Transitive Inference in Healthy Humans and Implications for Schizophrenia

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ABSTRACT

Transitive inference (TI) refers to inferences on relations between items based on other known relations of those items. Using a paradigm where participants first learn a series of four overlapping pairs that constitute the ordered sequence A>B>C>D>E and are then tested on the novel TI pair BD and non-TI pair AE, animal experiments demonstrated that intact function of the hippocampus is necessary for TI but not for non-TI. We performed three functional magnetic resonance imaging (fMRI) experiments to identify neural correlates of TI in healthy humans. First, we show hippocampal activation in learning overlapping pairs that constitute an ordered sequence but not non-overlapping individual pairs. Second, we demonstrate hippocampal recruitment in inferences on the ordered sequence of overlapping pairs (TI) but not on non-overlapping pairs (non-TI, e.g., if a>b and c>d then a>d). We then demonstrate the specificity of hippocampal activation to TI on pairs that are devoid of sequence end-items (e.g., B>D vs. A>C). The results support the relational flexibility account of hippocampal function. Under this account, the hippocampus plays a special role in declarative memory in that it acts to rapidly bind common features into a unified representation that supports flexible inferential memory expression. Other brain areas that were activated in TI included prefrontal cortex, pre-supplementary and supplementary motor areas, insula, anterior and posterior cingulate cortex, lateral temporal cortex, precuneus, posterior parietal cortex, cerebellum, thalamus, ventral striatum and midbrain (the TI network).

In schizophrenia, TI performance is impaired. Could this deficit be linked to hippocampal abnormalities in SZ? We used the findings from studies of TI in healthy participants to interpret an fMRI study of TI in SZ. In SZ, we confirmed the deficit in TI on pairs devoid of end-items (e.g., B>D) but not on pairs including an end-item (e.g., A>C) and linked it to reduced hippocampal activation. Further, we uncovered aberrant function in two points of the TI network - anterior cingulate and inferior parietal cortices - in SZ.

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Chapter 1: Background and introduction

Introduction

The motivation for the studies described in this thesis was to investigate the neural correlates of transitive inference (TI) in healthy human participants in order to elucidate the impairment of TI judgments observed in schizophrenia (SZ) and the neural basis of this impairment. TI refers to the inferences we make about relations between items based on other known relations of the items in question. For example, we can infer that Bob is taller than Jim if we have been told that Bob is taller than Tom and Tom is taller than Jim.

When TI impairments in SZ were first described (Titone et al., 2001), knowledge about the neural correlates of TI in humans was limited. The three neuroimaging experiments described in this thesis were aimed, therefore, at elucidating the neural mechanisms underlying TI in healthy participants. The results of these studies were then used to aid in the interpretation of a neuroimaging experiment that examined TI in SZ. The conclusions pointed to an aberrant function of the medial temporal lobe (MTL) and extra-MTL structures including anterior cingulate cortex (CC) and the parietal cortex. These areas play important roles in TI in healthy participants as demonstrated by the experiments described in this thesis.

The work presented here aims to contribute to the ongoing search for abnormalities that would be specific for and diagnostic of SZ. Although many structural and functional abnormalities of the brain have been reported for SZ, none of them has proved to be either completely specific or diagnostic. Similarly, many cognitive deficits have been

found, but we have yet to identify those that would separate this disease from the normal state or other disease entities.

In this background section, I will first introduce SZ, its symptoms and the burden it presents to the affected patients and society as a whole. I will then summarize known brain abnormalities, especially those of MTL, present in SZ. The TI impairment in SZ is considered a potentially specific deficit of memory and cognition in this disease. The next section will, therefore, provide an overview of the nature of cognitive deficits in SZ and memory dysfunction in particular. I will then describe the observed deficits of TI in SZ, which motivated this thesis research. Next, I will introduce TI and its neural mechanisms, especially the role of MTL, as uncovered in animal studies. I will then review the state of knowledge about the neural correlates of TI in humans that were known at the time this thesis research began. Then, I will present the particular motivation for studying TI in healthy participants and patients with SZ. TI may be an area of specific deficit in SZ that has been linked to intact structure and function of the hippocampal formation, an MTL structure with some of the best documented abnormalities in SZ.

Schizophrenia

Schizophrenia is a severe mental illness with a lifetime prevalence estimated between 0.5 (Jablensky, 2000) and 1.0% (Goff et al., 2001). Because of its chronic nature, its severity, and its early onset, SZ presents a major health expenditure in countries around the world. Direct costs of care in the United States alone were estimated at $ 17.3 billion in 1990 (Wong and Van Tol, 2003) and $ 33 billion when lost productivity was included (Goff et

al., 2001). This amounts to approximately 2.5% of all health expenditures in the US (Goff et al., 2001), with similar figures in the UK (Wong and Van Tol, 2003).

More importantly, the disease presents a significant and sometimes insurmountable psychological burden to those affected such that up to 12% of the patients commit suicide (Goff et al., 2001). The symptoms of SZ can be divided into three primary domains: (1) *positive* symptoms, including hallucinations, delusions, thought disorder and paranoia; (2) *negative* symptoms consisting of severe disturbances in social interactions, motivation, expression of affect, ability to experience pleasure, spontaneous speech typified as anhedonia, social withdrawal, and thought poverty; (3) *cognitive dysfunction* with pronounced deficits in executive function, attention, memory, and general intellectual function (Pearlson, 2000; Goff et al., 2001; Wong and Van Tol, 2003; Tamminga and Holcomb, 2005).

Cognitive dysfunction and memory impairment in schizophrenia

Although cognitive dysfunction is not the most striking feature of SZ, it presents one of the most debilitating aspects of the illness and may be a key factor in preventing SZ patients from holding jobs and integrating into society (Goff et al., 2001). Cognitive dysfunction is also a stable feature of the disease (Kuperberg and Heckers, 2000): it is evident up to 10 years before illness onset (Allin and Murray, 2002; Pantelis et al., 2005), it is resistant to conventional antipsychotic treatment (Davidson and Keefe, 1995; Gold and Weinberger, 1995; Arnold, 2000), and outlasts the patient's psychotic symptoms (Heinrichs, 2005). Thus, cognitive deficits are seen as core feature of the disease

(Kremen et al., 2001; Cirillo and Seidman, 2003), and the stability of cognitive deficits over time is evidence of the presence of static encephalopathy (Aleman et al., 1999). Indeed, some investigators suggest that cognitive deficits are the only research domain (compared with postmortem, MRI and fMRI studies) that distinguishes a majority of SZ patients from healthy people (Heinrichs, 2005).

The cognitive deficits affect a broad range of cognitive abilities. They are particularly pronounced in attention, social learning, processing speed, executive function and verbal and visual learning and memory, all in the context of preserved semantic knowledge (Kuperberg and Heckers, 2000; Muller et al., 2004; Nuechterlein et al., 2004; Tamminga and Holcomb, 2005; Wilk et al., 2005). It has been demonstrated that these cognitive deficits are not a consequence of a generalized intellectual decline. Even in studies where SZ patients and controls are matched on Full Scale IQ, age and education, the impairment is still detected (Wilk et al., 2005). Using the Wide Range Achievement Test-3 and maternal education as predictors, investigators found that cognitive function is below expected levels in 98% of SZ patients but only 42% healthy control participants (Keefe et al., 2005). In another study, neuropsychological function was diminished in schizophrenic participants compared to same-IQ healthy control participants. Thus, even though up to 27% of all SZ patients may fall within the normal range on neuropsychological assessments, they still show substantial cognitive compromise relative to their overall level of intellectual ability (Kremen et al., 2001).

Despite the general nature of the cognitive deficits, converging evidence indicates that executive function, and especially learning and memory, exhibit a differential and specific impairment in this disease (Harrison, 1999; Arnold, 2000; Kuperberg and Heckers, 2000; Antonova et al., 2004; Emilien et al., 2004; Muller et al., 2004). In several studies, SZ patients exhibited performance deficits in declarative memory that were beyond their intellectual decline (Seidman et al., 1998; Egeland et al., 2003; Muller et al., 2004) and beyond those seen in temporal lobe epilepsy (TLE) and major depressive disorder (Seidman et al., 1998; Egeland et al., 2003).

In an extensive review, Cirillo and Seidman (2003) observed that verbal declarative memory impairment relative to control participants could not be accounted for by differences in intelligence, attention, medications, symptoms, and age. They found SZ patients to be particularly poor at encoding operations, especially in spontaneously organizing information into semantic categories. On the other hand, retention was much better than in Alzheimer's disease (AD) and was comparable to TLE patients. Overall, rates of forgetting appear to be normal (Meeter et al., 2002). Patients were particularly impaired on free recall compared with recognition, which was interpreted as evidence for an encoding rather than a retrieval deficit (Cirillo and Seidman, 2003). In general, patients show impaired learning rates, failure to use semantic information to aid recall, and impaired episodic and recognition memory (Gold et al., 1992; Muller et al., 2004). They seem to be impaired to a similar extent on item and associative recognition tests (Pelletier et al., 2005). Unlike purely amnesic patients, however, SZ patients also exhibit abnormalities in short-term and working memory (Gold and Weinberger, 1995). They are

also unimpaired on implicit memory tasks, such as stem completing and procedural memory tasks (Danion et al., 2001). At least in part, the memory deficit seems to be associated more with the phenotype than the genotype of the disease because affected monozygotic twins show greater impairment than unaffected monozygotic twins (Bilder, 2001). Some researchers have argued, however, that a verbal memory impairment they documented is due to depression and slowness rather than a primary feature of the disease (Brebion et al., 2001). Together, these observations suggest a robust deficit in memory in SZ.

Cognitive abnormalities, however, do not appear at the time of onset of illness but precede it by many years. Retrospective studies of cognitive abnormalities displayed by children who developed SZ in adulthood support the neurodevelopmental rather than neurodegenerative account for SZ. Deficits in social and intellectual functioning and organizational ability were retrospectively identified in the childhood years of adult onset patients (Bilder, 2001). Decline in IQ between ages four and seven is predictive of SZ, although with a very low positive predictive value. The childhood of SZ patients is also characterized by reclusive social behavior: These children are more likely to play alone, tend to have fewer close friends, prefer to socialize in small groups, and are more sensitive. Males are less likely to have a girlfriend in adolescence (Lewis and Levitt, 2002).

The nature of cognitive and memory deficits in SZ leads to hypotheses about the involvement of particular brain regions in this disease. Deficits in attending to and

organizing information have been linked to aberrant function of prefrontal cortex (PFC) (Aleman et al., 1999; Kuperberg and Heckers, 2000; Cirillo and Seidman, 2003; Tamminga and Holcomb, 2005), whereas declarative memory deficits are believed to be consistent with dysfunction of MTL, in particular the hippocampus (Aleman et al., 1999; Arnold, 2000; Kuperberg and Heckers, 2000; Cirillo and Seidman, 2003). It has also been posited that memory deficits could be due to abnormal interaction between MTL and PFC (Davidson and Keefe, 1995; Gold and Weinberger, 1995; Kuperberg and Heckers, 2000; Pearlson, 2000; Kurachi, 2003) and also abnormal interactions between PFC and limbic regions, such as the anterior CC (Gold et al., 1992; Kuperberg and Heckers, 2000). It is probably no coincidence that these regions are those that figure figure among the most commonly observed brain abnormalities in SZ.

Brain abnormalities in schizophrenia

When first introduced by Kraepelin (Kraepelin, 1896), SZ was proposed to be a disease of the brain (Harrison, 1999). The following half century of research, however, produced such disparate findings that the disease was described as the "graveyard of neuropathologists" (Plum, 1972). Fortunately, further research on the neuropathological changes in this disorder, including postmortem and structural CAT and MRI studies, has provided a large number of findings that are now well established.

Postmortem studies have used four different approaches: cell cytoarchitecture (and cell orientation), neuropathological examination, volumetric and area measurements, and quantification of cell number. The inevitable confound of postmortem studies is the effect

of age because they typically use brains of old patients who were ill for a long time (Florencio and O'Driscoll, 1999). The major advantage of CAT and MRI scans is that they allow *in vivo* measurements in patients as well as their relatives and high-risk participants, although their resolution cannot match that of postmortem studies.

