DELINEATING THE EFFECTS OF LOAD ON TEMPORALLY DISSOCIABLE WORKING MEMORY COMPONENTS IN SCHIZOPHRENIA

by

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Abstract

This thesis is comprised of two functional MRI experiments, both of which used event-related designs to examine the patterns of activity associated with the temporally separate encoding, delay and response components of a variable-load working memory (WM) task. In experiment one, the patterns of load dependence associated with different components were assessed in healthy subjects. The majority of the regions activated during encoding were found to show a linear increase in activity with load. In contrast, the majority of the regions activated during responding did not show activity that increased linearly with load. In experiment two, we used the design developed in experiment one to delineate the effects of load on each WM component in patients with schizophrenia compared to non-patient controls. Contrasts for the average activity across memory load revealed that the two groups activated similar networks of regions for all components. For encoding, a reduced ability to increase activity with increasing load was found in the schizophrenic group. For the highest load delay condition, a reduced extent of activity was identified in the schizophrenic group in the left parietal cortex, a brain region implicated in short-term storage. For both phases, a greater degree of differential activity was associated with poorer performance, indicating that these activation differences are reflective of the neural abnormalities that underlie WM impairment. These findings support the notion that load dependent WM inefficiencies are present in schizophrenia.
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Delineating the effects of load on temporally dissociable working memory components in schizophrenia

Working memory (WM) impairment is considered a fundamental feature of schizophrenia. WM refers to a brain system that allows information to be temporarily held and manipulated in a short-term store in the service of guiding behaviour (Baddeley, 1992). The majority of WM tasks require subjects to register information that is then maintained in the ‘mind’s-eye’ in the absence of external stimuli so that it can be recalled after a short delay to allow a decision or response to be made. Although some WM tasks require the manipulation or processing of information while it is being maintained, other WM tasks, such as those used in the experiments described in this thesis, require very little manipulation beyond any that may be necessary for deciding on a response.

WM is thought to be an essential component of higher cognitive functions including language comprehension, learning, and reasoning (Baddeley, 1992). It is necessary for carrying out countless daily tasks such as remembering a phone number long enough to dial it or following a conversation. It has been proposed that many of the symptoms of schizophrenia are due to an inability to guide behaviour appropriately based on the contents of WM (Cohen et al., 1996; Goldman-Rakic, 1991). The physiological basis of WM dysfunction may be a fundamental component of the pathophysiological mechanisms underlying schizophrenia.

WM deficits have been well documented in schizophrenia (Keefe, 2000 for review). They have been identified in medicated, unmedicated and medication naive patients (Barch et al., 2001; Carter et al., 1996; Park & Holzman, 1992). Relative to psychotic symptoms, WM deficits are stable (Hughes, 2002), persistent (Park et al., 1999).
and resistant to pharmacological treatment (Goldberg & Weinberger, 1996), although some of the newer atypical antipsychotic medications do seem to offer a degree of WM improvement (Keefe et al., 1999; Meltzer and McGurk, 1999). Notably, in individuals with schizophrenia, impaired verbal WM function is a strong predictor of poor quality of life and poor long-term functional outcome (Green 1996). A better understanding of the nature and neural basis of WM impairments found in schizophrenia could lead to the development of pharmacological and/or psychological interventions to improve WM and perhaps in-turn improve the long-term prognosis for individuals who suffer from this disorder.

Neuroimaging studies have demonstrated that schizophrenic subjects show aberrant activity, relative to healthy subjects, in a number of brain regions during the performance of WM tasks. The most prominent finding has been that of differential prefrontal activation (Andreasen et al., 1992; Barch et al., 2001; Honey et al., 2002; Weinberger at al., 1986, 1988; Weinberger and Berman, 1996). This finding is in agreement with extensive clinical and neuropsychological evidence that has long implicated dysfunction of the frontal cortex in schizophrenia (Kraepelin, 1919; Piercy, 1964). Differential patterns have also been identified in other regions, including parietal and temporal regions (Callicott, 2000), subcortical regions including the basal ganglia, thalamus and cingulate gyrus, and in the cerebellum (Manoach et al., 2000; Mendrek et al., submitted 2002). It is widely agreed that WM deficits are likely to reflect dysfunctional neural circuitry rather than neuropathology in an isolated region (Manoach, 2003; Meyer-Lindenberg., 2001).
In healthy subjects, WM activity has been shown to vary with memory load. Using parametric designs where memory load is varied by increasing the number of items to be rehearsed in a graded fashion, neuroimaging studies have demonstrated that activity increases linearly with memory load during WM performance in many brain regions including prefrontal, parietal, premotor, supplementary motor, striatal, cingulate and cerebellar regions (Braver et al., 1997; Jonides et al., 1997; Rympa et al., 1999b). It has also been shown that when memory capacity is exceeded in healthy subjects, dorsolateral prefrontal cortex activity decreases (Callicott et al., 1999; Goldberg et al., 1998). Taken together these findings suggest that there is an ‘inverted-U’ relationship between dorsolateral prefrontal cortex activity and memory load, such that activity increases with load until WM capacity is reached, at which time it decreases.

It has been proposed that schizophrenic subjects have a reduced WM capacity and thus are inefficient at dealing with increases in memory load (Callicott et al., 2000; Monoaach 2003). This inefficiency means that as memory load increases they have to work harder in order to succeed, which is reflected by greater activation compared to healthy subjects. Their performance capacity would be reached sooner resulting in decreased activity relative to healthy subjects when the task becomes too difficult. Monoaach (2003) has proposed that schizophrenic subjects show the same inverted-U relationship between memory load and prefrontal activity as healthy subjects do, but that this curve is shifted to the left, reflecting peak activation at a lower memory load. In support of this model, many studies have found increased dorsolateral prefrontal activation in schizophrenic subjects compared to healthy subjects when memory demand is low and performance is only slightly impaired (Callicott et al., 2000; Jansma et al.,
There is evidence that schizophrenic subjects may show an inefficient response to increasing memory load in other brain regions besides the prefrontal cortex. In a study by Callicott et al., (2000) schizophrenic subjects were found to exhibit an exaggerated response to increasing load in the right dorsolateral prefrontal cortex, right parietal cortex and left anterior cingulate, and a reduced response to increasing load in numerous regions including the left parietal cortex, right anterior cingulate, right hippocampus and the cerebellum. These results suggest that there is a differing dynamic response to WM load in schizophrenia relative to healthy subjects across a widespread network of regions.

Although neuroimaging studies have been able to demonstrate that the degree and nature of the abnormal activity found in schizophrenia varies with WM load, they have not been able to clearly elucidate the stage of processing at which these abnormalities occur. WM tasks are complex; Even a simple delayed-response task can be separated into encoding, delay and response components or task phases. To complete a delayed-response task, subjects must first encode the information to be remembered; this involves attending to a stimulus, selecting it for entry into WM and activating its internal representation (Hartman et al., 2002). This encoded information must then be actively maintained over a delay. Finally, when the probe is presented, it must be compared with the remembered information, a decision must be made and a motor response must be prepared and executed. It is still unclear which of these specific WM components are impaired in schizophrenia. The identification of specific components that are selectively impaired in schizophrenia. The identification of specific components that are selectively impaired in schizophrenia.
impaired or spared could implicate more specific neural networks in WM dysfunction, and help to focus and refine rehabilitation strategies (Monoach, 2003).

Neuroimaging studies have not yet clearly delineated the patterns of activation associated with the encoding, delay and response components in healthy or schizophrenic subjects, as the majority of studies have used block designs which generate patterns of activity that are averaged over several trials and thus across components. Compared to block designs, event-related designs allow an increased resolution of temporally separated neural events (Zarahn et al., 1997). Event-related designs offer the possibility of using fMRI to demonstrate the patterns of activity associated with each of the WM components.

The current study is comprised of two fMRI experiments, both of which employed event-related designs to determine the patterns of activity associated with temporally separable components of a variable-load verbal WM task. In experiment one, the patterns of load dependence associated with different components were assessed in healthy subjects. In experiment two, we used the design developed in experiment one to delineate the effects of load on each WM component in patients with schizophrenia compared to non-patient controls.
Experiment One

Verbal working memory involves a complex brain system. Even the simplest WM tasks can be broken up into multiple processes or task phases. WM has been studied extensively in healthy subjects using neuroimaging techniques, and while there have been strides made in identifying which brain regions underlie various WM processes, the unique patterns of activity associated with the temporally separate encoding, delay and response components have not yet been clearly delineated.

Neuropsychological and functional imaging studies have implicated a distributed network of cortical and sub-cortical brain areas in verbal WM (for a review see Smith & Jonides, 1998). Included in this network are regions in the dorsolateral prefrontal cortex, bilateral superior and inferior parietal cortices, regions in the anterior cingulate, basal ganglia and cerebellum and left hemisphere frontal speech areas including the inferior frontal cortex, premotor cortex and supplementary motor area (SMA) (Petrides et al., 1993; Schumacher, 1996).

