on the data following baseline to examine the effect of hydrocortisone infusion. The a priori regions of interest (ROI) were the hippocampus, hypothalamus, amygdala and dorsolateral and ventrolateral prefrontal cortex, which express high concentrations of GR and MR (Young et al 1998). Within these areas we report as significant areas Family Wise Error (FWE) corrected peak level p(FWE)<0.05 for the region of interest. Other areas surviving p(FWE)<0.05 at a whole brain level are also reported for interest.

6.4 Results

6.4.1 Participant demographics

The mean age of patients was 44.9 ± 8.1 (range 26-58) versus mean age 36.5 ± 5.9 (range 31-55) for the healthy volunteers. The mean NART IQ score for the patient group was 109.7 ± 10.0 and for the healthy volunteers 113.3 ± 10.4. There was no significant difference between the patient group and healthy volunteer groups in either age or IQ (p=0.169 and p=0.152, respectively).

The baseline grid HAMD for the patient group was 24.4 ± 2.9 and 0.3 ± 0.5 for the healthy volunteers.

6.4.2 Hydrocortisone challenge phMRI

Following hydrocortisone infusion, compared with saline, there was no significant main effect in the healthy volunteers of any BOLD signal change in the a priori ROIs, namely the hypothalamus, hippocampus, amygdala, vlPFC and dlPFC. One non-hypothesised area was significant, however, with a significance decrease in the anterior cingulate (x=0, y = 32, z = 28, Z=4.62, p(FWE) (whole brain) =0.047, p_{unc}<0.001). No significant effect was
demonstrated in the patient group in this area. This area is illustrated in Figure 6-1 together with the other areas that were non-significant at both whole brain and peak level.

Figure 6-1: Shows a significant decrease of BOLD signal in the healthy volunteers. This non-hypothesised area was significant at whole brain level pFWE in the hydrocortisone group compared to the saline in the healthy volunteer main effect in the anterior cingulate (0, 32, 28), pFWE whole brain =0.047 during the infusion scan (Hydrocortisone-Saline, error bars indicated SE).

In the patient - healthy volunteers subtraction, hydrocortisone-saline and in the three way interaction between patient, drug and time (and in the opposite subtractions), there was no significant main effect in the healthy volunteers of any BOLD signal change in the a
priori ROIs, namely the hypothalamus, hippocampus, amygdala, vlPFC and dlPFC. There was also no significant BOLD signal change in non-hypothesised areas in the patient group and three way interaction groups (and in the opposite subtractions). However, there was a significant increase in the hydrocortisone minus saline subtraction in the medial frontal gyrus (BA9) \((x=15, y = 38, z = 28, Z=4.88, p_{\text{FWE}}(\text{whole brain})=0.011, p_{\text{unc}}<0.001)\), see Figure 6-2. Most the difference in the signal was accountable in the last 4 minutes after a sudden decrease in signal.

![Image of brain scan with highlighted areas](image1)

Figure 6-2: Shows a significant increase of BOLD signal in the medial frontal gyrus (BA9) in the healthy volunteers compared to the TRD group. This non-hypothesised area was significant whole brain \(p_{\text{FWE}}=0.011\) \((15, 38, 28)\) during the infusion scan (Hydrocortisone-Saline, Healthy volunteers-patients contrast, error bars indicated SE).
6.5 Discussion

We failed to demonstrate an effect in the hypothesised areas, namely the hippocampus, hypothalamus, amygdala and dorsolateral and ventrolateral prefrontal cortex. These are MR and GR dense areas and our previous study demonstrated a clear time dependent rise in the hippocampus (Symonds et al 2012) (see Chapter 4). The differences between the current study and our previous work will be further discussed, below. It is therefore difficult to interpret the meaning of the pFWE significant findings in this context. We demonstrated a main effect of the anterior cingulate in healthy controls with a significant decrease in BOLD signal over time. No effect was demonstrated in the patient group. The decrease in BOLD signal in response to an acute rise may represent the inhibitory role of the anterior cingulate in the HPA axis, as described in rat brain lesion studies (Diorio et al 1993). We showed an interaction between patient status, drug and time in BA9 with a statistically significant reduced BOLD signal in the patient group compared to controls, as hypothesised however this is difficult to interpret given the lack of involvement of the other limbic- hypothalamic - pituitary - adrenal axis areas. The decreased BOLD signal is in keeping with PET glucose metabolism studies following a psychosocial stress (Kern et al 2008). However, most of the significant difference between the healthy volunteers and the TRD group was accounted for by a sudden decrease in the BOLD signal over the last 4-8 minutes (see Figure 6-2). This shape is not typical of a physiological response to a hormone or drug (Anderson et al 2008b). It may well represent artefact, especially movement-related artefact, and therefore this result should be interpreted with caution.

The main limitation of this study was the small size of the sample making a Type II statistical error probable. Although our previous study had a similar number of healthy controls, that was a within-subject crossover design and the number of participants required for a parallel design is greater to obtain the same power (Louis et al 1984). One patient group only had 9 participants in it, which is below the usually accepted number of subjects required for a parallel group comparison (Amaro Jr and Barker 2006).

Another explanation as to the disparity between the current study and our previous work may lie in the assumption behind the hydrocortisone infusion phMRI technique. The
concept of a non-genomic effect of cortisol in the limbic system is relatively new. However, other groups have reported an effect both in rats (Ferris and Stolberg 2010) and humans (Henckens et al 2011; Lovallo et al 2009), although the latter study by Lovallo et al was underpowered and non-significant at the pFWE level. On balance, and in the wider context of current evidence, it is seems likely that hydrocortisone does cause an acute non-genomic effect on the hippocampus and other higher centres of HPA axis control, even though this study failed to demonstrate it.

Pharmacological challenge fMRI is a relatively new technique and it has been argued that it lacks robust test-retest reliability (Klomp et al 2013). Klomp et al recommended that phMRI experiments should be designed with a control condition and with a control group. In our current study, we used a saline infusion as the control condition, and age and sex matched healthy volunteers as controls. Nevertheless, confounding factors relating to other differences between the groups may have affected test-retest reliability. We did not measure physiological variables and Electroencephalography (EEG) during the scan which has been recommended in order to interpret the BOLD signal (Iannetti and Wise 2007). We did measure blood pressure, blood and oxygen saturation before and afterwards, with no deviation from normal parameters noted. Iannetti et al argued that as BOLD signal is not an absolute or quantitative measure and it is subject to many different variables, it is not a reliable technique. Other groups have successfully used this technique with appropriate control groups and tasks but these concerns remain.