One major contribution of postmortem studies has been the accumulation of overwhelming evidence against a neurodegenerative process in SZ. First, SZ brains are devoid of any obvious signs of neurodegeneration such as tangles, senile plaques, Lewy bodies, or GFAP-positive astrocytes that are seen in neurodegenerative disorders, such as Alzheimer's and Parkinson's disease (Arnold, 2000). Most findings, therefore, point to a neurodevelopmental abnormality. Alterations occur in the density and distribution of interstitial white matter (WM) in PFC and superior temporal gyrus (STG), presumed to be remnants of the embryonic cortical subplate (which is important in corticogenesis, neuronal migration and formation of corticothalamic connections) (Harrison, 1999; Eastwood and Harrison, 2005). The levels of reelin (a glycoprotein involved in migration and maintenance of neurons) are reduced in PFC, hippocampus, and cerebellum (Allin and Murray, 2002; Sawa and Snyder, 2002; Miyamoto et al., 2003), but, there is no evidence of gliosis (a marker of inflammation) (Bogerts, 1999; Harrison, 1999; Sawa and Snyder, 2002; Harrison, 2004).

Besides providing evidence for the neurodevelopmental hypothesis of SZ, postmortem studies have consistently identified a number of cytoarchitectural, synaptic, and neurochemical abnormalities. Grossly, brain weight and length are reduced in SZ

(Harrison, 1999). Findings consistently identify neuropathology in CC, PFC, thalamus and MTL structures, especially the hippocampus. The number of small interneurons in CC is reduced (Bogerts, 1999). Reductions in PFC neuropil are firmly established with associated loss of volume attributed to reductions of neuronal size rather than neuronal loss (Harrison, 1999; Harrison and Roberts, 2000; Bilder, 2001; Allin and Murray, 2002). In particular, neuropathological studies report decreased size of pyramidal neurons in layer III, an important target of cortico-cortical projections (Lewis and Lieberman, 2000; Innocenti et al., 2003). Moreover, these neurons have reduced axonal density and receive decreased input from GABAergic chandelier neurons (Bilder, 2001; Tamminga and Holcomb, 2005). The levels of synaptophysin, an important synaptic marker, are also reduced in PFC (Harrison, 1999; Lewis and Lieberman, 2000). Dorsolateral PFC (DLPFC), which is implicated by these studies, is connected to the hippocampus, amygdala, striatum, pallidum, substantia nigra, pons, the dorsomedial nucleus of thalamus and cingulate gyrus. It is also connected to parietal, temporal, and occipital cortices and CC ipsilaterally. This extensive connectivity is likely to make PFC function vulnerable to damage in these various parts of the brain and vice versa (Faraone et al., 2003). Overall, the neuropathology of PFC identified in SZ is believed to be one of the major contributors to the disease.

Abnormalities have also been found in the thalamus (Tamminga and Holcomb, 2005), particularly the mediodorsal nucleus (MD), which is the principle subcortical input to DLPFC (Popken et al., 2000). Total volume (Bogerts, 1999) and the number of neurons (Popken et al., 2000) are both reduced in MD (Harrison, 1999) (Cullen et al., 2003).

Overall, multiple lines of evidence indicate that reduced MD TH-PFC connectivity plays a role in the abnormal behavior of SZ patients (Lewis and Lieberman, 2000; Miyamoto et al., 2003).

The most robust neuropathological findings have been those in the MTL. In fact, some investigators posit that MTL structures, i.e., the hippocampus and the entorhinal, perirhinal, and parahippocampal cortices are central to the neuropathology and pathophysiology of SZ (Harrison, 2004). First, abnormal cytoarchitecture occurs in the entorhinal cortex (ERC), specifically poorly formed layer II and III neuron clusters and presence of laminar disorganization (Arnold, 2000; Harrison, 2004). Hippocampal volume is decreased overall. The cross sectional area of hippocampal pyramidal cell bodies is smaller (Harrison, 2004) but neuron numbers are not reduced, which separates SZ from MTL disease states, such as AD and TLE (Heckers and Konradi, 2002). Also unlike in AD, the CA1 subfield of the hippocampus is relatively spared, whereas the CA4 subfield is much more affected (Harrison, 2004). Cytoskeletal proteins, namely the microtubule-associated protein MAP2, are abnormally expressed in ERC and the subiculum of the hippocampus. Alterations in synaptic and dendritic markers have also been established (Bogerts, 1999; Arnold, 2000). Further, neurochemical changes occur in this region. Decreased activity of the inhibitory GABAergic neurons and decreased function of the AMPA glutamate receptor have been identified (Heckers and Konradi, 2002). MTL abnormalities, therefore, feature prominently in the neuropathology of SZ.

The neuropathological findings identified using postmortem studies, at least those concerned with abnormalities in the volumes and structure of the brain, have been mostly confirmed by *in vivo* structural MRI studies. Because these studies have identified structural abnormalities in high-risk, first-episode, and young as well as older patients, the abnormalities found in postmortem tissue are not due to potential confounds of life-long illness duration. On the contrary, brain abnormalities are central to the disease at the time of its onset and even at preceding times.

As in postmortem studies, the gross finding of reduced overall brain volume (by approximately 3%) has been reported in both chronic (Harrison, 1999; Harrison and Weinberger, 2005) and first-episode patients (Fannon et al., 2000; Miyamoto et al., 2003). The normal asymmetry of the frontal and occipital lobes (left larger than right) seems to be absent (Bogerts, 1999), but the opposite asymmetry in temporal lobe is preserved (Shenton et al., 2001). An older review ordered the commonly found brain abnormalities on the basis of their replicability by different groups as follows: cavum septum pellucidi (92%), lateral ventricle enlargement (80%), reduction of the volume of the amygdalo-hippocampal complex by approximately 4% (74%), third ventricle enlargement (73%), volume reduction of the basal ganglia (68%), superior temporal gyrus volume reduction (67% overall but 100% for gray matter alone), corpus callosum volume reduction (63%), reduced overall temporal lobe volume (61%), planum temporale abnormalities (60%), frontal and parietal volume reductions (60%), decrease in size of occipital lobe (44%) and volumetric reduction of the thalamus (42%) (Shenton et al., 2001). Reduced volumes have also been reported in anterior CC (Lewis and Lieberman,

2000). Some areas, therefore, show a differential reliability in the context of overall volume reduction in SZ.

A more recent meta-analysis of the findings reported on the basis of voxel-based morphometry (VBM) identifies the left superior temporal gyrus and left MTL as the structures with the most robustly demonstrated abnormalities. About half of the studies also report deficits in left inferior frontal gyrus, left medial frontal gyrus, right superior temporal gyrus, and left parahippocampal gyrus. Reductions of approximately 5% in overall MTL volume have been consistently reported, in line with the review cited above. The left MTL deficit was once again, however, not found to be either specific or definitive. About 30% of surveyed studies did not find any differences from control brains in left MTL (Honea et al., 2005). Some studies also showed reductions in the middle prefrontal gyrus and dorsomedial thalamus (McIntosh et al., 2004). VBM, therefore, confirms volume reductions in SZ.

In large part, the volume reductions in cortical regions can be attributed to cortical thinning. In a study comparing cortical thickness in temporal and PFC regions of interest (ROIs), cortical thickness maps showed decreases in orbitofrontal cortices bilaterally; inferior frontal, inferior temporal and occipitotemporal cortices on the left, and within medial frontal and medial temporal cortices on the right. Superior and parietal and primary somatosensory and motor cortices were spared (Kuperberg et al., 2003). Cortical thinning therefore most significantly affects MTL and PFC and explains volume reductions in these regions.

In accordance with the results of postmortem studies, *in vivo* MRI investigations also support the notion of SZ as a neurodevelopmental disorder. Although some MRI studies point to a subtle degenerative process following the illness onset, most structural abnormalities are found in first-episode patients, high-risk participants, and also relatives of the affected individuals. The degenerative processes one year after the illness onset include reduction in total brain volume (-1.2%) and gray matter volume of the cerebrum (-2.9%) as well as increase in lateral ventricle volumes (7.7%) (Cahn et al., 2002). More specifically, volume reductions shortly after illness onset have been reported in the medial temporal and orbital prefrontal regions. Hippocampal volumes, however, are relatively stable over time unlike ventricular volumes, which tend to increase (Pantelis et al., 2005).

In support of the neurodevelopmental hypothesis, reduced whole brain and cortical gray matter volume, especially in the left temporal cortex, along with enlargement of lateral and third ventricles have been documented in first episode patients (Fannon et al., 2000; Allin and Murray, 2002). Some studies have found decreased volumes specifically in right anterior CC, right medial frontal lobe, left medial temporal gyrus and the amygdala and hippocampus bilaterally (Job et al., 2002). Others find significant evidence of reduced thalamic volume as well (Miyamoto et al., 2003). Brain abnormalities were also present in early (childhood) onset SZ (Allin and Murray, 2002). MTL volume reductions have been identified in the offspring of SZ patients as well as their unaffected siblings (Bilder, 2001; Faraone et al., 2003; Harrison and Weinberger, 2005) and high-risk

individuals (Lawrie et al., 2003). Unaffected monozygotic but not dizygotic twins were found to have smaller left hippocampi than control twins (Narr et al., 2002). The combined evidence of brain abnormalities that precede the onset of SZ and some limited progressive components that follow its onset suggests that SZ as a neurodevelopmental disease, where an early lesion may render the brain vulnerable to anomalous development. Further, these anomalies may interact with other causative factors associated with the onset of psychosis (such as substance abuse and stress), which may have further neural sequalae, as evidenced by the presence of some limited brain changes following the onset of psychosis (Pantelis et al., 2005).

As in postmortem studies, abnormalities in MTL structures are among the most robust findings in structural MRI studies in SZ patients, their relatives and high-risk participants. As described above, volume reductions in MTL regions, especially the hippocampus, are among the most frequently observed abnormalities. Even though hippocampal volume reductions are not reported in every case, this omission could be due to different methods used (Geuze et al., 2005). Although the volume decrease is subtle (about 5%), it is found even in first episode patients, their first-degree relatives, at-risk children (Heckers and Konradi, 2002), and unaffected monozygotic twins (Narr et al., 2002). There is also increasing evidence for abnormalities in the hippocampal shape (Harrison, 2004; Harrison and Weinberger, 2005; Tamminga and Holcomb, 2005). Reduced hippocampal volumes were demonstrated in our own group, in a study where we did not find any anterior-posterior specificity of the reductions (Weiss et al., 2005). In another study conducted in our laboratory, we found significantly smaller MTL volumes overall (Sim et

al., 2005). Altogether, our results and those from other laboratories support the notion of hippocampal abnormalities as being central to the neuropathology of SZ (Harrison, 2004).

Putting the results of postmortem and *in vivo* studies of brain abnormalities in SZ together, it appears that the structural, cellular, and molecular abnormalities are most pronounced in regions that are of high connectional complexity, high plasticity, or prolonged maturation, like PFC, ERC, and hippocampus. This pattern may explain whyn why the predominant symptoms of SZ are in high-order cognitive, emotional and social domains rather than more basic neural functions (Arnold, 2000). It has been posited that the vast majority of the neuropsychological deficits described in the previous section can be explained in terms of a deficient neural system involving DLPFC and temporal lobe structures (Florencio and O'Driscoll, 1999). SZ is now often viewed as a condition of abnormal connectivity between disordered regions including PFC and MTL (Harrison and Owen, 2003), with further evidence of abnormal thalamic input into PFC (Innocenti et al., 2003). Some of the cognitive impairments could be linked to disturbances in one or more of these regions. Specifically, gray matter deficits in DLPFC may underlie working memory and executive dysfunction (Kuperberg et al., 2003). MTL, especially hippocampal, pathology likely underlies at least some of the cognitive deficits, given the central mnemonic roles of this region (Harrison, 2004). Specifically, abnormalities in this region are thought to lie behind the memory deficits observed in this disease (Florencio and O'Driscoll, 1999; Heckers and Konradi, 2002; Kuperberg et al., 2003)

Although structural abnormalities identified in postmortem and *in vivo* MRI studies coincide, SZ still cannot be diagnosed using a brain scan, a microscope (Harrison, 1999) or neuropsychological assessment. Heinrichs (2005) argues that the latter is the most promising avenue (Heinrichs, 2005).