Among the numerous potential processes involved in WM are those involved in perception, storage, subvocal rehearsal, manipulation, memory scanning and decision making. Posterior parietal regions appear to be involved in the short-term storage of information, left hemisphere language regions in subvocal rehearsal (Awh et al., 1996), and prefrontal regions are thought to regulate WM processes (Jonides, 1995). Although it was initially thought that the dorsolateral prefrontal cortex was only involved in the WM tasks that require manipulation (D'Esposito et al., 1999; Petrides, 1995), more recently, activation has also been found in this region during the performance of tasks that emphasize maintenance (Awh et al., 1999; Jansma et al, 2001; Zarahn et al., 1999).
Researchers have attempted to circumvent the poor temporal resolution allowed by block designs by having the delay phase be much longer in duration than the encoding or response phases or by using subtractive techniques. Subtractive methods utilize a control task that is assumed to contain all of the same processes as an activation task except for the cognitive process of interest (Posner at al., 1998). To obtain the activity associated with the processes of interest, the control task activity is subtracted from the activation task activity. One limitation of this method is that it relies on the assumption of pure insertion, which is that the neural activity underlying the irrelevant processes doesn’t change when the cognitive process of interest is added (Friston et al., 1996).

Event-related fMRI avoids some of the shortcomings of averaging and subtractive techniques. The increased temporal resolution allowed by event-related designs offers the ability to isolate the patterns of activation associated with sequential behaviors performed as they would naturally occur, in close temporal relation to one another.

Neuroimaging studies that have used block designs to study the delay phase have found activity that increases linearly with memory load primarily in prefrontal, parietal, premotor, supplementary motor, striatal, cingulate and cerebellar regions (Braver et al. 1997; Jonides et al., 1997). As the majority research has focused on identifying the brain structures which underlie the processes of maintenance and manipulation of information in the absence of external stimuli, the delay component of WM has been studied much more extensively than the encoding and response components. The activation patterns that correspond to the encoding and response phases are also of interest as they are an integral part of any WM task and likely also relevant to disorders in which WM deficits
are found. As encoding and responding have been less well studied than the delay, in the current study we employed a design that emphasized these phases.

The few studies that have attempted to separate the components of WM have focused on prefrontal regions (D'Esposito et al., 1999; Postle et al., 1999; Rowe et al., 2000). Rypma and colleagues (1999a, 2002) found that memory load exerts its effect on the extent of prefrontal activation mainly during the encoding phase, whereas subject factors, such as response time, are related to the extent of prefrontal activity mainly during the response phase. Sternberg's (1966) finding that response time increases linearly with memory load when subjects are required to judge whether a test symbol is contained in a memorized sequence suggests that physiological processes associated with responding may also be linearly dependent on load. This possibility is supported by the results of an fMRI study of memory retrieval and search processes completed by Pollman and colleagues (2000) in which the amplitude of the BOLD response in the SMA, a region associated with response preparation, was found to increase with memory load. These findings suggest, that in addition to the well documented load dependent changes associated with the delay phase, there may be other brain regions that show load dependent changes for encoding and response phases.

The current study explored the patterns of cerebral activity associated with the temporally separable encoding, delay and response components of a WM task in which memory load was varied. In contrast to previous event-related studies, which have focused on prefrontal regions, we completed whole brain analyses, in the hope of gaining a fuller understanding of the complex cognitive processes and neural networks that subserve WM. The design and analysis procedures were optimized to assess load
dependence during the encoding and response phases. To allow the activity associated with encoding and responding to be better separated from that associated with the delay the following measures were taken: firstly, memory load was modeled for encoding and responding but not for the delay and secondly, the duration of the delay was jittered. This novel design allowed us to identify both the brain regions that show load independent activity as well as those that show load dependent activity during encoding and responding.

Method

Subjects

Eighteen healthy people (10 female, ages 18-35, mean age 27.5) consented to participate. All participants were right-handed, had no personal history of mental illness or neurological conditions, no generalized medical conditions requiring treatment, no family history of psychotic disorders, and no contraindication for MR scanning. All procedures complied with University and Hospital ethics requirements. Estimates of intellectual functioning were determined using the NART (National Adult Reading Test; Sharpe and O'Carroll, 1991) and Quick Test (Ammons and Ammons, 1962). The demographic data collected, including age, IQ and parental socio-economic status, are summarized in Table 1.1.

Task Design

Two runs (9 min 48secs each) of a variable load WM task were completed (Figure 1.1). A modified version of the Sternberg Item Recognition Task (Sternberg, 1966) was programmed and presented on a personal computer using Presentation software (Neurobehavioral Systems). Stimuli were projected from an LCD projector onto a screen
mounted at the foot of the MRI table. An angled mirror reflected stimuli from the screen into the participants’ field of view. During a single trial of this task, subjects were presented with a string of 2, 4, 6 or 8 differing consonants in uppercase which they were instructed to remember over a short delay. Subjects were required to indicate if a single consonant in lowercase presented after this delay was the same as one of the remembered letters or different from all of the remembered letters. “Same” and “different” responses were indicated via a fiber-optic response device (Lightwave Medical, Vancouver, BC). The probability of the test letter having been in the remembered string was 0.5.

To encourage participants to maintain attention and motivation throughout the study, subjects received financial reward for each correct response for a total possible reward of 10 dollars (5 cents/2 letters; 10 cents/4 letters; 15 cents/6 letters; 25 cents/8 letters).

A rapid event-related design was used in order to maximize the number of trials presented for each memory load condition. Each of the four memory load conditions was presented 18 times. When using rapid event-related designs, it is important to ensure the conditions of interest are temporally uncorrelated in order that the unshared variance can be used to estimate the individual contribution of each condition to the overall pattern of activation. Design timing influences how well overlapping functions can be deconvolved, as the temporal pattern of onsets between conditions strongly affects the variance and the degree of correlation among these conditions (Ollinger et al., 2000). The general linear model is more efficient at separating overlapping functions when the correlations between covariates are low. The maximum correlation between any pair of covariates in our specified model was 0.23.
Multiple measures were taken to ensure that the correlations between the covariates in our specified model were low. Firstly, variable duration ITIs were used (3, 4 or 5 seconds). Secondly, 18 additional blank trials (20% of all trials) were randomly inserted. These two methods have previously been shown to increase the separability of overlapping functions associated with conditions which occur in a fixed temporal sequence within a trial of a rapid event-related design (Ollinger et al., 2000).

Thirdly, because we chose to focus on the encoding and response phases, we jittered the duration of the delay phase (3, 4 or 5 seconds) to introduce a temporal shift between the conditions of interest. The variance induced by a temporal jitter allows for more accurate signal estimation (Miezin et al., 2000). Finally, in order to determine load sensitive brain regions for the encoding and response phases, it was essential to model load for both of these phases. To minimize the correlation between covariates, load was not modeled for the delay phase.

**Imaging**

Echo-planar images (EPI) were collected on a standard clinical GE 1.5 Tesla system fitted with a Horizon Echo-speed upgrade. Conventional spin-echo T₁ weighted sagittal localizers were used to view the positioning of the participant’s head and to graphically prescribe the functional image volumes. Functional image volumes were collected with a gradient echo (GRE) sequence (TR/TE 3000/40 ms, 90° flip angle, FOV 24 x 24 cm, 64 x 64 matrix, 62.5 kHz bandwidth, 3.75 x 3.75 mm in plane resolution, 5.00 mm slice thickness, 29 slices, 145 mm axial brain coverage). This sequence is sensitive to the blood oxygen-level dependent (BOLD) contrast. Each stimulus run
consisted of 196 scans (encompassing the entire brain). The first 12 seconds collected at the beginning of each run were discarded, to avoid variation due to $T_1$ saturation effects.

**Data Processing**

Functional images were reconstructed offline. Statistical parametric mapping software (SPM99 – Wellcome Institute of Cognitive Neurology, London, UK) was used for image realignment, normalization into modified Talairach stereotaxic anatomical space (using affine and non-linear components, as implemented in SPM99), and smoothing using a Gaussian kernel (8 mm FWHM) to compensate for intersubject anatomical differences, and to optimise the signal to noise ratio. In the Talairach coordinate frame, the origin is at the midpoint of the anterior commissure, the x axis extends from left to right, the y axis from posterior to anterior passing through the posterior and anterior commissures, and the z axis from base of the brain to the vertex.