The main methodological difference between this study and our previous work was the introduction of prospective movement correction (PMC). This uses external measurements of the participants head movements without the need for post hoc regression of individual movement parameters (Maclaren et al 2013). These externally measured movements are factored in the signal at source, however if the BOLD signal of interest is similar in shape to the movement artefact, PMC may theoretically abolish neuronal signal as well as physiological and movement artefact without the ability to perform a separate sensitivity analysis with the raw data and movement data (Churchill et al 2012). This current study was the first phMRI study to be carried out using PMC on this particular 1.5T scanner and
further experimentation is needed to see whether this theoretical reason was the cause of the disparity in our results from our earlier findings.

The hydrocortisone phMRI technique potentially has a role in investigating the link between the HPA axis in psychiatric disorders, both from our previous work and the work of others (Ferris and Stolberg 2010; Henckens et al 2010). However, these current negative results show that refinements are necessary. Using the regression of movement parameters as proposed by Churchill et al (Churchill et al 2012) and as used in our previous study (Symonds et al 2012) instead of PMC, would address whether PMC use contributed to the lack of BOLD signal change in the ROI, or whether this is a problem integral to the technique of phMRI. This current study was under-powered and a larger, crossover, placebo controlled study is necessary. To maximise the tolerability of the long phMRI scan, a break may be necessary with a repeat of the T1 weighted structural scan. This would have the added benefit of reducing the participant’s fatigue and therefore reduce the patient movement to some degree. In addition, in order to understand the role of the HPA axis in depression, further work on the relationship between TRD patient’s hippocampal volumes and dynamic HPA function is also necessary.

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6.6 Role of the funding source

This work was supported by a clinical fellowship from NIHR Manchester Biomedical Research Centre and a grant from the Magnetic Resonance Imaging Facility, Manchester Wellcome Trust Clinical Research Facility; the funders had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. The ADD study was funded via a project grant was awarded by the Efficacy and Mechanism Evaluation (EME) Programme and is funded by the Medical Research Council (MRC) and managed by the National Institute for
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References


Chapter 7

Discussion
7.1 Summary of key findings

The role of the HPA axis in depression, especially its role in neurocognition and the higher control of the HPA axis (including via the hippocampus) is a complex one. The first research chapter, described in Chapter 3, was a systemic review with meta-analysis of the existing data on the effects of an acute rise of cortisol, induced by either exogenous drugs or psychological stress, on encoding and retrieval in healthy volunteers. It demonstrated that an acute rise in cortisol prior to retrieval, but not prior to encoding, led to a significant adverse effect on memory tasks. This adverse effect was most significantly seen when the subjects were recalling neutral words, and also following the administration of exogenous cortisol as opposed to following a stress task. The implications of the relationship between emotional memory and the HPA axis are not as clear, although the results suggest that the emotional content may be better encoded than the neutral content and therefore less vulnerable to the adverse effects of cortisol. The demonstrated effect of cortisol administered within four hours of testing on cognitive performance implies that this was a non-genomic action (Losel et al 2003).

The acute action of cortisol on the BOLD signal as a proxy for neurocognitive activity was also demonstrated in paper 2, described in Chapter 4. Although no behavioural effect of cortisol was demonstrated, hydrocortisone caused an increased signal in the ventrolateral prefrontal cortex and a decreased signal in the hippocampus during the n-back task during the n-back working memory task. Thirty five mins after receiving 100mg of hydrocortisone as a bolus, healthy volunteers demonstrated a lesser decrease in BOLD signal in the left hippocampus than was demonstrated following saline administration. This suggests acute hydrocortisone impairs the function of the working memory network related to decreased ability to switch attention to external stimuli or goals. This study also demonstrated an acute time-dependent increase in BOLD signal in the hippocampus when comparing the hydrocortisone infusion scans with the saline scans, demonstrating the localised, rapid effect of hydrocortisone. The maximal effect was demonstrated between 11-19 minutes.
post infusion consistent with an action through non-genomic signalling and with what we know histologically about the MRs and GRs in the hippocampus.

In contrast to these findings secondary to the acute administration, paper 3 (as described in Chapter 5) examined the fMRI correlates of neurocognition in a TRD population. Given that dysfunction in the HPA axis and hypercortisolaemia is a well documented finding in depressed individuals, especially those with more severe and prolonged disease (Porter et al 2004), and patients with depression also show deficits in cognition, it is hypothesised that these patients will demonstrate the chronic effects of hypercortisolaemia on their neurocognitive function. This study found no difference in the behavioural response in either the n-back working memory task or the emotional encoding retrieval task. It did, however, find there was a difference in terms of BOLD signal activation between the TRD group and the HV group, with a decreased BOLD signal in the dorsolateral prefrontal cortex during the n-back task in the TRD compared to the HV. In the emotional encoding task, the TRD patients showed a greater decrease in the posterior cingulate when encoding both the positive and neutral images and when retrieving both sets of images. They also showed an increased activation of the posterior insular cortex compared to HVs. The patient group showed a decrease in BOLD signal in the anterior cingulate, relative to controls, when comparing the response to retrieving positive images as compared to neutral images. This study suggests there is an alteration in the functioning of the cingulate and insular cortex in the encoding and retrieval of positive emotional memories in this group of patients with TRD.

The study described in chapter 6 (paper 4), describes the acute effect of hydrocortisone using challenge phMRI on TRD patients and healthy controls. In contrast with my previous findings, I did not find any signal change either in my hypothesised ROIs (the the hippocampus, hypothalamus, amygdala and dorsolateral and ventrolateral prefrontal cortex) in the main effect or in the comparison between the patients and HV; the comparison between the hydrocortisone and saline; and the interaction between patient group and the drug received. However, the main effect of hydrocortisone showed a significant decrease in the anterior cingulate. There was an effect of drug with the both patients and HV showing an increase in the medial frontal gyrus when comparing the
effect of hydrocortisone to saline. The patient group however, showed a significant lower response, in keeping with the hypothesis that depressed patient have reduced receptor sensitivity for cortisol and also have a down-regulation of corticosteroid receptors (Rupprecht et al 1991). However, given the failure to reproduce the previous findings, or detect a change in BOLD signal in the hypothesised areas known to be involved in the higher regulation of the HPA axis (de Kloet et al 2007), the significance and reliability of this result is unclear. Given the relative lack of power in this study, combined with possible problems with motion correction and the reliability of the technique, this result should be interpreted with caution.