Thus, the search for specific and diagnostic tests continues. It can be argued that a more powerful method for identifying a diagnostic abnormality would be to focus on areas of overlap of neuropathology and cognitive impairment. Given the robustness of memory deficits in SZ and differential abnormalities in MTL structures that are thought to underlie them, tasks that specifically probe subregional MTL function should be considered. One such task that seems to selectively recruite some MTL regions but not others, based on animal studies, and that seems to be impaired in SZ is TI.

Transitive inference: An introduction

Transitive inference refers to the ability to make novel inferences about relationships between items based on other relations among these items. Making TIs is essential for developing understanding of hierarchies and relationships in the world around us. A simple example of TI would be concluding that John is taller than Tom if we are told that John is taller than Sam and Sam is taller than Tom. Piaget (1928) described TI as an example of concrete operational thought because he presumed that children acquire the competence for TI once they become capable of mentally performing the physical manipulations needed to reach the correct answer (Piaget, 1928). Over time, however, it has become clear that TI is a basic animal skill because it has been demonstrated in apes,

monkeys, rats, and pigeons. Investigators have proposed that the capacity for TI in animals has developed as a beneficial strategy allowing them to infer the rank of animals within a habitat and thus increase their survival potential (Delius and Siemann, 1998). In fact, TI holds a special place among experimental paradigms probing declarative memory function in that it is readily translated from animal to human research. In that context, TI refers to the ability to infer relations between indirectly related items that have not been presented together, based on previous learning of a sequence of overlapping premise pairs (e.g., if A>B and B>C, then A>C). Using this definition, the capacity for TI has been demonstrated in birds (von Fersen et al., 1991; Bond et al., 2003), rodents (Davis, 1992; Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003), monkeys (McGonigle and Chalmers, 1986; Buckmaster et al., 2004) and humans (Greene et al., 2001; Martin and Alsop, 2004). Similarities between animal and human performance have also been clearly demonstrated (Colombo and Frost, 2001), further confirming similarity of TI in humans and animals.

Transitive inference in the context of neural correlates of memory function

In general terms, transitive inference relies on processes of memory-based decision making and relational reasoning. The general process of remembering is relatively well understood and is known to recruit parietal regions and prefrontal regions (especially DLPFC) in an effort-dependent fashion, regions within the sensory cortex responsible for the original encoding of the experience, and MTL (Squire, 1992), which rapidly binds neural representations associated with the experience and functions to reinstate these different representations during memory retrieval (Buckner and Wheeler, 2001). The

neural correlates of TI have been linked to MTL in particular (Dusek and Eichenbaum, 1997; Nagode and Pardo, 2002; Van Elzakker et al., 2003).

Because this thesis is primarily concerned with MTL function in TI, I will now focus on the role of MTL structures in memory. It is well accepted that MTL is essential for declarative memory (Squire and Zola-Morgan, 1991) as first demonstrated by patients with MTL lesions (Scoville and Milner, 1957; Corkin, 2002). The precise roles of the individual structures within this region, however, namely the hippocampus, the parahippocampal gyrus, entorhinal and perirhinal cortices, are still not established with absolute certainty (Brown and Aggleton, 2001), although the anatomical connections between them are well established. Nevertheless, evidence has emerged for differential contributions to declarative memory of the hippocampus and parahippocampal gyrus comprised of the entorhinal, perirhinal and parahippocampal cortices.

The parahippocampal gyrusin animals is concerned with familiarity or recency of individual stimulus items, whereas the hippocampus is needed to recollect relations among items (Cohen and Eichenbaum, 1993; Eichenbaum et al., 1994; Brown and Aggleton, 2001; Eichenbaum and Cohen, 2001). By this view, the parahippocampal gyrus, which receives convergent inputs from neocortical association areas and sends return projections to all of these areas, mediates the extended persistence of these cortical projections. The hippocampus, on the other hand, possesses the capacity to rapidly encode a sequence of events that make up an episodic memory, to retrieve that memory by re-experiencing one facet of the event, and to link the continuing experience to stored

episodic representations (Eichenbaum, 2000). In this model, hippocampus provides the linking of episodic memories into relational networks to abstract the common features among related memories and to mediate flexible memory expression. The hippocampus contributes to semantic memory by the construction of relational networks that coordinate memories stored in the cerebral cortex. It is believed to link memories in support of the flexibility of their expression through comparisons and generalizations across them and is, therefore, thought to be especially important for TI (Eichenbaum, 2004).

The hypotheses regarding differential contributions of the hippocampus and adjacent structures in MTL to declarative memory are supported by functional neuroimaging studies. In memory encoding, activations in the perirhinal cortex predict subsequent memory for all list words, whereas parahippocampal and anterior hippocampal roles in successful encoding may be limited to items that, for one reason or another, are treated as distinctive (Strange et al., 2002). Other studies have demonstrated that although the hippocampus and parahippocampal regions are similarly engaged during item-based working memory maintenance, the hippocampus differentially subserves the relational binding of items into an integrated memory trace (Davachi and Wagner, 2002). This result anticipates the importance of the hipocampus in TI.

Past functional studies aiming to disentangle the contributions of the different regions of MTL to declarative memory did not allow direct comparison to animal studies. Such a direct comparison and combination of analogous human and animal experiments would

be valuable because some of the most telling experiments elucidating these different roles have been carried out in animals. One of the major attractions of the TI phenomenon is precisely the fact that it is a memory process common to both humans and animals and thus allows bridging human and animal studies. Thus, we can learn about the nature of brain abnormalities contributing to TI deficit in SZ from observing the effect of brain lesions on TI performance in animals. An example of a TI experiment that can be carried out in both species is the ordered sequence experiment.

The ordered sequence experiment for transitive inference

The ordered sequence experiment for TI involves participants learning a sequence of stimuli that are arranged in a particular order and then making inferences on the relationships between stimuli from the sequence that have not been previously shown together. In the classical animal experiment, participants learn the ordered sequence A>B>C>D>E and are then presented with the novel pairs A>E (non-transitive inference because both sequence end-items are included) and B>D (NETI, i.e., non end-item TI because the items are embedded inside the sequence).

The ordered sequence experiment was used to demonstrate the irreplaceable role of the hippocampus in making TIs (Dusek and Eichenbaum, 1997). Rats were first taught a hierarchically ordered sequence of odors labeled A, B, C, D, E; such that A>B>C>D>E by exposing them to the individual pairs of odors, where one of the odors was associated with a food reward. The rats were deemed capable of TI if they picked B over D (the non end-item TI pair) when presented with this novel pair. Rats with lesions in the main

connections of the hippocampus (the fornix and the perirhinal and entorhinal cortices) were severely impaired in making this inference compared with normal rats. In fact, the lesioned rats performed no better than chance (50% correct) upon the first presentation of the BD pair, whereas normal rats performed significantly above chance levels (88%). On the other hand, the rats with hippocampal lesions performed as accurately as healthy rats on the A > E test, which did not require flexible manipulation of the sequence because it contained only the end items A and E (end-anchored non-transitive inference).

The authors concluded that the hippocampus is critical for the type of memory processing that underlies relational organization and declarative memory expression (as evidenced by the selective deficit on the NETI BD pair demonstrated by the lesioned animals). In contrast, the hippocampus is not critical for inferences that can be made by simple reference to previously learned information about the always reinforced item A and the never reinforced item E (as evidenced by intact performance on the non-transitive inference pair AE). Similar findings have been reported for monkeys (Buckmaster et al., 2004), where disconnection of the hippocampus from either its cortical or subcortical pathway resulted in the animals' inability to correctly chose B over D (NETI) whereas their ability to pick A over E (non-transitive inference) was spared. This finding has been perceived as key evidence for the flexible relational memory/logical inference account for the role of the hippocampus in animals (McGonigle and Chalmers, 1986; Eichenbaum, 1992; Squire, 1992; Cohen and Eichenbaum, 1993; Dusek and Eichenbaum, 1997; Burgess et al., 2002). By this account, hippocampal function is closely related to all declarative memory, including all explicit memory, but is especially crucial for relational

learning and flexible use of memory (Eichenbaum, 2004). Other accounts for TI performance demonstrated by animals exist, however. The coordination model (O'Reilly and Rudy, 2001) proposes that the training pairs are stored in memory (B>C, C>D) separately, and the two relevant training pairs are then recalled when a new pair is presented (B>D). Clearly, this model does not account for the ability to make correct inferences on novel pairs in which the individual stimuli are separated by more than one other stimulus in the ordered sequence (B>E).

In contrast to the relational flexibility theory and the coordination model, the excitatory strength/value transfer account posits that performance on TIs is guided by the absolute excitatory strength that each stimulus acquires during training, rather than by flexible manipulation of the sequence representation (von Fersen et al., 1991; Wynne, 1998; Frank et al., 2003; Van Elzakker et al., 2003). According to this account, each stimulus in the ordered sequence acquires an excitatory value during training dependent on its own history of reinforcement and partial generalization of the value of its partner stimulus. When faced with a novel pairing, the participant then simply chooses the item that has greater excitatory strength rather than relying on a logical sequence account. Based on this view, they argued that the B and D items in the original five-item (A through E) ordered sequence do not posses equal excitatory strengths. Similarly, the end items (A and E) do not have the same excitatory strengths either. Because E always loses, D only needs to be assigned a very weak excitatory value. On the other hand, A wins over B but B wins over C so B still has some excitatory value relative to A, thus lowering the absolute excitatory value of A. The notion of unequal excitatory strengths of the end

items A and E is supported by the observation that the accuracy of response on the training pair DE is significantly higher than on AB.

In support of the excitatory strength/value transfer account, healthy rats that were trained on a six-item sequence A>B>C>D>E>F failed to correctly choose B over D. They were capable of correctly selecting B over E, however. This result was explained by the greater difference in associative strengths between items B and E compared with items B and D. It was noted, however that this finding could be explained by the symbolic distance phenomenon, which is a variant of the relational flexibility account described above. The symbolic distance effect (SDE) refers to the greater ease of inference decisions the further apart the two items are on the relational continuum (Rapp et al., 1996; Acuna et al., 2002a; Bond et al., 2003; Frank et al., 2005). For example, decision on the novel pair B>D from the six-item sequence with a symbolic distance of one (i.e., one intervening item in the sequence) should require greater sequence manipulation than on the novel pair B>E with a symbolic distance of two (i.e., two intervening items in the sequence). Inferences on B>D should therefore elicit lower accuracies and/or longer latencies than inferences on B>E. Under the relational memory account of the hippocampus, more information has to be recalled about the sequence hierarchy in order to solve B>D than B>E. Therefore, if SDE is taken into consideration, the performance of rats on the six item sequence could still be accounted for by the relational flexibility account. Overall, the representational flexibility account for TI seems to be more plausible explanation for the performance exhibited by animals but open questions still exist about the exact mechanism by which TI takes place.

As noted above, the most attractive feature of this task is that it can be readily adapted for human studies (Greene et al., 2001; Martin and Alsop, 2004). Moreover, the experiments demonstrated some interesting features of the phenomenon in humans, including the fact that we are capable of TI without necessarily having conscious awareness of the hierarchy. In a recent study, the role of awareness in the 5-item ordered sequence TI task was evaluated (Greene et al., 2001). The authors found significantly better performance on the B>D trial in participants informed of the underlying sequence compared to those who were not informed. Their intriguing finding though was that successful performance on the TI task for uninformed participants did not depend on post-experimental awareness of sequence. They concluded that, contrary to previous theories, implicit tasks may rely on flexible representations. More significantly, they speculated that although declarative memory and relational learning are highly similar in function and structure, they are not interchangeable constructs. This view contradicts the previous conjecture that because relational learning and conscious forms of memory both depend on the hippocampal system, conscious awareness of learned contingencies is necessary and sufficient for relational learning (Schacter, 1998). The paradigm has also been adopted for a PET study where hippocampal activation was demonstrated in the comparison of training of bridging pairs (e.g., B>C and D>E) and independent pairs (e.g., A>B and C>D) (Nagode and Pardo, 2002). This experiment, however, suffered from several limitations and also failed to show any hippocampal involvement during TI judgments.