The BOLD response for the encoding phase was modeled as the convolution of a 4 second box-car (beginning at the onset of the letter string to be encoded) with a synthetic hemodynamic response function composed of two gamma functions (Josephs, Turner, & Friston, 1997). The delay phase was modelled as the convolution of a 3, 4 or 5 second boxcar (corresponding to the duration of the delay) with the synthetic hemodynamic response. Finally, the response phase was modelled as the convolution of a 1 second boxcar (beginning at the onset of the test letter) with the synthetic hemodynamic response. A high pass filter (cut-off period 89 secs) was incorporated into the model to remove noise associated with low frequency confounds (e.g. respiratory artefact). A notch filter (at the Nyquist frequency, with a 6 sec period) was also applied to remove noise associated with alternations of the applied radio frequency field. The
parameters of the modelled hemodynamic response were adjusted to fit the observed BOLD signal time course in each voxel, employing the General Linear Model, using SPM99.

Statistical Analysis

In the first level fixed effects analysis, eleven conditions were modeled: four memory load conditions (2, 4, 6, 8 letters) for both encoding and response and three duration conditions for the delay (3, 4 or 5 seconds). To examine the significance of specific effects of the conditions of interest, contrasts of the parameter estimates were used. For example, the average encoding activity was determined by weighting each encoding condition (2, 4, 6 and 8 letters respectively) equally with the contrast \( c = [1 \ 1 \ 1 \ 1 \ldots] \) being used (Figure 1.2a), whereas the load dependent encoding activity was determined by weighting each encoding condition in a parametric fashion with the contrast \( c = [3 -1 \ 1 \ 3 \ldots] \) being used (Figure 1.2b). Both contrasts return the sum of the parameter estimates for the column of interest weighted by the contrast weight. For the average analyses the significance of the average effects weighted across loads is tested at the second level. For the load dependent analyses the significance of the monotonic increase with load is tested at the second level (Friston, 1996).

In the second level mixed effects analysis, the following five contrasts were computed for each subject: 1) average encoding activation (across memory load) vs. implicit baseline, 2) average response activation vs. implicit baseline, 3) a linear regression of encoding activation and memory load, 4) a linear regression of response activation and memory load and 5) average delay activation (across phase duration) vs. implicit baseline. A one-sample t-test was computed on the individual subject contrast
images (one image per subject) to determine regions of significant activation over all subjects for each of the five contrasts. A height threshold of \( p<0.05 \) corrected for multiple comparisons across the whole brain was used unless otherwise specified.

Results

**Behavioral Performance**

Analysis of variance (ANOVA) of the mean reaction times (RTs) revealed a main effect of load \([F(3,51)=87.24, p<.001]\). Mean RTs for each load condition are shown in Figure 1.3a. *Post hoc* t-tests revealed that with each increase in memory load there was a significant increase in RT (2-4 letters, \( t(17)=4.75 \) \( p<.001 \); 4-6 letters, \( t(17)=6.70 \) \( p<.001 \); 6-8 letters, \( t(17)=2.66 \) \( p<.05 \)).

ANOVA of the error rates revealed a main effect of load \([F(2,51)=16.75, p<.001]\). Mean error rates for each load condition are shown in Figure 1.3b. *Post hoc* t-tests revealed that although 2 letter accuracy was not statistically different from 4 letter or 6 letter accuracy, there was a significant decrease in performance from 4 letters to 6 letters, \( t(17)=3.83, p<.001 \). In addition, performance accuracy was significantly worse for the 8 letter condition compared to each of the lower memory load conditions (2-8 letters, \( t(17)=4.87 \) \( p<.001 \); 4-8 letters, \( t(17)=6.78, p<.001 \); 6-8 letters, \( t(17)=4.96, p<.001 \)). The mean number of errors for all loads was significantly below chance (max 16.7% for the 8 letter condition).

**Imaging Data**

**Encoding**

The peak Talairach coordinate, extent and z-score of significant clusters for both the average activation and load dependent activation analyses of the encoding phase are
reported in Table 1.3. The contrast for the mean activity across all four memory loads
during encoding revealed activation in a widespread network of cortical and sub-cortical
regions (Figure 1.4a). Significant activation was identified bilaterally in the occipital
cortex, inferior and superior parietal lobules, precuneus, temporal lobes, basal ganglia,
cingulate motor area (CMA), middle frontal gyri, inferior frontal gyri and posterior lobe
of the cerebellum. Bilateral activation, that was greater in the left than the right
hemisphere, was identified in the precentral gyrus, SMA and midbrain. Significant
activity was also found in the left superior frontal gyrus.

As can be seen in Figure 1.4b, activity increased linearly with load in the majority
of areas that showed significant mean activity during encoding. The two contrasts
showed very few differences: Activity in the CMA was significant for the mean contrast,
but did not reach statistical significance for the linear load dependent contrast. The linear
load contrast revealed significant activity in bilateral cingulate and some subcortical
regions including the caudate, globus palidus and thalamus which did not meet
significance for the mean contrast.

Delay

No clusters reached our criteria for significance for the delay phase. At a lower
inclusion criterion ($p<0.05$ corrected for multiple comparisons at the cluster level; $p<.001$
uncorrected for multiple comparisons at the voxel level) significant clusters were
centered in the left superior frontal gyrus, extending medially through the left
SMA/CMA, and laterally into the left precentral gyrus, in the left occipital cortex,
extending into the right occipital lobe and left cerebellum, in the right superior frontal
gyrus, and in the left middle frontal gyrus, left precuneus and bilaterally in the insula (Table 1.3).

Response

The peak Talairach coordinate, extent and z-score of significant clusters for both average activation and load dependent activation analyses of the response phase are reported in Table 1.4. The largest significant clusters were in the SMA/CMA, left inferior parietal lobule, and the right anterior lobe of the cerebellum. Smaller significant clusters were also present in the primary motor cortex inferior frontal gyri, temporal lobes, left secondary motor cortex, left postcentral gyrus, right inferior parietal lobule, midbrain and posterior lobe of the cerebellum (Figure 1.5a).

In contrast to what was found for the encoding phase, response phase activity did not increase linearly with load in the majority of the regions that showed significant mean activity during responding (Figure 1.5b). Only the activity in the anterior SMA/CMA and small clusters in the right posterior lobe of the cerebellum reached statistical significance for both the linear load dependent contrast and for the mean contrast. An additional small cluster in the right middle frontal gyrus did not reach statistical significance for the mean contrast, but did reach significance for the linear load dependent contrast.

Discussion

The goal of the current study was to delineate the unique patterns of activity associated with the temporally separated encoding, delay and response components of a WM task, and to determine which of the regions involved in the encoding and responding exhibit activity that increases linearly with load. As discussed below, the patterns of
regional activity identified for each component are plausible in the light of previous research. Linear regression analyses revealed that the majority of regions activated during encoding show load dependence, or a linear increase in activity with load. In contrast, the majority of the regions activated during responding show load independent activity, that is, they do not show activity that increases linearly with load.

Behavioral Performance

As has been commonly found, response times increased significantly with each increase in memory load. Performance accuracy was significantly worse in the eight letter condition than in the lower memory load conditions, however the mean number of errors in the eight letter condition was still above chance, indicating that subjects remained engaged even when the task became more difficult.

Encoding

During encoding, the contrast for the mean activity across all four memory loads revealed significant activation in a distributed network of regions previously implicated in WM, including regions involved in visual pattern processing, subvocal rehearsal and storage of visual and verbal information. A linear regression of encoding activity and memory load revealed load dependent activity in a similar network of regions. In contrast to earlier WM studies that focused predominantly on the delay component, and reported activity confined to the left hemisphere in the precentral gyrus, basal ganglia and occipital cortex, we found bilateral activity in all of these regions. The most remarkable finding was the large extent of right and left occipital cortex that showed a linear increase in activity with load.
Attentional mechanisms may account for the extensive right hemisphere and bilateral occipital activity observed during encoding. Salient stimulus processing is an involuntary attentional mechanism that allows information that is most likely to be relevant to behaviour to be selected from the environment (Coull, 1998; Parasuraman, 1998). This attentional mechanism has been shown to involve relevant sensory association areas, comprising a right lateralized network of brain areas and bilateral occipital regions (Downar et al., 2000, 2001). Many of the regions which showed load dependent activity during encoding were those previously implicated in salient stimulus processing, including bilateral occipital regions, the SMA/CMA, premotor cortex, bilateral precuneus and the right cerebellum. If attentional mechanisms are more important during encoding than during maintenance or responding, studies which include a longer duration delay phase and average activity across an entire WM trial may be limited in their ability to detect right cortical activity specific to the encoding phase.