My hypotheses were that an acute rise in cortisol would have an adverse effect on cognition and cognitive processes, as measured by neurocognitive tests and fMRI. I hypothesized that this would represent a non-genomic action of cortisol. I demonstrated through meta-analysis and through fMRI that there was a rapid change in a time frame consistent with non-genomic action of cortisol via MRs and GRs. I also hypothesised that an acute rise in hydrocortisone would cause an increase in time-dependent BOLD signal in the hippocampus, and that this increase will be greater in healthy volunteers than for TRD patients, due to decreases sensitivity of GRs and MRs in the patient group. I demonstrated an increase in BOLD signal in HV; however this was not replicated in the second phMRI study so this hypothesis could not be proved. In addition, I hypothesised that the chronic effects of HPA dysfunction in TRD patients would result in hippocampal under activity during fMRI working memory and encoding-retrieval cognitive tasks, in keeping with the effects of acute cortisol. I did not demonstrate this. Also, I hypothesized that the anterior cingulate, insula, and dorsolateral prefrontal cortex would show relative under-activity compared to healthy volunteers, in keeping with previous literature, and I have proven this hypothesis.

7.2 The non-genomic action of corticosteroids on the hippocampus

One conclusion to be drawn from the findings of this thesis is that corticosteroids cause a rapid, demonstrable effect on behaviour that is consistent with the timings of the non-genomic action of corticosteroids on MRs and GRs (Losel et al 2003). Hydrocortisone also
causes a direct increase of BOLD signal on the hippocampus and also during fMRI neurocognitive tasks. It is accepted that cortisol’s action as a steroid hormone takes approximately 4 hours to demonstrate an effect, causing transcription of mRNA after the rise in cortisol (Joëls et al 2011), with the effect on nucleic DNA being detectable after 1 hour (Morsink et al 2006). The meta-analytic evidence summarised in Chapter 3 demonstrated that there is a behavioural response to an acute rise in cortisol, namely an impairment of the retrieval of words, especially of emotionally neutral words. This was further supported by the acute direct effects on the hippocampus and during cognitive tasks demonstrated in Chapter 4 (Symonds et al 2012). However, this was not replicated, leading to doubts about the methodology (see further discussion in Chapter 6, section 6.6 and in section 7.5, below). If patients with depression, especially more severe and treatment resistant forms of the disorder (Porter et al 2004), have abnormalities of the HPA axis including down-regulation and reduced sensitivity of MRs and GRs (Keeney et al 2006), then studying the non-genomic action of cortisol on the hippocampus could therefore provide evidence to support this theory. However, this thesis did not manage to provide direct evidence for or against the theory that the abnormalities in MR and GR sensitivity seen in depression are in part caused by an impairment of the non-genomic pathways. This was in part due to the problems with the phMRI technique used and described in Chapter 6. However this remains a promising area for future research given the potential role of anti-glucocorticoids as augmenting agents for antidepressants in TRD (Gallagher et al 2008; Jahn et al 2004) (see section 7.6) and the proposed model of reduced corticosteroid sensitivity as an aetiopathological factor in depression (Keeney et al 2006).

7.3 The validity for the actions of acute cortisol on the limbic system as a model for depression

This study chose to focus mainly on the acute actions of cortisol in the hippocampus, as well as the longer term effects of depression on cognition (see section 7.4, below). The challenge to this research is whether this model is applicable to depression. As discussed above (section 7.2), differences in the acute action of cortisol between patients with
depression and healthy volunteers may provide a model to support the theory that patients with depression have reduced sensitivity of their corticosteroid receptors (Keeney et al 2006). The limbic system, which plays a key role in depression, is rich in mineralocorticoid receptors, as well as glucocorticoid receptors (which are found more widely outside the limbic system) (Berardelli et al 2013). MRs have been most strongly linked to a rapid, non-genomic response than GRs, linking this MR rich area to a rapid response to the acute effects of corticosteroids (Olijslagers et al 2008). Hypercortisolaemia (due to iatrogenic or idiopathic causes) has been used as a model for depression in that it causes many similar symptoms, as well as being an abnormality that has been found in many patients with depression (Holsboer 2001). Depression is a multifactorial disorder and although acute cortisol, and the HPA axis as a whole, is unlikely to be the sole cause, anti-gluocorticoids have shown potential as antidepressants (Abercrombie et al 2011; Jahn et al 2004).

This thesis did not provide direct evidence of differences of receptor sensitivity. The indirect comparison between the neurocognitive effects of healthy volunteers given an acute bolus of hydrocortisone and the TRD population is not a valid one. I did not find a parallel between the findings in the non-genomic effects of cortisol on the n-back task in the TRD population. The experiment described in Chapter 6 aimed to provide a direct comparison between the non-genomic effects of hydrocortisone on the limbic-hypothalamic-adrenal system using phMRI. Unfortunately, the hypothesis that patients with TRD would demonstrate a reduced BOLD signal was not proven due to problems with the task, which were discussed in section 6, section 6.5. However, further experimentation with the hydrocortisone challenge phMRI technique may help provide direct evidence for or against the theory of reduced corticosteroid sensitivity in depression.

7.4 Neurocognition and depression

The work in this thesis investigated a convergence of ideas regarding neurocognitive function in depression and the role of the HPA axis. There is a demonstrable link between the HPA axis and cognition both in healthy individuals (Het et al 2005) as well as between depression and cognition (Gupta et al 2013). In addition, a link has been established
between cortisol levels in depressed patients and neurocognition using the cortisol awakening response (a technique used to examine the dynamic changes in the HPA axis caused by diurnal variation) (Hinkelmann et al 2013). In the work presented in this thesis, the exploration of the effects of cortisol on encoding and retrieval (see chapters 3 and 5), as well as on working memory (chapter 3 and 4), is explored. However, this thesis did not demonstrate a direct link between the HPA axis and neurocognition in depressed individuals.