The fact that intact MTL, especially hippocampal, function has been implicated in TI in animals and that the animal experiments can readily be adapted for use in humans opened the potential to use TI to study disorders with damage to MTL, such as SZ. Indeed, an important behavioral study of Titone et al. (Titone et al., 2004) demonstrated impairments on TI in this disease.

Transitive inference deficits in schizophrenia

As explained above, the search for specific and selective deficits in SZ is still inconclusive. A logical way of searching for such deficits is to focus on areas of overlap of neuropathology and cognitive impairment. One such area of overlap is presented by abnormalities in MTL structure and the memory functions it subserves. TI is a memory process that clearly depends on intact MTL function. Thus, TI hus presented a promising direction for investigation.

The ordered sequence experiment for probing TI function developed for animal studies was adopted for use in SZ by Titone et al. (2001, 2004), employing the original five item sequence A>B>C>D>E. They hypothesized that given the evidence of impaired explicit memory in SZ, and the association between explicit memory and relational processing, SZ patients should not differ from controls when memory requires only the encoding and expression of simple associative mappings (AB, BC, CD, DE, and the novel probe pair AE). In contrast, SZ patients should perform worse than controls when memory requires high-level relational memory organization. They, therefore, expected that SZ patients and controls would not differ in learning and remembering simple reinforcement histories for

a set of hierarchically structured stimuli (A>B, B>C, C>D and D>E). Second, SZ patients should perform worse than controls in responding to novel probe pairs that do not differ in terms of their simple reinforcement histories (e.g., BD: B and D are each correct 50% of the time). Patients' responses to AE probe pairs should be intact because these stimuli have consistently asymmetric reinforcement histories that put A (correct 100% of the time) at a clear advantage over E (never correct) (Titone et al., 2004).

The authors used a set of five visual patterns as the stimuli, and denoted them as items A through E. During training, the participants saw one pair at a time (from the four pairs AB, BC, CD, and DE) and were told that one of the pictures is hiding a "smiley face" and their task was to discover for each pair which item was hiding the smiley face. The training consisted of three distinct blocks of trials. In each block, the pairs of stimuli were presented in random order. The first training block was "front-loaded," such that adjacent pairs at the top of the hierarchy (AB and BC) were twice as likely (24 times each) to appear than the remaining two pairs CD and DE (12 times each). In the next training block, this loading was reversed. In the third training block, participants saw all pairs 12 times each. If they failed to reach criterion at this point, they were given one more training block. The testing phase consisted of two blocks. In the first, participants saw the original training pairs 12 times each without reinforcement. In the second, participants saw the original pairs and the inference pairs AE and BD 10 times each. The testing phase was followed by a de-briefing phase where awareness of the hierarchy was assessed.

The training criterion was passed by 25 out of 29 SZ patients and 16 out of 19 control participants. In the first testing block where only the original training pairs were shown, the two groups did not differ in accuracy. In the second testing block, SZ patients demonstrated lower accuracy on the original training pairs. Crucially, SZ patients but not controls showed diminished performance on BD compared with the pairs BC and CD. In contrast, SZ and controls performed similarly on AE as on AB and DE. SZ patients thus showed diminished performance on TI requiring flexible manipulation of the sequence (NETI pair BD), but not on inferences informed by the end items (non-transitive inference pair AE). Thus, the authors argued for a specific impairment in relational memory in SZ because intact performance on AE and on the reinforced learning trials indicated that a generalized deficit could not account for the results. These results demonstrated a potentially selective memory impairment in SZ. This impairment provides a promising lead for a specific area of memory dysfunction in this disease, all the more because TI has been linked to intact MTL function in animals and MTL structure and function is known to be abnormal in SZ as discussed earlier.

Motivation and outline

Given the apparent selectivity of TI impairment in SZ, the established role of MTL in TI in animals, and the reliably documented abnormalities in MTL structure in SZ, TI provides a promising lead for examining the link between specific areas of memory dysfunction and abnormalities of MTL structure and function in SZ. This confluence provided the motivation for the studies of TI described in this dissertation.

The thesis is devoted to functional magnetic resonance imaging (fMRI) experiments aimed at investigating the role of MTL structures in TI, along with its mechanisms, in humans. At the time this research project began, no study existed that examined the link between MTL function and TI in humans apart from the limited PET study of Nagode and Pardo (Nagode and Pardo, 2002). Thus, before investigating the link between TI deficit and MTL structure and function in SZ, it was necessary to establish the role of MTL in TI in healthy individuals. Three experiments are described. The first experiment was a block-design fMRI study of TI that demonstrated a role of MTL in TI in humans. The second experiment was an event-related fMRI experiment in which the specificity of MTL role in this process was investigated further. The third experiment used an event-related fMRI experiment to investigate MTL's role in the training that established the relations underlying TI.

The final section of the dissertation provides a general discussion of the three experiments and their implications for SZ. Specifically, the conclusions from the three experiements investigating TI in healthy participants guide an intepretation of the results of a recent neuroimaging study of TI in SZ conducted in the research group of which I am a member.

Research hypotheses and questions

The following questions and hypotheses are addressed in the three experiments comprising this thesis:

A. Experiment 1: Block-design fMRI study of TI in human participants
    a. Questions
        i. Is TI associated with MTL activation?
        ii. What other brain areas are recruited in TI in humans?
    b. Hypotheses
        i. Hippocampus is activated in transitive inference but not in inferences that are not transitive.
        ii. Previously identified network of cortical and subcortical brain regions, including PFC, pre-supplementary motor area, supplementary motor area, anterior and posterior CC, precuneus, posterior parietal cortex, thalamus and ventral striatum ("TI network") is recruited in TI but not in inferences that are not transitive.

B. Experiment 2: Event-related fMRI study of TI in human participants
    a. Questions
        i. What is the specific role of MTL and the TI network in TI?
        ii. What are the neural correlates of the symbolic distance effect in MTL and the TI network?

b. Hypotheses

i. Hippocampus is more activated in non end-item TI (e.g., B>D) than end-item TI (i.e., inferences involving one of the end-items A or F such as A>C).

ii. The TI network is more activated in non end-item than end-item transitive inferences.

iii. Hippocampus is more activated in judgments with smaller symbolic distance (e.g., B>D compared with B>E) because more information about the underlying hierarchy has to manipulated.

iv. The TI network, especially posterior parietal cortex previously implicated in comparisons, is more activated in judgments with smaller symbolic distance.

C. Experiment 3: Event-related study of training for TI in humans

a. Questions

i. What is the role of MTL and the TI network in learning relational contingencies?

b. Hypotheses

i. Hippocampus is more activated in training on the relations underlying transitive inferences (i.e., A>B, B>C, C>D, etc.) compared with training on simple non-overlapping relations (i.e., a>b, c>d, e>f, etc.).

ii. The TI network is more activated in training on the relationships underlying transitive inferences compared with training on simple non-overlapping relations.

References

Acuna BD, Sanes JN, Donoghue JP (2002) Cognitive mechanisms of transitive inference. Experimental Brain Research 146:1-10.

Aleman A, Hijman R, de Haan EHF, Kahn RS (1999) Memory impairment in schizophrenia: a meta-analysis. Am J Psychiatry 156:1358-1366.

Allin M, Murray R (2002) Schizophrenia: a neurodevelopmental or neurodegenerative disorder? Current Opinion in Psychiatry 15:9-15.

Antonova E, Sharma T, Morris R, Kumari V (2004) The relationship between brain structure and neurocognition in schizophrenia: a selective review. Schizophrenia Res 70:117-145.

Arnold SE (2000) Cellular and Molecular Neuropathology of the Parahippocampal Region in Schizophrenia. Annals New York Academy of Sciences:275-292.

Bilder R (2001) Schizophrenia as a neurodevelopmental disorder. Current Opinion in Psychiatry 14:9-15.

Bogerts B (1999) The neuropathology of schizophrenic diseases: historical aspects and present knowledge. Eur Arch Psychiatry Clin Neurosci:IV/2-IV/13.

Bond AB, Kamil AC, Balda RP (2003) Social complexity and transitive inference in corvids. Animal Behaviour 65:479-487.

Brebion G, Gorman JM, Malaspina D, Sharif Z, Amador XF (2001) Clinical and Cogntive Factors Associated With Verbal Memory Task Performance in Patients with Schizophrenia. Am J Psychiatry 158:758-764.

Brown MW, Aggleton JP (2001) Recognition memory: what are the roles of the perirhinal cortex and hippocampus? Nature Reviews Neuroscience 2.

Buckmaster CA, Eichenbaum H, Amaral DG, Suzuki WA, Rapp PR (2004) Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. The Journal of Neuroscience 24:9811-9825.

Buckner RL, Wheeler ME (2001) The cognitive neuroscience of remembering. Nature Reviews Neuroscience 2:624-634.

Burgess N, Maguire EA, O'Keefe J (2002) The human hippocampus and spatial and episodic memory. Neuron 35:625-641.

Cahn W, Hulshoff Pol H, Lems EBTE, Van Haren NEM, Schnack HG, Van der Linden JA, Schothorst PF, van Engeland H, Kahn RS (2002) Brain Volume Changes in First-Episode Schizophrenia. Arch Gen Psychiatry 59:1002-1010.

Cirillo MA, Seidman LJ (2003) Verbal Declarative Memory Dysfunction in Schizophrenia: From Clinical Assessment to Genetics and Brain Mechanisms. Neuropsychology Review 13:43-77.

Cohen NJ, Eichenbaum H (1993) Memory, amnesia, and the hippocampal system. Cambridge, MA: MIT Press.

Colombo M, Frost N (2001) Representation of serial order in humans: a comparison to the findings with monkeys (*Cebus apella*). Psychonomic Bulletin & Review 8:262-269.

Corkin S (2002) What's new with the amnesic patient H.M.? Nature Reviews Neuroscience 3:153-160.

Cullen TJ, Walker MA, Parkinson N, Craven R, Crow TJ, Esiri MM, Harrison PJ (2003) A postmortem study of the mediodorsal nucleus of the thalamus in schizophrenia. Schizophrenia Res 60:157-166.

Danion J-M, Meulemans T, Kauffmann-Muller F, Vermaat H (2001) Intact Implicit Learning in Schizophrenia. Am J Psychiatry 158:944-948.

Davachi L, Wagner AD (2002) Hippocampal contributions to episodic encoding: insights from relational and item-based learning. Journal of Neurophysiology 88:982-990.

Davidson M, Keefe RS (1995) Cognitive impairment as a target for pharmacological treatment in schizophrenia. Schizophr Res 17:123-129.

Davis H (1992) Transitive inference in rats (*Rattus norvegicus*). Journal of Comparative Psychology 106:342-349.

Delius JD, Siemann M (1998) Transitive responding in animals and humans: Exaptation rather than adaptation. Behavioural Processes 42:107-137.

Dusek JA, Eichenbaum H (1997) The hippocampus and memory for orderly stimulus relations. Proceedings of the National Academy of Sciences of the United States of America 94:7109-7114.

Eastwood SL, Harrison PJ (2005) Interstitial white matter neuron density in the dorsolateral prefrontal cortex and parahippocampal gyrus in schizophrenia. Schizophrenia Res 79:181-188.

Egeland J, Sundet K, Rund BR, Asbjornsen A, Hugdahl K, Landro NI, Lund A, Roness A, Stordal KI (2003) Sensitivity and Specificity of Memory Dysfunction in Schizophrenia: A Comparison with Major Depression. Journal of Clinical & Experimental Neuropsychology 25:79-93.

Eichenbaum H (1992) The hippocampus and declarative memory in animals. Journal of Cognitive Neuroscience 4:217-231.

Eichenbaum H (2000) A cortical-hippocampal system for declarative memory. Nature Reviews Neuroscience 1:41-50.

Eichenbaum H (2004) Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 44:109-120.

Eichenbaum H, Cohen NJ (2001) From conditioning to conscious recollection. Memory systems of the brain. Oxford: Oxford University Press.