The linear increase in activity with load in extensive occipital regions suggests that the role of the occipital cortex includes memory or attentional aspects in addition to perception. Alternatively, the load dependent increase in activity may be related to perceptual demand, as letter string length also varied with memory load in the current study. However, occipital activation has previously been shown to increase with memory load in an n-back fMRI study in which WM load for faces was varied but perceptual and motor demands were held constant (Druzgal & D’Esposito, 2000). This finding suggests that the bilateral increase in occipital cortex activation seen in the current study may have been at least partially due to increasing memory or attentional demands.
Delay

Our finding that left and right lateral prefrontal and left lateral parietal regions showed significant activation during the delay phase is consistent with previous research. Converging evidence from neuroimaging, single neuron recording and lesion studies have strongly implicated the lateral prefrontal cortex and the left parietal cortex in the maintenance of memory across a short delay (Cohen et al., 1997; Jonides 1997; Postle et al., 1999). In addition, our analysis revealed significant clusters of activity in left hemisphere premotor and supplementary motor areas and the left inferior frontal gyrus, regions that have been implicated in verbal rehearsal (Awh et al., 1996). Interestingly, bilateral occipital cortex activity was also identified during the delay component. The finding that occipital cortex is still active after the visual display has disappeared adds support to the notion that occipital cortex may contribute to abstract memory representation in addition to its role in visual perception.

Response

During the response phase, the contrast for mean activity across all four memory loads revealed significant activation in regions previously implicated in response preparation and execution (Jahanshahi & Frith, 1998; Requin et al., 1993; Rizzolatti et al., 1998), including the SMA/CMA, the left postcentral gyrus, left primary motor cortex and right cerebellum. In contrast to the encoding phase results, where the majority of significantly active regions were load dependent, very few of the regions active during the response phase exhibited significant load dependent activity.

Our results suggest that there are two functionally different stages of motor preparation during the response phase: a load dependent and a load independent stage.
Only the anterior SMA, CMA and small clusters in the midline and right hemisphere cerebellum, and right middle frontal gyrus (BA 46) showed significant load dependent activity. In contrast, the magnitude of activation in the left primary motor, premotor and parietal regions was independent of load - that is, these regions exhibited significant average activity but did not exhibit significant load dependent activity. These findings are in agreement with an fMRI study of memory retrieval and search processes completed by Pollman and colleagues (2000) in which the amplitude of the BOLD response was found to increase with memory load in the SMA but not in the primary motor cortex or the left posterior parietal cortex.

Leuthold and Jentzsch (2001) completed an event-related potential (ERP) study exploring the brain regions involved in preprogramming and execution of movements. They identified a medial dipole associated with the anterior SMA/CMA which exhibited activity that increased with the extent of advance motor preparation, unlike a later onset lateral dipole which was associated with the lateral premotor and primary motor cortex. Based on these findings and those of earlier electrophysiological studies (Ulrich et al., 1998), Leuthold and Jentzsch proposed that the SMA/CMA are responsible for the more abstract, higher order and load dependent aspects of response preparation, including assembly and selection of movement programs, whereas the lateral premotor cortex and the primary motor cortex are responsible for lower order and load independent aspects of response preparation, including more muscle-specific response preparation tasks.

The identification of anterior SMA/CMA load dependent activity and premotor and primary motor cortex load independent activity in the current study confirms the anatomical substrates suggested by electrophysiological studies and supports the notion
that regions responsible for higher motor functions, such as assembly and selection of a motor program, exhibit load dependent activity, whereas regions involved in lower order motor functions, such as response execution, exhibit load independent activity.

Conclusions

By selectively modeling memory load for distinct components (encoding and responding), and varying the duration of the intermediate component (delay), we were able to delineate the unique patterns of regional activity associated with the temporally separate encoding, delay and response components of a WM task. Linear regression analyses revealed extensive load dependent activity during encoding and more selective load dependent activity during responding. The analytical strategies employed in this study could be used to further delineate the component of working memory most severely compromised in clinical populations that have known working memory disturbances.
Experiment Two

Although neuroimaging studies have demonstrated aberrant activation in schizophrenic subjects during WM performance, the time frame of these abnormalities is not yet clear. The abnormal patterns observed could have occurred during any or all of the task phases. If patterns of aberrant activity can be isolated to a specific task phase(s), this may lead to the identification of specific WM processes that are impaired, which in turn may contribute to a better understanding of the dysfunctional neural circuitry that underlies WM impairment in schizophrenia.

The few behavioral studies that have attempted to separate out encoding and delay phase deficits have yielded conflicting results. Though some of these studies have provided evidence for impairment in both components (Lencz et al, 2003; Tek et al., 2002; Wexler et al, 1998), others have provided evidence that the impairments are isolated to either encoding (Hartman et al., 2002; Javitt et al., 1999) or to the delay (Goldberg et al., 1998). Hartman and colleagues (2002) found no group differences in performance under variable load delay conditions, if performance was first equated in a 0-delay condition by giving schizophrenic subjects more time to encode information. This finding was interpreted as evidence for isolated encoding impairment. In contrast, Goldberg at al., (1998) found disproportionately greater impairment on a six digit condition compared to a three digit condition in schizophrenic subjects even when the duration of presentation was identical for both conditions. This finding was interpreted as evidence for isolated delay phase impairment. None of these studies have assessed retrieval mechanisms independently of encoding and maintenance (Hartman et al., 2002).
In the current fMRI experiment, a variable load WM task, similar to that used in experiment one, was used to differentiate the patterns of activation corresponding to each phase of a WM task, in order to determine during which of the task phases differential activation occurs in patients relative to controls.

In experiment one, the majority of the regions activated during encoding were found to show load dependent activity while the majority of the regions activated during responding were found to show load independent activity. Previous research has indicated that many of the regions activated during the delay phase exhibit load dependent activity. In schizophrenia, the degree and nature of the abnormal WM activity found appears to vary with WM load. Based on these findings we predicted the following:

1. Patients with schizophrenia will show greater activation than controls for low load conditions.
2. The differences between patients and controls will be accentuated as load increases.
3. The difference will decrease or reverse as performance capacity is exceeded in the patient group
4. The differences will be greatest in the two load dependent phases (encoding and delay).

Method

Subjects

Fifteen patients with schizophrenia, recruited from outpatient community care teams (5 females, 10 males, mean age 32.6, age range 19-52), and 15 healthy volunteers
(5 females, 10 males, mean age 32.4, age range 19-50) participated. Only 1 patient and 1 healthy subject were left handed, as assessed by the Annette handedness scale (Annett, 1970). Participant groups did not differ significantly on the demographic variables of age, gender, parental socio-economic status (Hollingshead and Redlich, 1958), or on estimates of pre-morbid (National Adult Reading Test; Sharpe and O'Carroll, 1991) and current (Quick Test; Ammons and Ammons, 1962) intellectual functioning ($p < 0.05$; see Table 2.1). Participants provided written informed consent and were screened for MRI compatibility before entry into the scanning room. All experimental procedures met with University and Hospital ethical approval.

All patients were interviewed by a psychiatrist independent of the treatment team to confirm that they met diagnostic criteria for schizophrenia as defined in the Diagnostic and Statistical Manual of Mental Disorders (APA press, 1994). Symptom severity was assessed by the same psychiatrist using the Signs and Symptoms of Psychotic Illness scale (SSPI) (Liddle et al., 2002). The SSPI is a twenty item 5 point rating scale in which 0 represents no pathology, 1 represents questionable pathology and 2-4 represents increasing severity of clear pathology. The average duration of illness was 10.1 years (range 2-31).

All patients were taking oral neuroleptics prescribed by their community psychiatrist. One patient was taking the typical neuroleptic, haloperidol, and the rest were taking atypical neuroleptics. Seven patients were taking risperidone (mean dosage=3.4mg/day, range=1-8mg), 5 patients were taking clozapine (mean dosage=350mg/day, range=150-600mg), 4 Olanzapine (mean dosage= 20mg/day, range=8-35mg) and 1 Seroquil (200mg/day). In addition to neuroleptics, 8 patients were taking antidepressants,
2 benzodiazepines, 1 lithium carbonate, 3 anticonvulsants (likely prescribed as mood stabilizers) and 1 patient was taking Metformin HCl, an oral hypoglycemic medication.

**Task Design**

Though the task used for experiment two was similar to that used for experiment one, modifications were made to the stimulus presentation timing (Figure 2.1). To retain the option to model load dependence for the delay phase in a separate analysis procedure, the duration of the delay was changed from a jittered duration of 3, 4 or 5 seconds to a constant duration of 6 seconds. To ensure that the co-linearity of our variables remained low after removing the jitter and to provide a better estimate of the resting baseline activity, longer duration blank trials were used than those in experiment one to allow more time for the hemodynamic response to return to baseline. Specifically, rather then including 9 blank 11 seconds trials, 5 blank trials, 2 of 27 seconds in duration and 3 of 15 seconds in duration, were inserted at wide intervals throughout each run. These modifications increased the duration of each run from 9 minutes and 48 seconds (119 scans) to 10 minutes and 53 seconds (218 scans). Imaging and post processing procedures were identical to those used in experiment one.