This thesis demonstrated an under-activity in the ‘affective’ area of the anterior cingulate (BA33) in the TRD group compared to HV, which is in-keeping with abnormalities described in depressed individuals Devinsky et al (Devinsky et al 1995). Lesions in this area have been linked to the development of depression, and this current work re-enforces the importance of this area for emotional processing of memories in depression. Our findings with regard to a relative under-activity of the posterior cingulate when encoding emotional material is in keeping with some research but not all, as outlined in a recent review (Leech and Sharp 2013). There is no consensus on the action of the posterior cingulate; however, it is usually deactivated when attention is required to be switched to an external task. It is also one of the most highly connected areas of the human brain and is connected to the parahippocampus and hippocampus. This lack of difference in attention represented by a decreased activation of the posterior cortex represents a mechanism by which emotionally valenced cognitive biases may arise. The decreased BOLD signal in the dIPFC in the treatment resistant group is similar to other groups’ findings (Harvey et al 2005; Kerestes et al 2012). However, other groups have found medial rather than dorsolateral decrease in BOLD signal in depressed patients (Rose et al 2006a). This appears to be only present when a greater demand is made on working memory and this thesis did not find a difference in the behavioural response in any of the neurocognitive tasks. This was in contrast with previous studies (Gupta et al 2013; Harvey et al 2004; Oliveira et al 2013). However, this lack of difference in the behavioural response has also been reported by several other studies (Harvey et al 2005; Roiser and Sahakian 2013). The decreased activation of the dIPFC most likely represents decreased attention, as previous authors have theorised (Harvey et al 2005; Kerestes et al 2012).
This thesis adds to the understanding of the role of the prefrontal cortex and the cingulate cortex in the neurocognitive deficits commonly seen in depression. The suggestion from the literature is that the common feature is an underlying problem with attention and concentration, especially to emotionally valenced stimuli and when a greater demand is placed on working memory load.

### 7.5 Limitations of the research

In this thesis I set out to examine the role of the HPA axis in depression. I focused on the effect of cortisol and depression on the endocrine role of the hippocampus. I aimed to examine the evidence for acute effects of cortisol on cognition, both from performing a systematic review and meta-analysis and from experimental work with healthy volunteers and TRD patients, as well as examining the neurocognitive differences between HV and TRD patients using fMRI. This study was partially successful in achieving those aims and addressing our hypotheses, as described in section 7.1.

The main limitation was the number of patients in this study. The number of healthy volunteers was adequate but small with 15 in the crossover, within patient design study described in Chapter 4 and 30 HV recruited into the studies described in Chapters 5 and 6. For the fMRI study, there was an adequate but small sample of patients, however for the phMRI study; the individual comparison groups only had 12 people in them in a parallel group design which left the study underpowered compared to the previous within-subject design used in the study described in Chapter 4. This was one of the contributing factors in being unable to replicate the hydrocortisone challenge phMRI results from Chapter 4. This was partly due difficulties in recruit enough patients in the allotted time. However, the absence of any signal in the main effect suggests the change in technique to using prospective motion correction may have artefactually removed the signal.

Hydrocortisone challenge phMRI is a new technique and the parameters that affect its reliability and validity have not yet been determined. There are two controversial elements. The first is that phMRI is a relatively new technique and lacks test-retest reliability (Klomp et al 2013). Whether hydrocortisone is a suitable pharmacological agent
to use with phMRI has less acceptance. Apart from my earlier study, there are only two other studies published which demonstrated in humans and rats a change in BOLD signal secondary to an acute challenge with corticosteroids (Lovallo et al 2009), (Ferris and Stolberg 2010). Given that the theory of a rapid non-genomic action of cortisol which leads to a demonstrable action is a relatively new one with little evidence in humans, hydrocortisone may be seen as not compatible with a rapid pharmacological challenge technique.

The clinical significance of our results in regard the neurocognitive deficits is also limited by the fact they do not extend to an effect on the behavioural results. This is in keeping with many fMRI studies, where the behavioural difficulty should be controlled for (Amaro Jr and Barker 2006). I deliberately designed the neurocognitive tasks with this in mind. Whether these fMRI signs represent a meaningful difference between patients with treatment resistant depression and healthy volunteers is therefore open to debate. In addition, all the patients were on serotonergic antidepressant medication. This could be a potential confounding factor as it has been demonstrated that normal volunteers show an attenuated signal change to the Go/No Go task, enhancing the response in the lateral orbital frontal cortex and also enhancing the amygdala response to aversive faces in a facial emotion recognition task (Anderson et al 2002). Additionally, in a study comparing two depressed groups, a reduction in the response to faces with a fearful expression was demonstrated in those receiving a citalopram infusion in an out of scanner task (Bhagwagar et al 2004). However, my results showed that despite being on serotonergic drugs, the patients did not demonstrate an enhanced response to the stimuli in the neurocognitive tasks. Although it is important to be mindful to the possibility of potential confounding factors, our study did not replicate these findings and demonstrated significant deficits in this medicated, depressed population. This study compared TRD patients with HV, so no direct comparisons were made between first episode depression and those with a more severe and prolonged illness.
7.6 Future directions of research

Further validation of the hydrocortisone infusion phMRI technique is needed, specifically in regards to whether prospective motion correction was responsible for abolishing the BOLD signal in our a priori hypothesised areas (as outlined in Chapter 6, section 6.5). This is a potentially useful technique that could help us understand the acute reactivity of the neural areas involved in the higher control of the HPA axis and would go some way to prove whether patients with depression, especially TRD, do indeed have reduced sensitivity of their corticosteroid receptors, as has been theorised previously (Keeney et al 2006). Following further validation of the hydrocortisone challenge phMRI technique, future research could examine childhood adversity, a common aetiological factor for both HPA axis abnormalities and depression (Heim et al 2010), in relation to the effects of an acute rise in cortisol on neurocognition and cerebral functioning using the hydrocortisone phMRI challenge technique.

Another future direction of research would be to further explore the relationship between emotional memory, possible aetiological factors (such as the HPA axis), treatment and treatment resistant depression. The meta-analysis I undertook in Chapter 3, suggests that emotional memories may be relatively preserved, however too few studies existed to make a firm conclusion. Further experimental work with depressed patients with modulatory phMRI (i.e. following an acute bolus of corticosteroid) examining emotional memory would help us understand the role of the HPA axis and, perhaps in later experimentation using phMRI, other neurotransmitters on emotional memory, the key depressive symptom of cognitive bias towards negative memories could be targeted therapeutically. The cingulate cortex is an area of particular interest to be explored in future research.

7.7 Conclusions

There is a complex interaction between the acute and chronic effects of the HPA axis in relation to cognition; affective processing and emotional memory; and depression. This work attempted to develop a new technique to explore the relationship between the HPA
axis and depression, focusing on the hippocampus and was partly successful in its goals: it explored the difference in BOLD signal fMRI between TRD and HV, and imaged the acute effects of hydrocortisone on working memory and the hippocampus, but was unable to replicate the phMRI findings or examine the difference in glucocorticoid sensitivities in TRD directly.