Eichenbaum H, Otto T, Cohen NJ (1994) Two functional components of the hippocampal memory system. Brain and Behavioral Sciences 17:449-518.

Emilien G, Durlach C, Antoniadis E, Van Der Linden M, Maloteaux J-M (2004) Memory: neuropsychological, imaging and psychopharmacological perspectives. Hove and New York: Psychology Press.

Fannon D, Chinthis X, Doku VC, Tennakoon L, O'Ceallaigh S, Soni W, Sumich A, Lowe J, Santamaria M, Sharma T (2000) Features of Structural Brain Abnormality Detected in First-Episode Psychosis. Am J Psychiatry 157:1829-1834.

Faraone SV, Seidman LJ, Kremen WS, Kennedy D, Makris N, Caviness V, Goldstein J, Tsuang MT (2003) Structural brain abnormalities among relatives of patients with schizophrenia: implications for linkage studies. Schizophrenia Res 60:125-140.

Florencio PS, O'Driscoll GA (1999) The Medial Temporal Lobe and Schizophrenia. McGill Journal of Medicine 5:25-34.

Frank MJ, Rudy JW, O'Reilly RC (2003) Transitivity, flexibility, conjunctive representations, and the hippocampus. II. A computational analysis. Hippocampus 13:299-312.

Frank MJ, Rudy JW, Levy WB, O'Reilly RC (2005) When logic fails: Implicit transitive inference in humans. Memory & Cognition 33:742-750.

Geuze E, Vermetten E, Bremner AJ (2005) MR-based in vivo hippocampal volumetrics: 1. Review of methodologies currently employed. Molecular Psychiatry 10:147-159.

Goff DC, Heckers S, Freudenreich O (2001) Schizophrenia. In: The Medical Clinics of North America. Advances in the pathophysiology and treatment of psychiatric disorders: Implications for internal medicine (Nemeroff CB, ed), pp 663-689. Philadelphia: W.B. Saunders.

Gold JM, Weinberger DR (1995) Cognitive deficits and the neurobiology of schizophrenia. Curr Opin Neurobiol 5:225-230.

Gold JM, Randolph C, Carpenter CJ, Goldberg TE, Weinberger DR (1992) Forms of memory failure in schizophrenia. J Abnorm Psychol 101:487-494.

Greene AJ, Spellman BA, Dusek JA, Eichenbaum HB, Levy WB (2001) Relational learning with and without awareness: transitive inference using nonverbal stimuli in humans. Memory & Cognition 29:893-902.

Harrison PJ (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122:593-624.

Harrison PJ (2004) The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. Psychopharmacology 174:151-162.

Harrison PJ, Roberts GW, eds (2000) The neuropathology of schizophrenia, 1. Edition. Oxford: Oxford University Press.

Harrison PJ, Owen MJ (2003) Genes for schizophrenia? Recent findings and their pathophysiological implications. Lancet 361:417-419.

Harrison PJ, Weinberger DR (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Molecular Psychiatry 10:40-68.

Heckers S, Konradi C (2002) Hippocampal neurons in schizophrenia. Journal of Neural Transmission 109:891-905.

Heinrichs RW (2005) The Primacy of Cognition in Schizophrenia. American Psychologist 60:229-242.

Honea R, Crow TJ, Passingham D, Mackay CE (2005) Regional Deficits in Brain Volume in Schizophrenia: A Meta-Analysis of Voxel-Based Morphometry Studies. Am J Psychiatry 162:2233-2245.

Innocenti GM, Ansermet F, Parnas J (2003) Schizophrenia, neurodevelopment and corpus callosum. Molecular Psychiatry 8:261-274.

Jablensky A (2000) Epidemiology of schizophrenia: the global burden of disease and disability. Eur Arch Psychiatry Clin Neurosci 250:274-285.

Job DE, Whalley HC, McConnell S, Glabus M, Johnstone EC, Lawrie SM (2002) Structural Gray Matter Differences between First-Episode Schizophrenics and Normal Controls Using Voxel-Based Morphometry. Neuroimage 17:880-889.

Keefe RSE, Eesley CE, Poe MP (2005) Defining a Cognitive Function Decrement in Schizophrenia. Biol Psychiatry 57:688-691.
Kraepelin E (1896) Psychiatrie. Ein Lehrbuch für Studirende und Aerzte. Fünfte, vollständig umgearbeitete Auflage., 5. vollständig umgearbeitete Auflage Edition. Leipzig: Johann Ambrosius Barth.
Kremen WS, Seidman LJ, Faraone SV, Tsuang MT (2001) Intelligence Quotient and Neuropsychological Profiles in Patients with Schizophrenia and in Normal Volunteers. Biol Psychiatry 50:453-462.
Kuperberg G, Heckers S (2000) Schizophrenia and cognitive function. Current Opinion in Neurobiology 10:205-210.
Kuperberg GR, Broome MR, McGuire PK, David AS, Eddy M, Ozawa F, Goff D, West WC, Williams SCR, van der Kouwe AJW, Salat DH, Dale AM, Fischl B (2003) Regionally Localized Thinning of the Cerebral Cortex in Schizophrenia. Arch Gen Psychiatry 60:878-888.
Kurachi M (2003) Pathogenesis of schizophrenia: Part I. Symptomatology, cognitive characteristics and brain morphology. Psychiatry and Clinical Neurosciences 57:3-8.
Lawrie SM, Whalley HC, Job DE, Johnstone EC (2003) Structural and Functional Abnormalities of the Amygdala in Schizophrenia. Annals New York Academy of Sciences 985:445-460.
Lewis DA, Lieberman JA (2000) Catching up on schizophrenia: natural history and neurobiology. Neuron 28:325-334.
Lewis DA, Levitt P (2002) Schizophrenia As A Disorder of Neurodevelopment. Annu Rev Neurosci 25:409-432.
Martin N, Alsop B (2004) Transitive inference and awareness in humans. Behavioral Processes 67:157-165.
McGonigle B, Chalmers M (1986) Representations and strategies during inference. In: Reasoning and discourse processes (Myers T, Brown K, McGonigle B, eds), pp 141-164. London: Academic Press.
McIntosh AM, Job DE, Moorhead WJ, Harrison LK, Forrester K, Lawrie SM, Johnstone EC (2004) Voxel-Based Morphometry of Patients with Schizophrenia or Bipolar Disorder and Their Unaffected Relatives. Biol Psychiatry 56:544-552.
Meeter M, Talamini LM, Murre JMJ (2002) A computational approach to memory deficits in schizophrenia. Neurocomputing 44:929-936.
Miyamoto S, LaMantia AS, Duncan GE, Sullivan P, Gilmore JH, Lieberman JA (2003) Recent Advances in the Neurobiology of Schizophrenia. Molecular Interventions 3:27-39.
Muller BW, Sartory G, Bender S (2004) Neuropsychological Deficits and Concomitant Clinical Symptoms in Schizophrenia. European Psychologist 9:96-106.
Nagode JC, Pardo JV (2002) Human hippocampal activation during transitive inference. Neuroreport 13:939-944.
Narr KL, van Erp TGM, Cannon TD, Woods RP, Thompson PM, Jang S, Blanton R, Poutanen VP, Huttunen M, Lonnqvist J, Standerksjold-Nordenstam CG, Kaprio J, Mazziotta J, Toga AW (2002) A Twin Study of Genetic Contributions to Hippocampal Morphology in Schizophrenia. Neurobiology of Disease 11:83-95.

Nuechterlein K, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK (2004) Identification of separable cognitive factors in schizophrenia. Schizophrenia Res 72:29-39.

O'Reilly RC, Rudy JW (2001) Conjunctive representations in learning and memory: principles of cortical and hippocampal function. Psychology Reviews 108:311-345.

Pantelis C, Yucel M, Wood SJ, Velakoulis D, Sun D, Berger G, Stuart GW, Yung A, Phillips L, McGorry PD (2005) Structural Brain Imaging Evidence for Multiple Pathological Processes at Different Stages of Brain Development in Schizophrenia. Schizophrenia Bull 31:672-696.

Pearlson GD (2000) Neurobiology of Schizophrenia. Ann Neurol 48:556-566.

Pelletier M, Achim AM, Montoya A, Lal S, Lepage M (2005) Cognitive and clinical moderators of recognition memory in schziophrenia: a meta-analysis. Schizophrenia Res 74:233-252.

Piaget J (1928) The Language and Thought of the Child. New York: Harcourt Brace.

Plum F (1972) Prospects for research on schizophrenia. 3. Neurophysiology. Neuropathological findings. Neurosci Res Program Bull 10:384-388.

Popken GJ, Bunney Jr. W, Potkin SG, Jones EG (2000) Subnucleus-specific loss of neurons in medial thalamus of schizophrenics. Proc Natl Acad Sci U S A 97:9276-9280.

Rapp PR, Kansky MT, Eichenbaum H (1996) Learning and memory for hierarchical relationships in the monkey: effects of aging. Behavioral Neuroscience 110:887-897.

Sawa A, Snyder SH (2002) Schizophrenia: Diverse Approaches to a Complex Disease. Science 296:692-695.

Schacter DL (1998) Memory and awareness. Science 280:59-60.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. Journal of Neurology, Neurosurgery & Psychiatry 20:11-21.

Seidman LJ, Stone WS, Jones R, Harrison RH, Mirsky AF (1998) Comparative effects of schizophrenia and temporal lobe epilepsy on memory. Journal of the International Neuropsychological Society 4:342-352.

Shenton ME, Dickey CC, Frumin M, McCarley RW (2001) A review of MRI findings in schizophrenia. Schizophrenia Research 49:1-52.

Sim K, DeWitt I, Ditman T, Zalesak M, Greenhouse I, Goff D, Weiss AP, Heckers S (2005) Hippocampal and Parahippocampal Volumes in Schizophrenia:A Structural MRI Study.

Squire L (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychological Review 99:195-231.

Squire LR, Zola-Morgan S (1991) The medial temporal lobe memory system. Science 253:1380-1386.

Strange BA, Otten LJ, Josephs O, Rugg MD, Dolan RJ (2002) Dissociable human perirhinal, hippocampal, and parahippocampal roles during verbal encoding. Journal of Neuroscience 22:523-528.

Tamminga CA, Holcomb HH (2005) Phenotype of schizophrenia: a review and formulation. Molecular Psychiatry 10:27-39.

Titone D, Warner JL, Levy DL, Holzman PS (2001) The hippocampal memory system in schizophrenia: evidence from an ordered transitivity task. Schizophrenia Res 49:S122-123.

Titone D, Ditman T, Holzman PS, Eichenbaum H, Levy DL (2004) Transitive inference in schizophrenia: impairments in relational memory organization. Schizophrenia Res 68 (2-3):235-247.

Van Elzakker M, O'Reilly RC, Rudy JW (2003) Transitivity, flexibility, conjunctive representations, and the hippocampus. I. An empirical analysis. Hippocampus 13:334-340.

von Fersen L, Wynne CDL, Delius JD, Staddon JER (1991) Transitive inference formation in pigeons. Journal of Experimental Psychology: Animal Behavior Processes 17:334-341.

Weiss AP, DeWitt I, Goff D, Ditman T, Heckers S (2005) Anterior and posterior hippocampal volumes in schizophrenia. Schizophrenia Res 73:103-112.

Wilk CM, Gold JM, McMahon RP, Humber K, Iannone VN, Buchanan RW (2005) No, It Is Not Possible to Be Schizophrenic Yet Neuropsychologically Normal. Neuropsychology 19:778-786.

Wong AHC, Van Tol HHM (2003) Schizophrenia: from phenomenology to neurobiology. Neuroscience and Biobehavioral Reviews 27:269-306.

Wynne CDL (1998) A minimal model of transitive inference. In: Models of action (Wynne CDL, Staddon JER, eds), pp 269-307. Mahwah, N.J.: Lawrence Erlbaum Associates.