As amotivation is a prominent feature in schizophrenia (Schmand et al., 1994), the interpretation of activation differences in the context of poor performance is confounded by the possibility that schizophrenic subjects are not engaged in the task (Price & Friston, 1999). To encourage participants to maintain attention and motivation throughout the study, subjects received financial reward as described in experiment one.
Statistical Analysis

fMRI data

When memory load is modeled for all three phases the maximum correlation between any two covariates is $r=.55$. In order to maintain the lowest possible correlation between the covariates of interest, two separate fixed effect models were assessed. Model one was similar to that used in experiment one. Four memory load conditions were specified for the encoding and response phases, whereas one condition (the average activation across load) was specified for the delay phase. The BOLD response for the encoding phase was modeled as the convolution of a 4 second box-car (beginning at the onset of the letter string to be encoded) with a synthetic hemodynamic response function composed of two gamma functions. The delay phase was modelled as the convolution of a 6 second boxcar (beginning when the encode stimuli was turned off) with the synthetic hemodynamic response. The response phase was modelled as the convolution of a 1 second boxcar (beginning at the onset of the test letter) with the synthetic hemodynamic response. The maximum correlation between any two covariates was $r=.16$.

In model two, four memory load conditions were specified for the delay phase whereas one condition (the average activation across load) was specified for each of the encoding and response phases. The BOLD response was modeled as the convolution of a box car of the previously specified onset and duration for each phase and the synthetic hemodynamic response function. In this second model, the encoding activity was temporally correlated with the response phase activity (max correlation: $r=.58$), but the delay phase activity was not highly correlated with the activity from either the encoding or response phase (max correlation: $r=.18$). The results from model two are presented as
an examination of load specific delay activity. The mean delay results from the two analyses were compared for equivalency to explore the validity of comparison between these models.

For model one, in the second level mixed effects analysis, the following five contrasts were computed for each subject: 1) average encoding vs. implicit baseline, 2) delay vs. implicit baseline, 3) average response vs. implicit baseline, 3) a linear regression of encoding activation and memory load, and 4) a linear regression of response activation and memory load. For model two, in the second level mixed effects analysis, the following two contrasts were computed for each subject: 1) average delay vs. implicit baseline and 2) a linear regression of delay activation and memory load. For both models, contrasts for the linear regressions were computed using the parameter estimates -3, -1, 1, and 3 for the 2, 4, 6 and 8 letter conditions, respectively. For each group, a one-sample t-test was computed on the individual subject contrast images (one image per subject) to determine regions of significant activation over all subjects for each of the contrasts.

Independent sample t-tests were used to assess differences between patients and controls in BOLD activity for the average contrast, linear regression contrast and load specific contrasts for each phase. For whole brain analyses, an inclusion criterion of \( p<0.001 \) (uncorrected for multiple comparisons) was chosen, with only clusters that reached the cluster level significance of \( p<0.05 \) (corrected for multiple comparisons) being reported. This threshold was lower than that chosen for experiment one, given the smaller sample size and more variable population studied in experiment two.
Region of interest Analysis

A binary mask was created of regions found to increase significantly with load during encoding in healthy subjects in experiment one (height threshold t=4.70, p<.05 corrected for multiple comparisons, cluster extent (k)=2158). The number of above-threshold voxels (height threshold of t=1.65, p<0.05 uncorrected for multiple comparisons) within the load dependent encoding mask was determined for each subjects' load specific encoding contrasts.

Results

Symptom Severity Ratings

The mean SSPI score for the schizophrenic patients was 7.4 (SD=3.6) consistent with mild to moderate symptomatology (Liddle et al., 2002).

Task Performance

The mean RTs for each memory load condition are shown for both groups in Figure 2.2a. RT data were analyzed using a Group (healthy participants, schizophrenic patients) x Load (2, 4, 6 and 8 letters) ANOVA. RTs from incorrect trials were excluded. The analysis revealed a main effect of load [F(2,84)=33.98, p<.001], with both groups showing the expected linear increase in RT with load. Though the main effect of Group did not reach statistical significance [F(1,28)=2.31, p=.14], the mean RT was longer in the schizophrenic group for each memory load condition, with differences of more than 100ms for the three higher memory load conditions. There was no significant Group x Load interaction [F(2,84)=1.35, p=.27].

The mean percent correct for each memory load condition are shown for both groups in Figure 2.2b. All of the participants performed above chance in all four memory
load conditions. (Minimum percent correct for the 8 letter load: schizophrenic patients 71.9% (SD=16); healthy participant 81.9%, (SD=15)). Accuracy data were analyzed using a Group x Load ANOVA. The analysis revealed a main effect of Load \[F(2,84)=37.56, \ p<.001\], with both groups showing the expected linear decrease in accuracy with increasing load. Though the differences were not statistically significant (main effect of Group \[F(1,28)=1.35, \ p=.25\]), the mean percent correct was lower in the schizophrenic group than in the healthy group for each memory load condition, with the largest difference of 10% for the highest memory load condition. There was a trend towards a Group x Load interaction \[F(2,84)=2.52, \ p=.08\].

**Imaging Data: Whole Brain Analyses**

**Load Independent Analyses**

Contrasts for the average encoding activity revealed that the healthy and the schizophrenic groups both activated a network of regions previously implicated in encoding (Figure 2.3a). In both groups the peak clusters were located in bilateral occipital, bilateral striatal, left inferior frontal, left precentral and supplementary and cingulate motor areas. The total extent of mean activation was greater for the schizophrenic group (k=6031) than for the healthy group (k=4199), but a subtraction contrast revealed no significant differences between groups.

In both groups the contrast for mean delay activity revealed peak clusters in the left inferior frontal gyrus, left precentral gyrus and in the SMA. In the control group, above threshold clusters were also identified in the left inferior parietal lobule, bilateral striate, CMA, right middle frontal gyrus (BA 6) and in the right temporal lobe (Figure 2.4a). In contrast to the pattern observed for the encode phase, the total extent of mean
activation was greater for the healthy group \( (k=771) \) than for the schizophrenic group \( (k=140) \), but a subtraction contrast revealed no significant differences between groups. The pattern of average delay activity derived from model two (appendix A.2) was very similar to that derived from model one (appendix A.1).

Contrasts for the mean response activity revealed similar patterns of activity in the healthy and schizophrenic groups (Figure 2.5a). Extensive mean activity was found in both groups, but the peak clusters were identified in regions previously implicated in responding (experiment one) including the left postcentral gyrus, SMA/CMA and the right cerebellum. Although the total extent of mean response activity was greater for the healthy group \( (k=8716) \) than for the schizophrenic group \( (k=5339) \), the subtraction contrast revealed no significant differences between groups.

**Load Dependent Analyses**

**Encoding**

In healthy subjects, the contrast for load dependent encoding activity revealed that activity increased linearly with load in a similar network of regions as was identified in the load independent analysis. The schizophrenic group showed the same regions of peak activity as the healthy group for the load dependent encoding contrast (in bilateral occipital, precentral, SMA/CMA and right superior parietal cortex), but the extent of activation extended into the bilateral dorsolateral prefrontal, bilateral inferior frontal, cerebellar, subcortical and left parietal regions only in the healthy group (Figure 2.3b).

In the healthy group, the extent of encoding activation was greater for the load dependant contrast than for the load independent contrast. In contrast, in the schizophrenic group, the extent of encoding activation was greater for the load
independent contrast than for the load dependent contrast. Although the subtraction contrast revealed no significant differences between groups, the total extent of load dependent encoding activation was much greater for the healthy group \(k=8738\) than it was for the schizophrenic group \(k=943\). While the extent of activation was much greater for the load dependant analysis than the load independent analysis in controls, the opposite pattern was seen in the patients. Load specific contrasts yielded no significant group differences.

\textit{Delay}

The contrast for load dependent delay activity revealed activity in a similar network of brain regions in the healthy and schizophrenic group (Figure 2.4b). In both groups regions of peak activity were identified in bilateral parietal, left precentral, right dorsolateral prefrontal, and in medial supplementary and cingulate motor areas. In the healthy group, but not the schizophrenic group, above-threshold activation was also identified in the left dorsolateral prefrontal cortex and in subcortical regions. In both groups, the extent of delay activation was much greater for the load dependent contrast than for the load independent contrast. The total extent of load dependent delay activation was greater for the healthy group \(k=3012\) than for the schizophrenic group \(k=1075\), but the subtraction contrast revealed no significant differences between groups.