Further work is required to further develop hydrocortisone challenge phMRI and to explore other modalities of investigating patients with treatment-resistant depression such as exploring the role of emotional memory networks through connectivity analysis, as arguably they are the ones who will most benefit from therapeutic advances arising from research in this area.

7.8 References


8 References


Appendix 1 - Mini Screen for SCID

Patient Name: _________________________________ Date of Interview: __________________
Date of Birth: ____________________

If YES, go to the corresponding S.C.I.D module ↓

Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?
NO YES → A1

Has there ever been a time in the past when you have been consistently depressed or down, most of the day, nearly every day, for at least two weeks?
NO YES → A12

In the past two weeks have you been less interested in most things or less able to enjoy the things you used to enjoy most of the time?
NO YES → A1

Has there ever been a time in the past when you have been less interested in most things or less able to enjoy the things you used to enjoy most of the time lasting for at least two weeks?
NO YES → A12

Have you felt sad, low or depressed most of the time for the past two years? NO YES → A38

In the past month did you think that you would be better off dead or wish you were dead?
NO YES →

SUICIDE RISK

Have you ever had a period when you were feeling ‘up’ or ‘high’ or so full of energy or full of yourself that you got into trouble or that other people thought you were not your usual self? (Do not consider times when you were intoxicated on drugs or alcohol)
NO YES → A18

Have you ever been persistently irritable, for several days, so that you had arguments or verbal or physical fights, or shouted at people outside your family?
Have you or others noticed that you have been more irritable or over reacted compared to other people, even in situations that you felt you were justified?
NO YES → A18

Have you on more than one occasion, had spells or attacks when you suddenly felt anxious, frightened, uncomfortable or uneasy, even in situations where most people would not feel that way? Did the spells peak within 10 minutes?
CODE YES ONLY IF THE SPELLS PEAK WITHIN 10 MINUTES.
NO YES → F1
Do you feel anxious or uneasy in places or situations where you might have a panic attack or panic like symptoms, or where help might not be available or escape might be difficult: like being in a crowd, standing in a line (queue), when you are away from home or alone at home, or when crossing a bridge, travelling in a bus, train or car?

NO YES → F7

In the past month were you fearful or embarrassed of being watched, being the focus of attention or fearful of being humiliated? This includes things like speaking in public, eating in public or with others, writing while someone watches, or being in social situations?

NO YES → F11

In the past month have you been bothered by recurrent thoughts, impulses, or images that were unwanted, distasteful, inappropriate, intrusive or distressing? (e.g. the idea that you were dirty or contaminated or had germs, or fear contaminating others or fear of harming someone even though you didn’t want to or fearing you would act on impulse or fear or superstitious that you would be responsible for things going wrong or obsessions with sexual thoughts, images or impulses or hoarding collecting or religious obsessions.)

NO YES → F20

In the past month, did you do something repeatedly without being able to resist doing it, like washing or cleaning excessively, counting or checking things over and over, or repeating, collecting or arranging things, or other superstitious rituals?

NO YES → F20

Have you ever experienced or witnessed or had to deal with an extremely traumatic event that included actual or threatened death or serious injury to you or someone else?

EXAMPLES OF TRAUMATIC EVENTS INCLUDE SERIOUS ACCIDENTS, SEXUAL OR PHYSICAL ASSAULT, A TERRORIST ATTACK, BEING HELD HOSTAGE, KIDNAPPING, FIRE, DISCOVERING A BODY, SUDDEN DEATH OF SOMEONE CLOSE TO YOU, WAR, OR NATURAL DISASTER.

NO YES → F25

Did you respond to the trauma with intense fear, helplessness or horror? NO YES → F25

During the past month, have you re-experienced the event in a distressing way (such as, dreams, intense recollections, flashbacks or physical reactions)?

NO YES → F25

In the past 12 months have you had 3 or more alcoholic drinks within a 3 hour period on more than 3 occasions?

NO YES → E1

Now I am going to show you / READ THE LIST BELOW of street drugs or medicines. In the past 12 months did you take any of these drugs more than once to get high, to feel better or to change your mood?

Amphetamines Speed Crystal Meth Dexedrine Ritalin, Diet Pills Cocaine Crack Freebase Codeine, Percodan, OxyContin
Heroine Morphine, Methadone Opium Dermerol Ecstasy LSD Mescaline PCP MDMA Steroids
Inhalants Glue Ether GHB Barbiturates, Valium, Xanax, Ativan THC, Marijuana Cannabis,
Hashish Grass

NO YES → E9

How tall are you? _____Feet ____ Inches

What was your lowest weight in the past three months? ______Lbs

IS THE PATIENT’S WEIGHT LOWER THAN THE THRESHOLD CORRESPONDING TO HIS/HER
HEIGHT? SEE TABLE BELOW

Females 4’10 4’11 5’0 5’1 5’3 5’4 5’5 5’6 5’7 5’8 5’9
Weight 85 86 87 89 94 97 99 102 104 107 110
Males 5’3 5’4 5’5 5’6 5’7 5’8 5’9 5’10 5’11 6’ 6’1
Weight 108 110 111 113 115 118 120 122 125 127

NO YES → H1

In the past three months, did you have eating binges or times when you ate a very large
amount of food within a 2-hour period?

NO YES → H4

In the last 3 months, did you have eating binges as often as twice a week? NO YES → H4

Have you worried excessively or been anxious about several things over the past 6 months?

NO YES → F31

Are there any other things that you have been especially afraid of like flying, seeing blood,
heights, closed spaces or certain types of animals or insects?

NO YES → F16
SUICIDE RISK ASSESSMENT

In the past month did you:

C1  Think that you would be better off dead or wish you were dead?  NO  YES  1
C2  Want to harm yourself?  NO  YES  2
C3  Think about suicide?  NO  YES  6
C4  Have a suicide plan?  NO  YES  10
C5  Attempt suicide?  NO  YES  10

In your lifetime:

C6  Did you ever make a suicide attempt?  NO  YES  4

IS AT LEAST 1 OF THE ABOVE CODED YES?