Chapter 2: Experiment 1 – Neural correlates of transitive inference in humans

Introduction

At the time when the transitive inference (TI) experiment that demonstrated a selective deficit in schizophrenia (SZ) was conducted (see Background/Introduction), the link between successful TI performance in animals and intact MTL function had been firmly established. No such link, however had been established in humans although similarities between animal and human performance had been clearly demonstrated in behavioral studies (Colombo and Frost, 2001). The only study that investigated the role of MTL in TI was a study conducted using positron emission tomography (PET), which focused more on the training with the pairs constituting an ordered sequence rather than on transitive inference testing (Nagode and Pardo, 2002). Thus, before investigating the neural correlates of the presumed deficit in TI in schizophrenia, we used fMRI to investigate the neural underpinnings of this phenomenon in healthy participants. Our goal was to test the hypothesis that TI in humans is associated with MTL structures, specifically the hippocampus. We also wanted to identify other neural systems that might be involved in this process.

As outlined in the Introduction, TI refers to the ability to infer relations among indirectly related items that have not been presented together, based on previous learning of a sequence of overlapping premise pairs (e.g., A>C, if A>B and B>C). As previously discussed, using a hierarchically ordered set of five odors (A>B>C>D>E), Dusek and Eichenbaum (1997) demonstrated that disconnection of the hippocampus from either its cortical or subcortical pathway prevents rats from inferring the proper order for odors B and D (Dusek and Eichenbaum, 1997). This and subsequent experiments in rodents

(Fortin et al., 2002; Van Elzakker et al., 2003) and monkeys (Buckmaster et al., 2004) supported the view that the hippocampus is necessary to establish a flexible representation of memory (Eichenbaum, 2004). No such evidence for hippocampal role existed in humans for hippocampal recruitments during performance of TI tasks. The only previous neuroimaging study that investigated the neural correlates of TI in human participants covered brain areas outside of the MTL (Acuna et al., 2002b).

We used a 2x2 factorial design to study the effects of inference (novel versus previously learned pairings) and stimulus sequence (overlapping versus non-overlapping pairs) (Figure 1). Initially, participants were trained to discriminate arbitrary visual stimulus pairs. One set of 8 stimuli created four non-overlapping pairs (labeled "P" in Figure 1); another set of 5 stimuli created four overlapping pairs (labeled "S"). We then studied the ability to infer a relation between items that had not been presented together, based on previous learning of a sequence of overlapping pairs (TI) or non-overlapping pairs (non-transitive inference). We hypothesized that TIs about novel pairings derived from the overlapping stimulus set (labeled "IS") would be associated with hippocampal activation, whereas non-transitive inferences about novel pairings derived from the non-overlapping stimulus set (labeled "IP") would not.

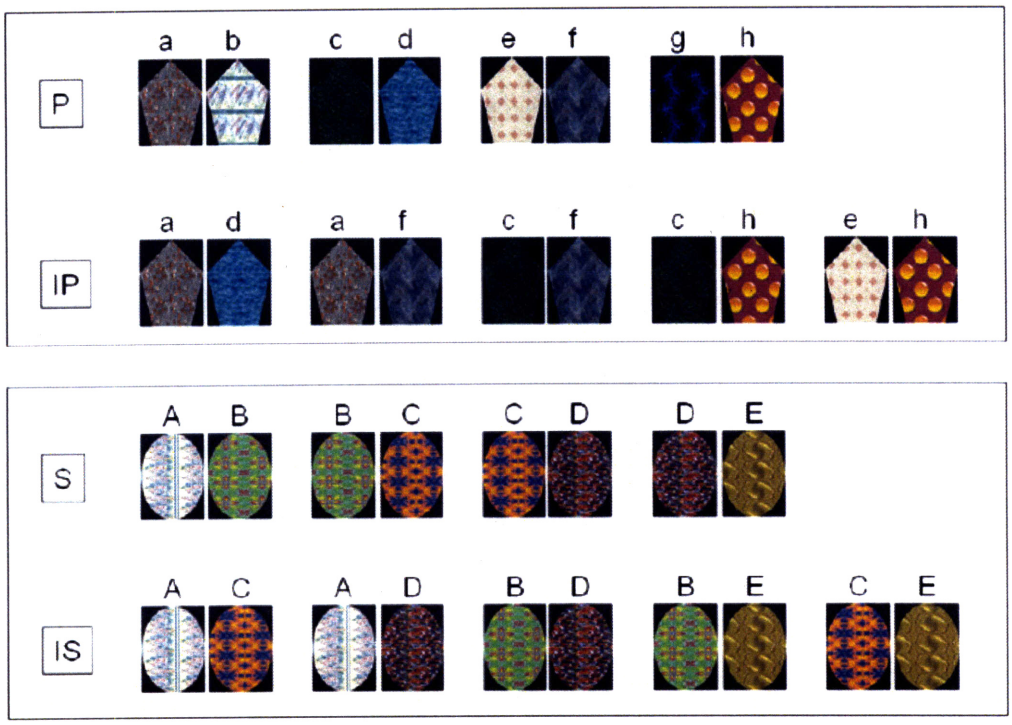

Figure 1: **Stimulus set and experimental conditions**. Prior to scanning, participants were trained to discriminate non-overlapping pairs (P) and an overlapping sequence of pairs (S). The reinforced item within each pair is shown on the left. During scanning, participants were asked to recollect the correct response for previously seen pairs (P and S) and to infer the correct response for five novel pairings of non-overlapping pairs (IP) and overlapping pairs (IS). No letters were shown in the experiment, and the presentation of the pairs and the position of the two stimuli within each pair were randomized (Heckers et al., 2004a).

Experimental procedures

Participants

We studied 16 healthy participants (8 female and 8 male, ages 21-28, mean age 23.9, average verbal IQ (Blair and Spreen, 1989) = 114) who gave informed consent in a manner approved by the institutional review board of the Massachusetts General Hospital. No participant had a history of significant medical, neurological, or psychiatric illness.

Stimuli and paradigm

Stimuli

From pattern fills provided by CorelDraw, we selected 13 visually exemplars. Two sets of pattern fills (8 for the non-overlapping pairs, and 5 for the overlapping pairs) were randomly assigned to pairs of pentagon and ellipsoid shapes.

Training prior to scanning

Participants were told that they would see pairs of visual patterns on a computer screen, and that one pattern in each pair would always hide a "smiling face" (e.g., ☺). They were then shown each of the pairings, along with the correct answer in each case, and instructed to remember the location of the smiling face. Left/right position of individual patterns for each pair was counterbalanced; participants indicated their response by pressing "1" for the stimulus on the left and "2" for the stimulus on the right. When participants made a correct guess during training, the selected visual pattern would move to reveal the smiling face reinforcer. When participants made an incorrect guess, the selected visual pattern would move but the smiling face would not appear.

Participants were first trained and tested on the non-overlapping pairs, next on the overlapping pairs, and then on a mixture of non-overlapping and overlapping pairs. This final testing session was similar in format to that used in the non-inference conditions during scanning, with one difference: stimulus pairs were presented randomly, rather than in blocked sessions, such that on each trial participants were equally likely to be

presented with a pair of stimuli from the overlapping set as they were to be presented with a pair of stimuli from the non-overlapping set. The training procedure, identical for non-overlapping and overlapping pairs, was broken into three blocks. The training lasted approximately 1 hour altogether and was carried out immediately prior to scanning. The first training block consisted of 60 trials that contained twice as many of two of the four stimulus pairs. For example, during training of the overlapping stimulus set, participants saw 20 instances of AB and BC, and 10 instances of CD and DE. Thus, the sequential stimuli during the first training block were "front loaded". During training of the non-overlapping stimulus set, participants saw 20 instances of ab and cd, and 10 instances of ef and gh. The second training block also consisted of 60 trials that contained twice as many of two of the four stimulus pairs from each set. In this second block, however, the pairs were "back loaded". Thus, participants saw 20 instances of CD and DE, and 10 instances of AB and BC. During the second block of training of the non-overlapping stimulus set, participants saw 20 instances of ef and gh, and 10 instances of ab and cd. The third training block consisted of 24 trials that contained equal numbers of the four stimulus pairs. Thus, for the training of both overlapping and non-overlapping pairs, participants saw 6 instances of each stimulus.

Overall, participants were presented with an equal number of each of the pairs in the overlapping and non-overlapping stimulus sets. This method of training ensured that all participants would not only be able to learn the correct response for each pairing but would also be likely to hierarchically encode the overlapping stimulus set. Previous behavioral studies in humans suggested that the initial front loading of pairs was

necessary for healthy participants to correctly respond to the novel BD trial during the inference test trials (Titone et al., 2004).

Recognition task during fMRI scan

Participants took part in two fMRI scans, each lasting 5 minutes. Each scan started and ended with 30 seconds of fixation trials. In between, blocks of 10 trials of four different types (P, S, IP, IS) were presented in the following sequence: P, S, IP, IS, P, S, IP, IS. For each trial, participants were instructed to indicate by pressing a button which pattern they associated with reinforcement, based on the previous training session. During scanning, however, the smiling face, used for reinforcement during training, was not presented.

To avoid bias associated with particular object shapes and/or patterns, we rotated the position of the fills within the two sets for each participant (a total of 16 fills was used, each subject saw 13 of the 16 fills) and the two shapes across all participants (8 participants saw non-overlapping pairs as pentagons and 8 participants saw overlapping pairs as pentagons) (Figure 1).

Functional imaging

Participants were scanned in a Siemens 1.5 Tesla Sonata high-speed echo-planar imaging device (Munich, Germany). They rested on a padded scanner bed in a dimly illuminated room and wore ear plugs. Foam padding was used to stabilize the head. Stimuli were generated using Presentation software (Neurobehavioral Systems) on a personal

computer, projected onto a screen, and viewed inside the scanner by the participants via a tilted mirror placed in front of their eyes.

Functional scanning began with an initial sagittal localizer scan. The two functional series lasted 5:10 min each. The first 10 sec of each series were discarded to allow for T1 equilibration. During the remaining time in each series, 120 BOLD functional brain images were collected to capture 80 trials lasting 3 sec each, bracketed by 2 blocks of fixation trials, lasting 30 sec each (TE/TR = 40/2500 ms; 25 coronal slices, perpendicular to the anterior commisure – posterior commisure line and starting anteriorly at the frontal pole, 5 mm thickness, 1 mm skip; voxel size 3.1x3.1x5 mm, FOV = 200 mm; flip angle = 90 degrees),.

Data analysis

Behavioral data

The accuracy data were analyzed using a repeated measures 2 (Sequence type: overlapping, non-overlapping) x 2 (Inference type: present, absent) ANOVA. The latency of correct responses was also analyzed using a repeated measures 2 x 2 ANOVA.

Functional neuroimaging data

All functional data were transformed into a common reference space (MNI Talairach brain) and corrected for head motion using SPM99 (Wellcome Department of Cognitive

Neurology, London, UK). Functional images were smoothed using an 8 mm full-width-half-maximum (FWHM) Gaussian filter.

Functional images were analyzed in two stages in a mixed-effects model. First, general linear models were created for each subject, which included the effects of session (1, 2) and condition (P, S, IP, IS) to explain the variance of BOLD signal change at each voxel. We tested for the main effects of inference ([IP+IS] versus [P+S]) and sequence ([S+IS] versus [P+IP]) and their interaction ([IS versus IP] versus ([S versus P]) across the two functional imaging sessions. Second, we pooled all individual contrast images for the main effects and interactions into a one-sample t-test for within group effects. Activations were considered significant at a voxel extent threshold of $\geq$50 voxels, with $p<0.0001$, uncorrected for multiple comparisons. To disambiguate significant results of the main effects analysis we followed up with analyses that included only two conditions (simple effect analysis). Activations were considered significant at $p<0.0001$, uncorrected for multiple comparisons.

Results

Behavioral data

Accuracy

The pattern of accuracy for each of the four conditions (P, S, IP, and IS) was as follows: 98.0 $\pm$ 4.0% (mean $\pm$ SD) for P; 95.9 $\pm$ 6.9% for S; 94.5 $\pm$ 15.0% for IP; and 91.7 $\pm$ 10.4% for IS. Responses to non-overlapping pairs of items were not significantly different from responses to overlapping pairs (main effect of sequence: $F(1, 15) = 3.3$, p

= .09). Responses to previously learned pairs were not significantly different from responses to novel pairings (main effect of inference: $F(1, 15) = 1.8$, $p = .20$). The interaction between the two main effects, i.e., sequence and inference, was not significant ($F(1, 15) = 0.8$, $p = .78$).