Load specific contrasts for the delay phase yielded no significant differences for the 2, 4, and 6 letter conditions, but a significant difference was found for the 8 letter condition. Controls exhibited significantly greater activity than patients in the left parietal lobe \(k=29\); peak activation \(x=-44, y=-24, z=44\) while maintaining eight letters over the delay (Figure 2.6). This finding was in agreement with our prediction of larger
differences for higher memory load conditions than for lower memory load conditions, but the difference was in the opposite direction than expected.

Response

In both groups, the extent of response activation was much greater for the load dependent contrast than for the load independent contrast. As we previously found in healthy subjects, activity did not increase linearly with load in either group in the majority of regions that showed significant mean activity during the response phase. In both groups the region of peak activity was located in the SMA/CMA. In the healthy group, but not the schizophrenic group, small above threshold clusters were also found in the bilateral inferior and middle frontal, right cerebellar and left precentral cortex (Figure 2.5b). Although the total extent of load dependent response activation was greater for the healthy group (k=335) than for the schizophrenic group (k=71), again the subtraction contrast revealed no significant differences between groups. Load specific contrasts also yielded no significant group differences.

Imaging Data: Region of Interest Analyses

Load Dependent Encoding Mask

The mean number of above threshold voxels within the load dependent encoding mask (from experiment 1) is shown for each load specific encoding condition for both groups in Figure 12. A Group x Load ANOVA revealed a main effect of Load $[F(2,84)=60.69, p<.001]$, with an increase in extent following each load increase in both groups. There was a main effect of Group $[F(1,28)=4.65, p=.04]$ and a trend towards a Group x Load interaction $[F(2,84)=2.38, p=.10]$. Post hoc t-tests revealed that the schizophrenic group showed significantly greater activation than the healthy group for
the low load conditions (2 letters $t(28) = -2.70, p = .01$; 4 letters $t(28) = -2.36, p = .03$) but a similar extent of activation for the high load conditions (6 letters $t(28) = -.75, p = .46$; 8 letters $t(28) = .32, p = .75$).

Relation of Activation Extent to WM Performance

Pearson correlations were used to assess the relationship between performance and the extent of activation within two regions - the load dependent encoding mask derived from experiment one and the left parietal region cluster in which a significant difference was found between patients and controls during the delay phase for the eight letter load condition. In the schizophrenic group an increased extent of activity within the encoding mask was significantly related to decreased accuracy for both the two letter ($r = -.57, p = .03$) and four letter conditions ($r = -.74, p = .002$). There were no significant correlations between extent of activation and accuracy in the control group. For both groups, the extent of encoding activation was not significantly correlated with RT for any load condition (see Table 2.2 for correlation coefficients).

A binary mask was created of the left parietal region ($p < .05$ uncorrected for multiple comparisons, $k = 323$) was found to be significantly more active in the controls than in the patients during the maintenance of eight letters. The number of above threshold voxels within this mask was determined in each subject for the eight letter delay condition (height threshold of $t = 1.65, p < 0.05$ uncorrected for multiple comparisons). In both groups, a greater extent of left parietal activation while maintaining eight letters was related to higher eight letter accuracy. This relationship reached significance for the schizophrenic group ($r = .61, p = .02$) and approached significance for the control group ($r = .41, p = .12$). The extent of eight letter left parietal
activation was not found to be related to eight letter RT in either group (schizophrenic
$r=-.04, p=.44$; healthy $r=-.21, p=.22$).

**Discussion**

Contrasts for the average activity across memory load revealed that schizophrenic and healthy control subjects activate similar networks of regions during the encoding, delay and response components of a WM task. However, load dependent differences were found during both the encoding and delay phases. The load dependent patterns of encoding activation found in the schizophrenic subjects indicate that relative to healthy subjects, they have a reduced ability to increase encoding activity as load increases. For the highest load delay condition, a reduced extent of activity was identified in the schizophrenic group in the left parietal cortex, a brain region implicated in short-term storage. For both phases, a greater degree of differential activity was associated with poorer performance, indicating that these activation differences are reflective of the neural abnormalities that underlie WM impairment.

That few differences were found overall is not surprising, given that the patients did not perform significantly worse than controls. Differences in magnitude and extent of cerebral activation found between schizophrenic subjects and controls during WM performance have been shown to decrease when the two groups are matched for performance (Manoach, 1999).

*Behavioral Performance*

As expected, response times and errors increased with memory load in both healthy and schizophrenic subjects. The mean number of errors for the highest memory load condition was well above chance in both groups, indicating that subjects remained
engaged as task difficulty increased. There were no significant differences between groups in reaction time or accuracy, perhaps because the patients participating in this study were all stably medicated, living in the community and relatively well at the time of assessment.

Encoding

Whereas the two groups showed similar extent and patterns of activation for the load independent encoding contrast, a much greater extent of activation was found in the controls for the load dependent encoding contrast than was found in the patients. The voxel count analysis within the load dependent regions derived from the first study confirmed the pattern suggested by the whole brain results, that is, activity did not increase to the same degree with increasing load in the schizophrenic group as it did in the controls. As predicted, the patients showed a greater extent of activation than the controls for the low load condition but these differences decreased rather than increased with load. The patients activated more than controls when memory load was low, but with each additional increase in load the extent of activation increased to a lesser degree in the patients than it did in the controls.

One possible explanation for this finding is that in order to perform as successfully as controls, patients would have to be able activate encoding regions to a greater extent than controls for any memory load condition, but they are unable to do this as demand increases. The schizophrenic subjects in this study did activate more than controls for the low load conditions but not for the high load conditions. As the load increased the schizophrenic subjects may have approached a ceiling effect whereby they were unable to further increase activation in response to increasing demand.
Alternatively, perhaps control subjects are better able to anticipate or recognize that they need to exert greater effort as memory load increases and longer strings needed to be remembered. Either possibility would lead to a reduced encoding capacity that would contribute to impaired WM performance as memory load increases. The finding that the extent of encoding activation was positively correlated with the number of errors for low load conditions, in the patient group, supports the proposition that patients find the low load conditions more difficult than the controls do and therefore activate encoding regions to a greater extent.

*Delay*

Left parietal activation was found to increase with load during the delay in both groups, but for the highest memory load condition control subjects activated this region to a significantly greater extent than patients. The left posterior parietal cortex has long been implicated in the short-term storage of verbal information (Smith & Jonides, 1998). Recently, it has been demonstrated that sustained delay phase activity is necessary for successful WM performance in a network of regions which include the left posterior parietal cortex (Peossa et al., 2002). That a greater extent of activation in the left parietal cortex during the eight letter condition was positively correlated with accuracy supports the idea that this region is necessary for the maintenance of verbal information. Our finding that non-patient controls activate left parietal cortex significantly greater then patients with schizophrenia for the eight letter condition, but not for the lower load conditions supports Hartman et al.,’s (2002) proposal that the WM deficits found in schizophrenia may be due at least in part to a reduced storage capacity.
The observed parietal difference during the delay phase could be reflective of some members of the patient group exceeding their storage capacity at the eight letter condition. Alternatively, the observed difference in performance and decreased activation during the delay phase may be a direct consequence of the patients group’s inability to increase activation sufficiently in encoding regions as the task difficulty increases.

Response

No activation differences were found between groups for the response phase. That differences were found for the encoding and delay phases, but no differences were found for the relatively load independent response phase, supports our hypothesis that it is load dependent activity that is compromised in schizophrenia. It may be that the response phase is not impaired or only minimally impaired relative to the encoding and delay phases.

Conclusions

These findings support the notion that load dependent WM inefficiencies are present in schizophrenia. That the nature of the differences found varied considerably with task phase underscores the importance of using methods that allow WM components to be studied separately.