IF YES, ADD THE TOTAL NUMBER OF POINTS FOR THE ANSWERS (C1-C6) CHECKED "YES" AND SPECIFY THE LEVEL OF SUICIDE RISK AS FOLLOWS:

<table>
<thead>
<tr>
<th>Points</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>Low</td>
</tr>
<tr>
<td>6-9</td>
<td>Moderate</td>
</tr>
<tr>
<td>≥ 10</td>
<td>High</td>
</tr>
</tbody>
</table>

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Appendix 2 - Edinburgh Handedness Inventory

A2.1 Version used in Chapter 4

Handedness Questionnaire

Instructions

For each of the ten activities below, please tell us:

1. Which hand do you prefer for that activity? Right Left Either
2. Do you ever use the other hand for the activity? Yes No

<table>
<thead>
<tr>
<th>Activity</th>
<th>Which hand do you prefer when?</th>
<th>Do you ever use the other hand?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Writing:</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
<tr>
<td>Drawing:</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
<tr>
<td>Throwing:</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using Scissors:</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using a Toothbrush:</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using a Knife (without fork):</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using a Spoon:</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
<tr>
<td>Using a Broom (upper hand):</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
<tr>
<td>Striking a Match:</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
<tr>
<td>Opening a Box (lid):</td>
<td>R L E</td>
<td>Yes No</td>
</tr>
</tbody>
</table>

Thank you for your responses

The Oldfield rating method - Scores range from +20 (RH) to -20 (LH)

Left = -2  Right = +2  Either = 0

(Use of other hand scores ± 1, unless there is no preference)
A2.2 Version use in Chapter 5 and 6

Edinburgh Handedness Inventory (revised)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Always Left</th>
<th>Usually Left</th>
<th>No Preference</th>
<th>Usually Right</th>
<th>Always Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Writing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throwing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scissors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toothbrush</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knife (without fork)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spoon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Match (when striking)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Computer Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please mark the box that best describes which hand you use for the activity in question.

- Always left = -50
- Usually left = -25
- No preference = 0
- Usually right = +25
- Always right = +50

Handedness:

- Left = < -200
- Mixed = -200 to +200
- Right = > +200

Score:

To code score:

- 0 = Mixed
- 1 = L
- 2 = R
Appendix 3 - Massachusetts General Hospital Staging Method to Classify Treatment Resistant Depression

EME-08/43/39
Antiglucocorticoid augmentation of anti-depressants in Depression - The ADD Study

Healthy Volunteer Screening

<table>
<thead>
<tr>
<th>Centre</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Volunteer Number</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td></td>
</tr>
</tbody>
</table>

**MASSACHUSETTS GENERAL HOSPITAL STAGING METHOD TO CLASSIFY TREATMENT RESISTANT DEPRESSION**

Has the volunteer ever received any treatment with ANTIDEPRESSANT medications

1 = Yes  2 = No  If yes – Discuss with PI may not be eligible

If yes
1) On the grid below in column A, tick any antidepressants the volunteer has taken at the minimum dose for at least 6 weeks during THIS episode of depression.

2) For antidepressants ticked in column A, put another tick in column B if the treatment continued for at least 10 weeks.

3) Tickle column C if the volunteer has taken the drug at a dose equal to or greater than the maximum dosage listed for that medication (there is no extra score for doses higher than the maximum.)

4) If the volunteer has been prescribed any of the drugs listed here during the same time period to boost the antidepressant effect, write the name in column D. (Citalopram, Bupropion, Lithium, Mirtazapine, Glucocortec, Olanzapine, Risperdal and any second Antidepressant).

<table>
<thead>
<tr>
<th>Genetic name</th>
<th>Min dose (mg/day)</th>
<th>At least 6 wks</th>
<th>At least 10 wks</th>
<th>Max dose (mg/day)</th>
<th>Equal or greater</th>
<th>Name of drugs added to augment this antidepressant (second antidepressants should be scored here not column A)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desipramine</td>
<td>150</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlomipramine</td>
<td>150</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxapine</td>
<td>150</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>75</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tranylcypromine</td>
<td>150</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipramine</td>
<td>150</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxepin</td>
<td>75</td>
<td>150</td>
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</tbody>
</table>

Did the volunteer ever receive ECT treatment

Note: To score this test, please refer to the separate information sheet

Total MGH score.

Rater’s Initials

Monester’s Initials

Page 125
Massachusetts General Hospital (MGH)

(1) Non-response to each adequate (at least 6 weeks of an adequate dose of antidepressant) trial of a marketed antidepressant generates an overall score of resistance

  ° score 1 point per antidepressant trial

(2) Optimization of dose, optimization of duration, and augmentation/combination of each trial (based on the MGH or Antidepressant Treatment Response Questionnaire) increases the overall score

  ° score 0.5 points per optimization/strategy for each trial

(3) ECT* increases the overall score by 3 points

*ECT, electroconvulsive therapy.
Appendix 4 - Results from piloted Encoding/Retrieval Scenes Task

A4.1 Behavioural Results

There was no significant difference between the performance in correctly identifying scenes after hydrocortisone infusion or after placebo (p=0.650). This is illustrated in Table A1 below.

<table>
<thead>
<tr>
<th></th>
<th>Hydrocortisone</th>
<th>Saline</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number correct</td>
<td>55.4 (±8.3)</td>
<td>54.1 (±7.4)</td>
<td>0.650</td>
</tr>
<tr>
<td>Mean(+/-SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage correct (total)</td>
<td>71.0(±10.6)</td>
<td>69.4 (±9.5)</td>
<td>0.650</td>
</tr>
<tr>
<td>Number omitted</td>
<td>2.8 (±5.1)</td>
<td>2.2 (±3.6)</td>
<td>0.489</td>
</tr>
<tr>
<td>Percentage attempted correct (no correct/total-no omitted)</td>
<td>73.4(±8.4)</td>
<td>71.5 (±9.2)</td>
<td>0.549</td>
</tr>
</tbody>
</table>

Table A-1: effect of hydrocortisone vs. placebo on the encoding/retrieval scenes task (in scanner)

A4.2 Functional MRI Results

A4.2.1 Main effect of task

No effect was seen on the hippocampus in encoding (encode-rest task or rest task-encoding, see Figure ). There is no effect seen on the hippocampus in retrieval-rest task (see Figure A4-3). Significant effects were seen in the visual cortex in both task (p task (p<0.001)
Figure A4-1: This illustrates the main effect of encoding, as calculated from an encoding-rest task subtraction (placebo and hydrocortisone scans combined)

Figure A4-3: This illustrates the main effect of retrieval, as calculated from a retrieval-rest task subtraction (placebo and hydrocortisone scans combined)
A4.2.2 Effects of drug

There was a significant effect on the hippocampus during encoding, \( P<0.001 \) on a saline-cortisol encoding-rest task subtraction. This is illustrated in Figure A4.3.