Response Latency

Response latency (in msec) for each of the four conditions (P, S, IP, and IS) was as follows: 871.2 ± 194.7 (mean ± SD) for P; 1138.3 ±234.6 for S; 868.4 ±289.0 for IP; and 1330.4 ±334.2 for IS (Figure 2). Responses to overlapping pairs were significantly slower than responses to non-overlapping pairs (main effect of sequence: $F(1, 15) = 67.6$, $p < .0001$). Responses to novel pairings were significantly slower than responses to previously learned pairs (main effect of inference: $F(1, 15) = 8.9$, $p = .009$). Further, the increase in reaction time associated with inferential judgments was significantly greater for the overlapping pairs than the non-overlapping pairs (sequence-by-inference interaction: ($F(1, 15) = 19.9$, $p < 0.001$). Thus, judgments that did not require transitive processing resulted in no increase in reaction time but TIs did (Figure 2).

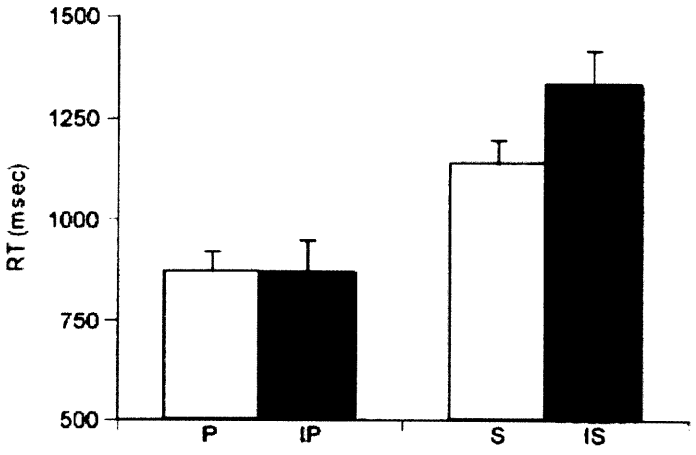

Figure 2: **Reaction times:** Mean (± SD) for the four conditions: non-overlapping pairs (P), novel non-overlapping pairs (IP), overlapping pairs (S), and novel overlapping pairs (IS) (Heckers et al., 2004a).

I will refer to this significant sequence-by-inference interaction as the *TI effect*. In this experiment, therefore, the TI effect denotes the contrast between the difference in making inferences on pairs from a sequence compared with individual pairs and the difference in recognition of the original pairs from the sequence and recognition of the original non-overlapping pairs (i.e., a difference of differences).

fMRI data

TI

We investigated the *TI effect* by testing which voxels showed a significant sequence-by-inference interaction (contrast: [IS > IP] > [S > P]). This analysis revealed significant right anterior hippocampal activation during TI (Table 1 and Figure 3). Activation of the same right anterior hippocampal region during TI was confirmed in the simple comparison between transitive and non-transitive inferences [IS>IP] and the comparison between TI and judgments on overlapping items [IS>S] (Table 1).

Table 1: Hippocampal activation during TI. Letters in square brackets refer to the four conditions as indicated in Figure 1. Coordinates are given in mm and refer to the MNI305 stereotactic space, an approximation of Talairach space (Talairach and Tournoux, 1988).

Effect	Brain region	Z score	MNI Talairach (x, y, z)
[IS>IP]>[S>P]	R Anterior hippocampus	4.44	34, -14, -16
[IS]>[IP]	R Anterior hippocampus	4.22	36. -6. -22
		4.13	34, -10, -16
[IS]>[S]	R Anterior hippocampus	3.89	34, -4, -14

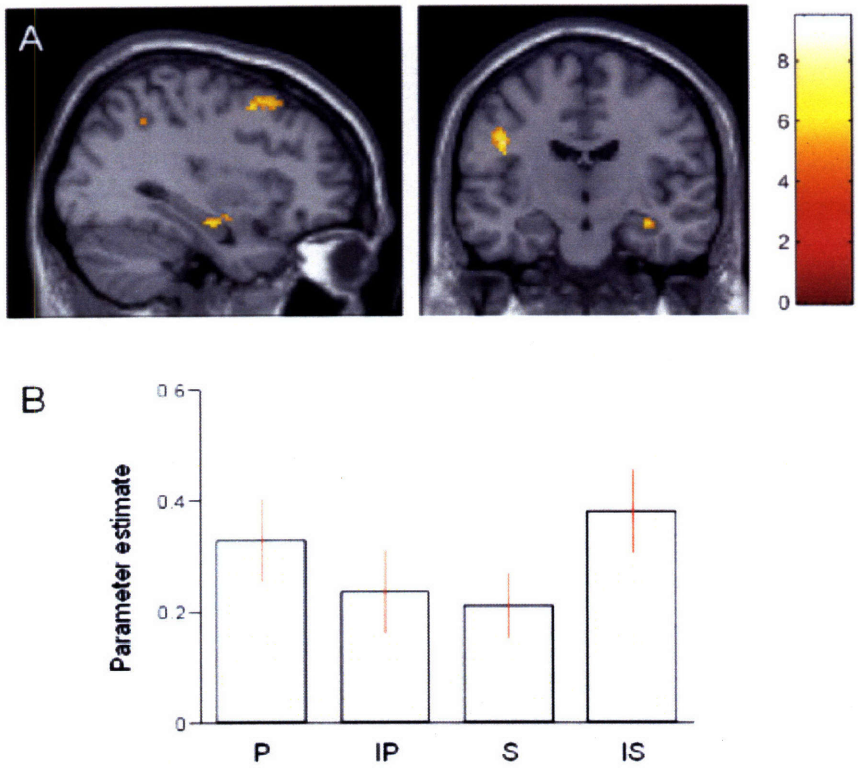

Figure 3: **Right anterior hippocampal activation during TI.** A: Areas of significant activity in 16 participants ($p < 0.0001$, uncorrected) are mapped onto a template structural MRI of a single participant. The bar indicates the t values of activated voxels. B: Parameter estimates (± standard error) for the magnitude of the haemodynamic response (relative to a baseline fixation condition) in right anterior hippocampus for the four experimental conditions (i.e., previously seen (P) and novel (IP) non-overlapping pairs and previously seen (S) and novel (IS) overlapping pairs). The parameter estimates are collapsed across sessions within participants and are averaged across participants (Heckers et al., 2004a).

We explored the neural circuitry underlying TI by identifying all voxels with a significant sequence-by-inference interaction. A distributed network of brain regions, including the pre-supplementary motor area (pre-SMA), bilateral frontal cortex, bilateral parietal cortex, bilateral posterior temporal cortex, and pulvinar showed significant activation associated with TI (Table 2 and Figure 4).

Table 2: TI network (Heckers et al., 2004a). Coordinates are given in mm and refer to the MNI305 stereotactic space, an approximation of Talairach space (Talairach and Tournoux, 1988). H denotes hemisphere.

Brain region	H	z score	MNI Talairach coordinates (x, y, z)
Pre-SMA	R	5.32	8, 18, 50
Inferior temporal gyrus (BA 37)	L	5.14	-44, -52, -6
	R	4.80	54, -50, -12
Middle temporal gyrus (BA 21)	L	5.04	-58, -30, -8
Premotor cortex (BA 6)	L	4.99	-32, 2, 62
	R	4.98	30, 10, 50
Inferior frontal gyrus (BA 47)	L	4.95	-44, 36, -8
	L	4.42	-30, 20, -12
Inferior parietal cortex (BA 40)	L	4.87	-42, -54, 46
	R	4.72	46, -50, 42
Anterior cingulate cortex (BA 24)	L	4.79	-4, 6, 26
Pulvinar	L	4.44	-8, -24, 10
Hippocampus	R	4.44	34, -14, -16

To disambiguate the two effects that contributed to the TI effect in these brain regions, we studied the main effects of inference (novel pairs > previously learned pairs) and sequence (overlapping pairs > non-overlapping pairs). We found significant main effects of inference and sequence in pre-SMA (peak activation at coordinates –4, -20, -52; $\underline{z}$ = 5.64 and 2, 12, 56; $\underline{z}$ = 4.41 respectively), left prefrontal cortex (–52, 18, 34; $\underline{z}$ = 5.76 and –46, 28, 32; $\underline{z}$ = 4.58 respectively), and left parietal cortex (–46, -54, 50; $\underline{z}$ = 4.86 and –38, -52, 58; $\underline{z}$ = 5.47 respectively). In addition, a main effect of sequence was observed in right prefrontal cortex (46, 4, 54; $\underline{z}$ = 4.33), right parietal cortex (38, -48, 44; $\underline{z}$ = 5.16), and bilateral temporal cortex (-20, -58, -12; $\underline{z}$ = 5.22 and 26, -52, -20; $\underline{z}$ = 4.87). In contrast, neither of these two main effects was present in the hippocampus.

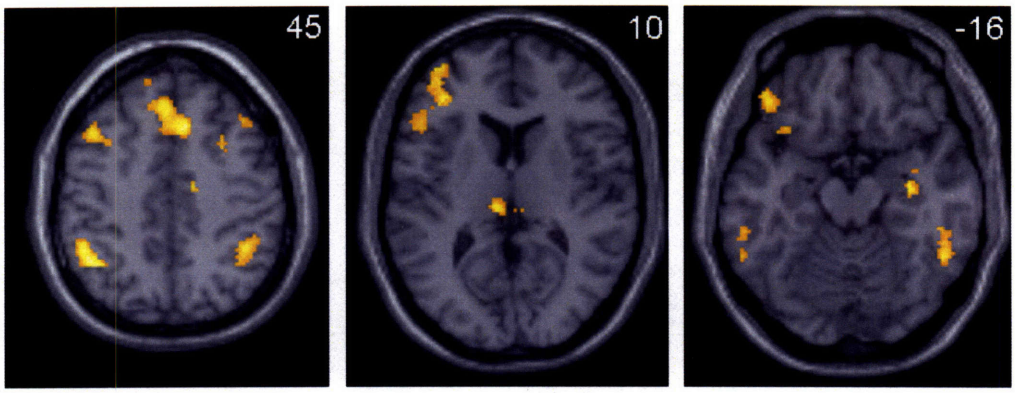

Figure 4: **TI network.** Horizontal slices through a template structural MRI of a single participant display areas of significant activity in 16 participants ($p < 0.0001$, uncorrected) in bilateral frontal cortex, pre-SMA, and bilateral parietal cortex (at 45 mm above the AC-PC axis), left prefrontal cortex, pulvinar (+10 mm), left inferior frontal cortex, bilateral inferior temporal cortex, and right hippocampus (-10 mm) (Heckers et al., 2004a).

Discussion

In this experiment, we demonstrate a role for the hippocampus in TI in humans. We also show the recruitment of a network of extra-hippocampal brain regions. In the discussion that follows, I will consider the respective roles of these different brain regions.

Role of the hippocampus in TI

Our results indicate that the hippocampus is part of the neural circuitry underlying TI for arbitrary visual stimulus patterns in humans, similarly to its role in animals that has been previously demonstrated (Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003; Buckmaster et al., 2004).

Rodents and monkeys trained to discriminate the overlapping stimulus set A>B>C>D>E cannot correctly discriminate the novel stimulus pair BD after hippocampal lesion, but

are still able to correctly discriminate the novel pairing of the two end items A and E (Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003; Buckmaster et al., 2004). Our study design differs from the animal studies in that we employed two sets of stimuli: a set of overlapping pairs that constituted the ordered sequence and a set of non-overlapping individual pairs. We then compared TIs on the ordered sequence with non-TIs on the individual pairs. The latter were equivalent to the AE pair, which did not require TI in the animal studies. In the contrast between TI and non-TI, analogous to the contrast between the novel BD and AE trials in animals, we demonstrated hippocampal activation in humans.