Possibilities for future research include investigating whether the reduced degree of increase in encoding activity with load found in the schizophrenic group was due to a ceiling effect or to an impaired ability to anticipate when greater effort is needed. If the latter is the case, psychological interventions could be developed to help patients anticipate when they need to concentrate more.
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Table 1.1

*Experiment One: Demographic Data*

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.50</td>
<td>(9.22)</td>
</tr>
<tr>
<td>NART</td>
<td>118.60</td>
<td>(7.61)</td>
</tr>
<tr>
<td>Quick Test</td>
<td>107.40</td>
<td>(11.34)</td>
</tr>
<tr>
<td>Parental SES</td>
<td>2.97</td>
<td>(2.29)</td>
</tr>
</tbody>
</table>

Note. NART = National Adult Reading Test
       SES = Socio-economic status
### Table 1.2

**Encoding Phase: Anatomical Regions Showing Significant Activation**

<table>
<thead>
<tr>
<th>Anatomical Region (Brodmann’s area)</th>
<th>Average across all loads</th>
<th>Linear regression with load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z-score</td>
<td>x   y   z</td>
</tr>
<tr>
<td>L precentral gyrus (6)</td>
<td>109</td>
<td>-12  4</td>
</tr>
<tr>
<td>R precentral gyrus (6)</td>
<td>77</td>
<td>-20  6</td>
</tr>
<tr>
<td>L SMA (6)</td>
<td>50</td>
<td>-12  0</td>
</tr>
<tr>
<td>R SMA (6)</td>
<td>27</td>
<td>4    12</td>
</tr>
<tr>
<td>L superior frontal gyrus (6)</td>
<td>18</td>
<td>-4   0  60</td>
</tr>
<tr>
<td>L middle frontal gyrus (6)</td>
<td>22</td>
<td>-32  -4</td>
</tr>
<tr>
<td>R middle frontal gyrus (6)</td>
<td>29</td>
<td>4    60</td>
</tr>
<tr>
<td>L inferior frontal gyrus (44)</td>
<td>20</td>
<td>-52  4</td>
</tr>
<tr>
<td>R inferior frontal gyrus (9)</td>
<td>17</td>
<td>52   4</td>
</tr>
<tr>
<td>L inferior parietal lobule (40)</td>
<td>26</td>
<td>-44  -44</td>
</tr>
<tr>
<td>R inferior parietal lobule (40)</td>
<td>20</td>
<td>32   -56</td>
</tr>
<tr>
<td>L precuneus</td>
<td>63</td>
<td>-24  -72</td>
</tr>
<tr>
<td>R precuneus</td>
<td>83</td>
<td>28   -72</td>
</tr>
<tr>
<td>L superior parietal lobule (7)</td>
<td>7</td>
<td>-28  -56</td>
</tr>
<tr>
<td>R superior parietal lobule (7)</td>
<td>36</td>
<td>28   -76</td>
</tr>
<tr>
<td>L temporal Lobe</td>
<td>37</td>
<td>-48  -64</td>
</tr>
<tr>
<td>R temporal Lobe</td>
<td>28</td>
<td>40   -52</td>
</tr>
<tr>
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<td>24   -96</td>
</tr>
<tr>
<td>R occipital lobe</td>
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<td>-32  -96</td>
</tr>
<tr>
<td>L CMA (32/24)</td>
<td>7</td>
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</tr>
<tr>
<td>R CMA (32/24)</td>
<td>28</td>
<td>4    16</td>
</tr>
<tr>
<td>L anterior cingulate</td>
<td>43</td>
<td>-24  4</td>
</tr>
<tr>
<td>R putamen</td>
<td>38</td>
<td>20   12</td>
</tr>
<tr>
<td>L caudate</td>
<td>8</td>
<td>-16  16</td>
</tr>
<tr>
<td>L globus palidus</td>
<td>16</td>
<td>12   16</td>
</tr>
<tr>
<td>R globus palidus</td>
<td>11</td>
<td>-12  0</td>
</tr>
<tr>
<td>L thalamus</td>
<td>12</td>
<td>16   0</td>
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<tr>
<td>R thalamus</td>
<td>15</td>
<td>-12  -8</td>
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<tr>
<td>R midbrain</td>
<td>21</td>
<td>16   -8</td>
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<tr>
<td>L cerebellum posterior lobe</td>
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<tr>
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<tr>
<td>L cerebellum posterior lobe</td>
<td>39</td>
<td>-12  -80</td>
</tr>
<tr>
<td>R cerebellum posterior lobe</td>
<td>44</td>
<td>28   -64</td>
</tr>
</tbody>
</table>

Note. Random effects voxel level statistics, p<0.05 corrected for multiple comparisons
Table 1.3

*Delay Phase: Anatomical Regions Showing Significant Activation across All Loads*

<table>
<thead>
<tr>
<th>Anatomical Region (Brodman’s area)</th>
<th>KE</th>
<th>Z-score</th>
<th>x</th>
<th>y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. precentral gyrus (6)</td>
<td>38</td>
<td>6.56</td>
<td>-44</td>
<td>-8</td>
<td>56</td>
</tr>
<tr>
<td>L. SMA (6)</td>
<td>50</td>
<td>7.05</td>
<td>-4</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>R. SMA (6)</td>
<td>28</td>
<td>6.23</td>
<td>-4</td>
<td>12</td>
<td>49</td>
</tr>
<tr>
<td>L. superior frontal gyrus</td>
<td>30</td>
<td>8.85</td>
<td>-4</td>
<td>8</td>
<td>52</td>
</tr>
<tr>
<td>R. superior frontal gyrus</td>
<td>16</td>
<td>7.57</td>
<td>-4</td>
<td>8</td>
<td>52</td>
</tr>
<tr>
<td>L. middle frontal gyrus</td>
<td>77</td>
<td>5.90</td>
<td>-16</td>
<td>8</td>
<td>64</td>
</tr>
<tr>
<td>R. middle frontal gyrus</td>
<td>19</td>
<td>6.01</td>
<td>36</td>
<td>44</td>
<td>28</td>
</tr>
<tr>
<td>L. inferior frontal gyrus</td>
<td>5</td>
<td>4.24</td>
<td>-48</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>L. precuneus (19)</td>
<td>14</td>
<td>5.11</td>
<td>-28</td>
<td>-64</td>
<td>40</td>
</tr>
<tr>
<td>L. occipital lobe (17)</td>
<td>56</td>
<td>6.44</td>
<td>-20</td>
<td>-96</td>
<td>8</td>
</tr>
<tr>
<td>R. occipital lobe (17)</td>
<td>101</td>
<td>6.44</td>
<td>16</td>
<td>-92</td>
<td>0</td>
</tr>
<tr>
<td>L. CMA (32)</td>
<td>11</td>
<td>4.90</td>
<td>-4</td>
<td>20</td>
<td>44</td>
</tr>
<tr>
<td>R. CMA (32)</td>
<td>16</td>
<td>5.07</td>
<td>4</td>
<td>20</td>
<td>44</td>
</tr>
<tr>
<td>L. putamen</td>
<td>6</td>
<td>4.75</td>
<td>-20</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>R. putamen</td>
<td>2</td>
<td>4.45</td>
<td>16</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>R. cerebellum - posterior lobe</td>
<td>29</td>
<td>6.39</td>
<td>28</td>
<td>-64</td>
<td>32</td>
</tr>
</tbody>
</table>

Note. Random effects cluster level statistics, p<0.05 corrected for multiple comparisons.
### Table 1.4

**Response Phase: Anatomical Regions Showing Significant Activation**

<table>
<thead>
<tr>
<th>Anatomical Region (Brodman's area)</th>
<th>Average across all loads</th>
<th>Linear regression with load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peak Talairach Coordinate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$K_{tr}$</td>
<td>Z-score</td>
</tr>
<tr>
<td>L precentral gyrus 1° (4)</td>
<td>2</td>
<td>8.17</td>
</tr>
<tr>
<td>R precentral gyrus 1° (4)</td>
<td>1</td>
<td>7.90</td>
</tr>
<tr>
<td>L precentral gyrus 2° (6)</td>
<td>4</td>
<td>9.06</td>
</tr>
<tr>
<td>R SMA / CMA (6/32)</td>
<td>47</td>
<td>9.86</td>
</tr>
<tr>
<td>L inferior frontal gyrus (47)</td>
<td>3</td>
<td>8.16</td>
</tr>
<tr>
<td>R inferior frontal gyrus (47)</td>
<td>1</td>
<td>8.41</td>
</tr>
<tr>
<td>R middle frontal gyrus (46)</td>
<td>134</td>
<td>12.12</td>
</tr>
<tr>
<td>R inferior parietal lobule (40)</td>
<td>1</td>
<td>8.41</td>
</tr>
<tr>
<td>R postcentral gyrus (2)</td>
<td>4</td>
<td>7.85</td>
</tr>
<tr>
<td>L middle temporal lobe</td>
<td>6</td>
<td>9.07</td>
</tr>
<tr>
<td>R middle temporal lobe</td>
<td>5</td>
<td>10.11</td>
</tr>
<tr>
<td>Midbrain</td>
<td>1</td>
<td>10.11</td>
</tr>
<tr>
<td>Cerebellum - anterior lobe</td>
<td>11</td>
<td>10.62</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>10.91</td>
</tr>
<tr>
<td>Cerebellum - posterior lobe</td>
<td>4</td>
<td>8.91</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8.64</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.94</td>
</tr>
</tbody>
</table>

Note. Random effects voxel level statistics, $p<0.05$ corrected for multiple comparisons
Table 2.1

*Experiment Two: Demographic Data*

<table>
<thead>
<tr>
<th></th>
<th>Healthy Participants</th>
<th>Schizophrenic Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>(SD)</td>
</tr>
<tr>
<td>Age</td>
<td>32.40</td>
<td>10.09</td>
</tr>
<tr>
<td>NART (Pre-morbid intellectual functioning)</td>
<td>117.30</td>
<td>5.29</td>
</tr>
<tr>
<td>Quick Test (Current intellectual functioning)</td>
<td>109.16</td>
<td>7.21</td>
</tr>
<tr>
<td>Parental SES (Hollingshead)</td>
<td>3.60</td>
<td>1.69</td>
</tr>
</tbody>
</table>