Figure A4-3: Graphic shows reduced activation in the left hippocampus during the Encoding phase of encoding/retrieval task, \( p<0.001 \). It consisted of a series of emotionally neutral images presented with interpolated control task (counting), followed by the Retrieval phase (cued recall). Also shown is the results are shown as percentage BOLD signal change per group derived from random effects analysis. Error bars reflect 90% confidence interval. BOLD Signal was significantly reduced after administration of hydrocortisone in the hippocampus.
## Appendix 5 - Supplementary material relating to Chapter 3

<table>
<thead>
<tr>
<th>Trial</th>
<th>Participants</th>
<th>Intervention</th>
<th>Methods</th>
<th>Time pre-encode</th>
<th>Time pre-retrieve</th>
<th>Reason for rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andreano et al (2006)</td>
<td>Healthy volunteers (male &amp; female) age 18-25 n=36 males and 46 females</td>
<td>Cold pressor</td>
<td>‘War of the Ghosts’ paragraph test (‘relatively neutral’). Free recall one week after encoding. Parallel design</td>
<td>7 days. stress 2 mins after encoding</td>
<td>Greater than the 4 hours pre-retrieval necessary for inclusion</td>
<td></td>
</tr>
<tr>
<td>Abercrombie et al (2010)</td>
<td>Healthy males n=53</td>
<td>Viewing IAPS pictures</td>
<td>63 (21 neutral, 21 positive, 21 negative) IAPS pictures viewed Parallel design</td>
<td>48 hours</td>
<td>No control group. Memory task was also stressor.</td>
<td></td>
</tr>
<tr>
<td>Beckner et al (2006)</td>
<td>Healthy males &amp; females volunteers age 18.8 n=101.</td>
<td>Public speaking</td>
<td>WAIS paragraph test B (neutral material). Free recall 48 hours after learning material. Parallel design</td>
<td>45 mins</td>
<td>Rejected after sensitivity analysis - task too dissimilar and accounted for 82% of variance in analysis</td>
<td></td>
</tr>
<tr>
<td>Cornelisse et al (2011)</td>
<td>n=64 healthy volunteers</td>
<td>spironolactone 400mg and Trier Stress test or placebo and Trier stress test</td>
<td>24 words with recall and recognition. Parallel design</td>
<td>120 mins (drug)</td>
<td>Combination of stress and drug as intervention did not fit with design of this meta-analysis</td>
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<tr>
<td>Elzinga et al (2005)</td>
<td>Healthy volunteers (female) n=16</td>
<td>Social stress challenge</td>
<td>Word list. Recall. No control group.</td>
<td>20 mins</td>
<td>Rejected as no control task</td>
<td></td>
</tr>
<tr>
<td>Errico et al (2002)</td>
<td>Recovering Alcohol dependent n=48 HV=30</td>
<td>Cold Pressor</td>
<td>Wechsler paragraph test (recall and recognition). Parallel design</td>
<td>40 mins</td>
<td>Rejected as participants not similar enough to rest of meta-analysis population</td>
<td></td>
</tr>
</tbody>
</table>

Table A5-1: Characteristics of papers rejected
<table>
<thead>
<tr>
<th>Trial</th>
<th>Participants</th>
<th>Intervention</th>
<th>Methods</th>
<th>Time pre-encode</th>
<th>Time pre-retrieve</th>
<th>Reason for rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coluccia et al (2008)</td>
<td>n=24 patients rheumatoid arthritis age 41-65</td>
<td>Prednisolone 5mg</td>
<td>Word list of neutral words with free recall, Parallel design</td>
<td>60 mins</td>
<td></td>
<td>‘Normal volunteer’ group also had rheumatoid arthritis - too dissimilar to other studies.</td>
</tr>
<tr>
<td>Ganguli et al (1994)</td>
<td>Healthy volunteers (n=7) &amp; patients with schizophrenia (n=7)</td>
<td>hydrocortisone</td>
<td>Word list. Recall. Parallel design</td>
<td>2 mins</td>
<td></td>
<td>No control task for healthy group.</td>
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<tr>
<td>Grossman et al (2006)</td>
<td>PTSD patients (n=15) &amp; Healthy controls (n=12)</td>
<td>hydrocortisone 17.5mg</td>
<td>Paragraph recall from Wechsler (recall). Parallel design</td>
<td>30 mins</td>
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<td>No control task for healthy group.</td>
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<tr>
<td>Hopper et al (2004)</td>
<td>Cocaine dependent patients</td>
<td>hydrocortisone 0.2mg/kg or 0.5mg/kg</td>
<td>12 categorical words (all neutral). Recognition memory. parallel (2 groups no placebo)</td>
<td>20, 60 and 100 mins</td>
<td></td>
<td>No control group and group too dissimilar to healthy volunteers</td>
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<tr>
<td>Hsu et al (2003)</td>
<td>Healthy male volunteers age 18-30 n=18</td>
<td>hydrocortisone 100mg or placebo</td>
<td>Word list (neutral). Recall and recognition 60 mins after encoding. Crossover design</td>
<td>60 mins</td>
<td></td>
<td>No means and SD of delayed recall available - just a composite score</td>
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<tr>
<td>Kuhlman et al (2006)</td>
<td>Healthy Volunteers</td>
<td>Trier stress test and hydrocortisone 30mg</td>
<td>15 neutral words and 15 negative words. Recall. Parallel study</td>
<td>60 mins</td>
<td></td>
<td>Combination of stress and drug did not fit with design of this meta-analysis</td>
</tr>
</tbody>
</table>