Note. NART = National Adult Reading Test, SES = Socio-economic status
Table 2.2

*The Relation of the Extent of Encoding Activation to Performance*

<table>
<thead>
<tr>
<th>Load</th>
<th>Group</th>
<th>Encoding Region of Interest</th>
<th>Reaction Time</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Letters</td>
<td>Healthy</td>
<td></td>
<td>r = -.16</td>
<td>p = .57</td>
</tr>
<tr>
<td></td>
<td>Schizophrenic</td>
<td></td>
<td>r = -.13</td>
<td>p = .65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>r = -.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = .002</td>
<td></td>
</tr>
<tr>
<td>4 Letters</td>
<td>Healthy</td>
<td></td>
<td>r = .18</td>
<td>p = .52</td>
</tr>
<tr>
<td></td>
<td>Schizophrenic</td>
<td></td>
<td>r = -.15</td>
<td>p = .59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>r = -.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = .03</td>
<td></td>
</tr>
<tr>
<td>6 letters</td>
<td>Healthy</td>
<td></td>
<td>r = .09</td>
<td>p = .75</td>
</tr>
<tr>
<td></td>
<td>Schizophrenic</td>
<td></td>
<td>r = .33</td>
<td>p = .23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>r = .45</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = .09</td>
<td></td>
</tr>
<tr>
<td>8 letters</td>
<td>Healthy</td>
<td></td>
<td>r = .18</td>
<td>p = .52</td>
</tr>
<tr>
<td></td>
<td>Schizophrenic</td>
<td></td>
<td>r = .48</td>
<td>p = .07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>r = .37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = .18</td>
<td></td>
</tr>
</tbody>
</table>

Note. Extent within the load dependant encoding mask from Experiment One.

*a*Significant *p*<0.05  
*b*Trend *p*<0.10
Variable Load Working Memory Task

Figure 1.1. Experiment One timing sequence for a single trial.
Figure 1.2. Contrasts and design matrices for the encoding analyses.

(A) Average encoding activity across memory load.

(B) Linear regression of encoding activity with memory load.
Figure 1.3. Experiment One working memory performance.

(A) Reaction times increased linearly with memory load.

(B) Performance accuracy was significantly lower for eight letters compared to each of the lower memory load conditions ($p<0.05$).
Figure 1.4. Experiment One encoding phase activation.

(A) Brain regions exhibiting activity on average across all memory load conditions.

(B) Brain regions exhibiting activity that increases linearly with memory load.

Note. Height threshold $T=6.64 \ p<0.05$, corrected for multiple comparisons.
Figure 1.5. Experiment One response phase activation.

(A) Brain regions exhibiting activity on average across all memory load conditions.

(B) Brain regions exhibiting activity that increases linearly with memory load.

Note. Height threshold $T=6.64 \ p<0.05$, corrected for multiple comparisons.
Figure 2.1. Experiment Two timing sequence for a single trial.
Figure 2.2. Experiment Two working memory performance. Bar graphs of (A) mean reaction time (RT) and (B) percent correct responses with standard error bars in each condition for the healthy and schizophrenic groups.
Figure 2.3. Experiment Two encoding phase activation.

(A) Brain regions exhibiting activity on average across all memory load conditions

(B) Brain regions exhibiting activity that increases linearly with memory load

Note. Height threshold \( T=3.79, p<0.05 \), corrected for multiple comparisons at the cluster level
Figure 2.4. Experiment Two delay phase activation.

(A) Brain regions exhibiting activity on average across all memory load conditions
(B) Brain regions exhibiting activity that increases linearly with memory load

Note. Height threshold $T=3.79$, $p<0.05$, corrected for multiple comparisons at the cluster level
Response Phase

A) Average Over Load

B) Linear Regression With Load

Figure 2.5. Experiment Two response phase activation.

(A) Brain regions exhibiting activity on average across all memory load conditions

(B) Brain regions exhibiting activity that increases linearly with memory load

Note. Height threshold $T=3.79, \ p<0.05$, corrected for multiple comparisons at the cluster level
Figure 2.6. Delay phase, eight letter group comparison. While maintaining eight letters over a delay, the healthy group showed significantly greater activation than the schizophrenic group in a left parietal region.
Figure 2.7. Load dependent encoding region of interest analysis.

A) Mask of the regions found to show activity that increased with load during encoding in Experiment One.

B) When memory load was low, schizophrenic subjects showed significantly greater activation within the load dependent encoding mask than healthy subjects.

Note. Height threshold $T=1.65$, $p<0.05$, uncorrected for multiple comparisons
Figure 2.8. The relation of the extent of encoding activation to accuracy. In the schizophrenic group, but not in the healthy group, there was a significant negative correlation between accuracy and the extent of encoding activation for both the (A) two letter and (B) four letter condition.

Note. Extent within the load dependent encoding mask from Experiment One (k=2158).
Height threshold $T=1.65, p<0.05$, uncorrected for multiple comparisons
Figure 2.9. The relation of the extent of left parietal activation to accuracy. In the schizophrenic group, but not in the healthy group, there was a significant positive correlation between accuracy and the extent of delay activation for the eight letter condition.

Note. Extent within the left parietal mask (k=323). Height threshold T=1.65, p<0.05, uncorrected for multiple comparisons.
Healthy Group, Delay [mean], Model 1

**SPM results:**
- Height threshold $T = 3.79$
- Extent threshold $k = 13$ voxels

**Design matrix**

**Statistics:**
- **Volume summary** ($p$-values corrected for entire volume)

<table>
<thead>
<tr>
<th>$p$</th>
<th>$c$</th>
<th>$P_{corrected}$</th>
<th>$\frac{k}{\text{uncorrected}}</th>
<th>P_{uncorrected}$</th>
<th>$t$</th>
<th>$Q_p$</th>
<th>$P_{corrected}$</th>
<th>$P_{uncorrected}$</th>
<th>x, y, z (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>6</td>
<td>0.000</td>
<td>224</td>
<td>0.000</td>
<td>0.000</td>
<td>16.50</td>
<td>(6.49)</td>
<td>0.000</td>
<td>-36 8 -6</td>
</tr>
<tr>
<td>0.000</td>
<td>102</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>6.49</td>
<td>(6.49)</td>
<td>0.000</td>
<td>12 16 -4</td>
</tr>
<tr>
<td>0.000</td>
<td>184</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>7.45</td>
<td>(4.60)</td>
<td>0.000</td>
<td>22 16 -4</td>
</tr>
<tr>
<td>0.016</td>
<td>28</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>6.94</td>
<td>(4.36)</td>
<td>0.000</td>
<td>32 26 -8</td>
</tr>
<tr>
<td>0.000</td>
<td>184</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>5.56</td>
<td>(3.37)</td>
<td>0.000</td>
<td>24 49 16</td>
</tr>
<tr>
<td>0.000</td>
<td>61</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>6.49</td>
<td>(6.49)</td>
<td>0.000</td>
<td>-4 4 48</td>
</tr>
</tbody>
</table>

*Table shows at most local maxima > 8.0 mm apart per cluster.*

- Height threshold: $T = 3.79$, $p = 0.001$ (1.000 corrected)
- Degrees of freedom $= 10.14.0$
- Extent threshold: $k = 13$ voxels, $p = 0.056$ (3.056 corrected)
- Smoothness FWHM $= 12.32.8$ mm
- Search volume $= 1333376$ mm$^3$ $= 2395.9$ voxels
- Expected number of voxels $= 693.6$ rese
- Expected number of voxels $= 693.6$ rese
- Expected number of clusters $= 1.01$
- Expected number of clusters $= 1.01$
- Expected number of clusters $= 1.01$
- Expected number of clusters $= 1.01$
- Expected number of clusters $= 1.01$
- Voxels size $= 4.0, 4.0, 4.0$ mm $= 29.81$ voxels

*Figure A.1. Load independent delay analysis: Model 1*
Healthy Group, Delay [mean], Model 2

**SPM results:**

Height threshold: \(T = 3.79\), \(p = 0.000\) corrected
Extent threshold: \(k = 13\) voxels, \(p = 0.000\) corrected

---

**Design matrix**

**Expected voxels per cluster, \(<k>\):** 1.998

**Search volume:** \(S = 1533376\) mm\(^3\), \(33552\) voxels

**Voxel size:** \([4.0, 4.0, 4.0]\) mm, \(1\) resel = 29.38 voxels

---

**Figure A.2.** Load independent delay analysis: Model 2