Table A5-1: Characteristics of papers rejected
<table>
<thead>
<tr>
<th>Trial</th>
<th>Participants</th>
<th>Intervention</th>
<th>Methods</th>
<th>Time pre-encode</th>
<th>Time pre-retrieve</th>
<th>Reason for rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupien et al (1997)</td>
<td>Healthy elderly age 62-83 (male and female) n=14</td>
<td>Social stress task</td>
<td>Neutral word list. Recall. Within subject no reverse order.</td>
<td>2 mins</td>
<td></td>
<td>Older age group and no control for effect of time / order on recall.</td>
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<tr>
<td>Lupien et al (2002a)</td>
<td>Healthy male volunteers (age 20-30) n=14</td>
<td>hydrocortisone and metyrapone</td>
<td>Neutral word list - paired learning with interrupted encoding</td>
<td>30 mins</td>
<td></td>
<td>Control group had metyrapone and task dissimilar to others</td>
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<tr>
<td>Lupien et al (2002b)</td>
<td>Healthy male &amp; female volunteers (mean age 68.4) n=14</td>
<td>hydrocortisone and metyrapone</td>
<td>Neutral word list - paired learning with interrupted encoding</td>
<td>30 mins</td>
<td></td>
<td>Control group had metyrapone and task dissimilar to others</td>
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<td>Maheu et al (2005)</td>
<td>Healthy volunteers (male) age 18-34 n=42</td>
<td>Metyrapone and Trier stress test</td>
<td>Paragraph learning</td>
<td>5 mins</td>
<td></td>
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<td>Porter et al (2002)</td>
<td>Healthy elderly age 69-82</td>
<td>hydrocortisone 20mg</td>
<td>Rey AVLT</td>
<td>1 hour</td>
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<td>Oei et al (2006)</td>
<td>Healthy volunteers (male) mean age 21.8 n=20</td>
<td>Trier stress test</td>
<td>Paragraph learning</td>
<td>30 mins</td>
<td></td>
<td>No means and SD of delayed recall available - just a composite score</td>
</tr>
<tr>
<td>Trial</td>
<td>Participants</td>
<td>Intervention</td>
<td>Methods</td>
<td>Time pre-encode</td>
<td>Time pre-retrieve</td>
<td>Reason for rejection</td>
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<td>Preuß et al (2009)</td>
<td>Healthy volunteers age 19-28</td>
<td>Trier Stress Test</td>
<td>Emotional pictures, recall, parallel design</td>
<td>5 mins</td>
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<td>Pictures not words - judged too dissimilar</td>
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<td>Pruessner et al (2007)</td>
<td>Healthy male volunteers</td>
<td>Trier Stress Test</td>
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<td>10 mins</td>
<td>10 mins</td>
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<td>Metyrapone</td>
<td>Emotional paragraph learning, recall, crossover</td>
<td>210 mins</td>
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<td>To dissimilar intervention</td>
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<td>Schlosser et al (2010)</td>
<td>Patients with depression&amp; healthy controls n=36</td>
<td>Hydrocortisone</td>
<td>Emotional cued recall of autobiographical content</td>
<td>10 mins</td>
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<td>Task too dissimilar and no placebo control</td>
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<td>Schwabe et al 2009 (Schwabe et al 2009a)</td>
<td>Healthy volunteers</td>
<td>Socially evaluated</td>
<td>Emotional pictures. Recall and recognition 24 hours after encoding (23 hours after stress). Parallel design</td>
<td>24 hours</td>
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<td>Time frame beyond remit of this meta-analysis and task too dissimilar</td>
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<td>Schwabe et al 2009 (Schwabe et al 2009b)</td>
<td>Healthy volunteers</td>
<td>Trier stress test</td>
<td>Emotional words. Recall. parallel</td>
<td>30 mins</td>
<td></td>
<td>Conditions were stress test + placebo or stress test + propranolol so excluded</td>
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</table>

Table A5-1: Characteristics of papers rejected after scrutiny of the paper content. More papers were rejected during earlier stages of the literature search (See Figure A5-1)
Supplementary Figure A5- 1: Risk of bias graph - review authors’ judgments about each risk of bias item presented as percentages across all included studies.
## Appendix 6 - Supplementary results for Chapter 4

<table>
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<th>Area</th>
<th>BA</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>K</th>
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<th>pFWE</th>
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<tr>
<td>Medial parietal cortex</td>
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<td>-63</td>
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<td>27</td>
<td>4.82</td>
<td>0.010&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
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<td>7</td>
<td>L</td>
<td>-8</td>
<td>-63</td>
<td>55</td>
<td>24</td>
<td>4.82</td>
<td>0.010&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>3.99</td>
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<td>-15</td>
<td>20</td>
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<td>0.009</td>
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<td>63</td>
<td>5</td>
<td>25</td>
<td>4.58</td>
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<tr>
<td></td>
<td>9</td>
<td>R</td>
<td>3</td>
<td>53</td>
<td>40</td>
<td>18</td>
<td>4.50</td>
<td>0.035&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>53</td>
<td>30</td>
<td>4.81</td>
<td>0.010&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Supplementary Table S1: Effect of n-back task under saline in a priori ROIs.

<sup>1</sup> Significant at a whole brain level (FWE=Family wise error, ROI = region of interest analysis, BA = Brodmann Area, K=cluster size at $p_{unc}<0.005$). The areas shown in italics failed to meet predefined significance but is included for information as it suggests bilateral activation.
Appendix 7 - Main effect of encoding-retrieval task (Chapter 5)

### Whole Brain Encode- Rest

<table>
<thead>
<tr>
<th>Area</th>
<th>BA</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z</th>
<th>K</th>
<th>p(FWE-corr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occipital lobe</td>
<td>19</td>
<td>R</td>
<td>37.5</td>
<td>-77</td>
<td>-5</td>
<td>&gt;10</td>
<td>2250</td>
<td>&lt;0.001</td>
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<tr>
<td>Occipital lobe</td>
<td>19</td>
<td>R</td>
<td>34</td>
<td>-84</td>
<td>5</td>
<td>&gt;10</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>18</td>
<td>R</td>
<td>23.5</td>
<td>87.5</td>
<td>-5</td>
<td>&gt;10</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lateral fronto-orbital gyrus</td>
<td>47</td>
<td>L</td>
<td>-43</td>
<td>24.5</td>
<td>-10</td>
<td>7.13</td>
<td>493</td>
<td>&lt;0.001</td>
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<tr>
<td>precentral (middle frontal)</td>
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<td>10.5</td>
<td>30</td>
<td>5.21</td>
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<td>0.001</td>
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<td>middle frontal gyrus</td>
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<td>L</td>
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### Region of Interest (ROI) Encode- Rest

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Table A7-1: Shows the main effect of the encoding task (encode-rest subtraction) areas only, both at whole brain level and following ROI Analysis (FWE=Family wise error, BA = Brodmann Area, K=cluster size at punc<0.005). MNI= Montreal Neurological Institute Space. All other subtractions did not show significant results.
Figure A7-4: Array illustrating areas of the main effect of the encoding task (encode-rest contrast)
### Whole Brain Retrieve- Rest

#### Co-ordinates (MNI) & Cluster

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<th>x</th>
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<th>Z</th>
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#### Table A7-2: Shows the main effect of the retrieval task (encode-rest subtraction) areas only, both at whole brain level and following ROI Analysis (FWE=Family wise error, BA = Brodmann Area, K=cluster size at punc<0.005). MNI= Montreal Neurological Institute Space. All other subtractions did not show significant results.
Figure A7-2: Array illustrating areas of the main effect of the retrieval task (retrieve-rest contrast)