The only previous study that investigated hippocampal function in TI in humans reported hippocampal activation during learning of overlapping pairs rather than during TI (Nagode and Pardo, 2002). Previous fMRI studies of relational memory in humans have demonstrated hippocampal activation during encoding (Rombouts et al., 1997; Sperling et al., 2001; Davachi and Wagner, 2002) and recognition (Stark and Squire, 2001) of inter-item associations. Our results extend these reports by showing that relation judgments that require a flexible representation of a hierarchically ordered set of stimuli are associated with significantly greater hippocampal activation compared to a simple association between two items. This result provides support for a relational memory account of hippocampal function. Under this account, the hippocampus supports all declarative memory, but is especially crucial for its flexible representation and manipulation (Eichenbaum, 2004).

One limitation of the present study related to the block-design, is that novel pairs requiring different degrees of TI were grouped together. Specifically, novel pairs that included an end-item and could thus be solved by referring to the end-item (the four pairs AC, AD, BE and CE) and the novel pair BD that is devoid of end-items and therefore required full manipulation of the sequence, were examined together. If the relational memory account of hippocampal function that we subscribe to applies, one would expect that non end-item TI would engage the hippocampus to a greater degree than end-item TI. Further, our sequence contained only five items (A through E), with only one hard inference trial, BD, available for analysis. We were, therefore, unable to investigate the neural correlates of the symbolic distance effect, which refers to the greater ease of decision on hard inference pairs the further apart the two items in the pair are on the hierarchical continuum. This issue could be investigated by separately analyzing the inference pairs BD and BE if one used a six-item (A-F) ordered sequence. These limitations will be addressed in Experiment 2 in the next chapter.

Role of the extra-hippocampal network in TI

Besides investigating the specific role of the hippocampus in TIs and verifying its similarity to its role in animals, we also set out to identify other brain regions in this process. Previously, a distributed network including prefrontal cortex, cingulate cortex, pre-supplementary and supplementary motor areas, parietal cortex, thalamus, and ventral striatum was implicated in TI (Acuna et al., 2002b). The results from this experiment confirm the previous findings and suggest a distributed but specific network of brain

regions that underlies TI in humans. We found significant activation associated with TI in the pre-supplementary motor area (Brodmann area 6) and bilateral parietal, prefrontal, and inferior temporal cortices. This pattern is similar to that described by Acuna (Acuna et al., 2002b), who trained healthy control participants on an hierarchically ordered set of 11 different unicolored shapes. The authors report significant brain activation in bilateral prefrontal cortex, pre-supplementary motor area, premotor area, insula, precuneus, and lateral posterior parietal cortex during the recognition of novel pairs of visual stimulus items. The slice selection used by Acuna et al did not cover the medial temporal lobe. Other recent studies of TI using verbal premise pairs (Goel and Dolan, 2001) or iconic stimuli (Dickins et al., 2001) also reported significant prefrontal and parietal cortex activation. Taken together, studies in humans have established an important role of frontal and parietal multimodal association cortices, both of which are closely connected with the hippocampus via the entorhinal cortex, for TI.

The main effects analysis revealed that pre-SMA, left parietal cortex, and left prefrontal cortex contributed not only to the TI effect, but to all trials that required inferences (transitive as well as non-transitive) and overlapping pairs (previously seen as well as novel). Further, right parietal, right prefrontal, and bilateral temporal cortex activation was greater for all overlapping pairs. In contrast, right hippocampal activation was not seen in the main effects analyses, but only in the interaction and the simple effects of TI. This indicates that the hippocampus contributes uniquely to the ability to infer relationships between a sequence of items, while pre-SMA, parietal, and prefrontal cortex

contribute more broadly to inferential judgments and the recognition of overlapping pairs of visual stimuli. The specific roles of these brain areas will be discussed further in Chapters 2 and 4.

References

Acuna BD, Sanes JN, Donoghue JP (2002a) Cognitive mechanisms of transitive inference. Experimental Brain Research 146:1-10.

Acuna BD, Eliassen JC, Donoghue JP, Sanes JN (2002b) Frontal and parietal lobe activation during transitive inference in humans. Cerebral Cortex 12:1312-1321.

Aizenstein HJ, Stenger VA, Cochran J, Clark K, Johnson M, Nebes RD, Carter CS (2004) Regional Brain Activation during Concurrent Implicit and Explicit Sequence Learning. Cerebral Cortex 14:199-208.

Aleman A, Hijman R, de Haan EHF, Kahn RS (1999) Memory impairment in schizophrenia: a meta-analysis. Am J Psychiatry 156:1358-1366.

Allin M, Murray R (2002) Schizophrenia: a neurodevelopmental or neurodegenerative disorder? Current Opinion in Psychiatry 15:9-15.

Alvarez-Jaimes L, Betancourt E, Centeno-Gonzalez M, Feliciano-Rivera MZ, Rodriguez D, Pena de Ortiz S, Maldonado-Vlaar CS (2004) Spatial learning in rats is impaired by microinfusions of protein kinase C-γ antisense oligodeoxynucleotide within the nucleus accumbens. Neurobiology of Learning & Memory 81:120-136.

Ansari D, Garcia N, Lucas E, Hamon K, Dhital B (2005) Neural correlates of symbolic number processing in children and adults. Neuroreport 16:1769-1773.

Antonova E, Sharma T, Morris R, Kumari V (2004) The relationship between brain structure and neurocognition in schizophrenia: a selective review. Schizophrenia Res 70:117-145.

Arnold SE (2000) Cellular and Molecular Neuropathology of the Parahippocampal Region in Schizophrenia. Annals New York Academy of Sciences:275-292.

Aron AR, Shohamy D, Clark J, Myers C, Gluck MA, Poldrack RA (2004) Human midbrain sensitivity to cognitive feedback and uncertainty during classification learning. Journal of Neurophysiology 92:1144-1152.

Ashburner J, Friston K (1997) Multimodal Image Coregistration and Partitioning - A Unified Framework. Neuroimage 6:209-217.

Atallah HE, Frank MJ, O'Reilly RC (2004) Hippocampus, cortex, and basal ganglia: insights from computational models of complementary learning systems. Neurobiology of Learning & Memory 82:253-267.

Ban TA (2004) Neuropsychopharmacology and the genetics of schizophrenia. A history of the diagnosis of schizophrenia. Progress in Neuro-Psychopharmacology & Biological Psychiatry 28:753-762.

Benes FM (1995) Altered glutamatergic and GABAergic mechanisms in the cingulate cortex of the schizophrenic brain. Arch Gen Psychiatry 52:1015-1018; discussion 1019-1024.

Berns GS, McClure SM, Pagnoni G, Montague PR (2001) Predictability Modulates Human Brain Response to Reward. The Journal of Neuroscience 21:2793-2798.

Bilder R (2001) Schizophrenia as a neurodevelopmental disorder. Current Opinion in Psychiatry 14:9-15.

Bischoff-Grethe A, Goedert KM, Willingham DT, Grafton ST (2004) Neural Substrates of Response-based Sequence Learning using fMRI. Journal of Cognitive Neuroscience 16:127-138.

Blair J, Spreen O (1989) Predicting premorbid IQ: a revision of the national adult reading test. The Clinical Neuropsychologist 3:129-136.

Boecker H, Dagher A, Ceballos-Baumann AO, Passingham RE, Samuel M, Friston KJ, Poline J-B, Dettmers C, Conrad B, Brooks DJ (1998) Role of the Human Rostral Supplementary Motor Area and the Basal Ganglia in Motor Sequence Control: Investigations With H_2O^{15} PET. Journal of Neurophysiology 79:1070-1080.

Bogerts B (1999) The neuropathology of schizophrenic diseases: historical aspects and present knowledge. Eur Arch Psychiatry Clin Neurosci:IV/2-IV/13.

Bond AB, Kamil AC, Balda RP (2003) Social complexity and transitive inference in corvids. Animal Behaviour 65:479-487.

Bor D, Cumming N, Scott CEL, Owen AM (2004) Prefrontal cortical involvement in verbal encoding strategies. European Journal of Neuroscience 19:3365-3370.

Brebion G, Gorman JM, Malaspina D, Sharif Z, Amador XF (2001) Clinical and Cogntive Factors Associated With Verbal Memory Task Performance in Patients with Schizophrenia. Am J Psychiatry 158:758-764.

Brown MW, Aggleton JP (2001) Recognition memory: what are the roles of the perirhinal cortex and hippocampus? Nature Reviews Neuroscience 2.

Buckmaster CA, Eichenbaum H, Amaral DG, Suzuki WA, Rapp PR (2004) Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. The Journal of Neuroscience 24:9811-9825.

Buckner RL, Wheeler ME (2001) The cognitive neuroscience of remembering. Nature Reviews Neuroscience 2:624-634.

Burgess N, Maguire EA, O'Keefe J (2002) The human hippocampus and spatial and episodic memory. Neuron 35:625-641.

Cahn W, Hulshoff Pol H, Lems EBTE, Van Haren NEM, Schnack HG, Van der Linden JA, Schothorst PF, van Engeland H, Kahn RS (2002) Brain Volume Changes in First-Episode Schizophrenia. Arch Gen Psychiatry 59:1002-1010.

Casey BJ, Thomas KM, Davidson MC, Kunz K, Franzen PL (2002) Dissociating striatal and hippocampal function developmentally with a stimulus-response compatibility task. The Journal of Neuroscience 22:8647-8652.

Chen N-K, Dickey CC, Yoo SS, Guttmann CRG, Panych LP (2003) Selection of voxel size and slice orientation for fMRI in the presence of susceptibility field gradients: application to imagin of amygdala. Neuroimage 19:817-825.

Christoff K, Prabhakaran V, Dorfman J, Zhao Z, Kroger JK, Holyoak KJ, Gabrieli JD (2001) Rostrolateral Prefrontal Cortex Involvement in Relational Integration during Reasoning. Neuroimage 14:1136-1149.

Cirillo MA, Seidman LJ (2003) Verbal Declarative Memory Dysfunction in Schizophrenia: From Clinical Assessment to Genetics and Brain Mechanisms. Neuropsychology Review 13:43-77.

Clark D, Dedova I, Cordwell S, Matsumoto I (2006) A proteome analysis of the anterior cingulate cortex gray matter in schizophrenia. Molecular Psychiatry 11:459-470.

Cohen NJ, Eichenbaum H (1993) Memory, amnesia, and the hippocampal system. Cambridge, MA: MIT Press.

Colombo M, Frost N (2001) Representation of serial order in humans: a comparison to the findings with monkeys (*Cebus apella*). Psychonomic Bulletin & Review 8:262-269.

Cordes D, Turski PA, Sorenson JA (2000) Compensation of susceptibility-induced signal loss in echo-planar imaging for functional applications. Magnetic Resonance Imaging 18:1055-1068.
Corkin S (2002) What's new with the amnesic patient H.M.? Nature Reviews Neuroscience 3:153-160.
Cullen TJ, Walker MA, Parkinson N, Craven R, Crow TJ, Esiri MM, Harrison PJ (2003) A postmortem study of the mediodorsal nucleus of the thalamus in schizophrenia. Schizophrenia Res 60:157-166.
Cusack RB, Osswald K (2003) An evaluation of the use of magnetic field maps to undistort echo-planar images. Neuroimage 18:127-142.
Danion J-M, Meulemans T, Kauffmann-Muller F, Vermaat H (2001) Intact Implicit Learning in Schizophrenia. Am J Psychiatry 158:944-948.
Daselaar SM, Rombouts SARB, Veltman DJ, Raaijmakers JGW, Jonker C (2003) Similar network activated by young and old adults during the acquisition of a motor sequence. Neurobiology of Aging 24:1013-1019.
Davachi L, Wagner AD (2002) Hippocampal contributions to episodic encoding: insights from relational and item-based learning. Journal of Neurophysiology 88:982-990.
Davidson M, Keefe RS (1995) Cognitive impairment as a target for pharmacological treatment in schizophrenia. Schizophr Res 17:123-129.
Davis H (1992) Transitive inference in rats (*Rattus norvegicus*). Journal of Comparative Psychology 106:342-349.
Deichmann R, Josephs O, Hutton C, Corfield DR, Turner R (2002) Compensation of Susceptibility-Induced BOLD Sensitivity Losses in Echo-Planar fMRI Imaging. Neuroimage 15:120-135.
Delius JD, Siemann M (1998) Transitive responding in animals and humans: Exaptation rather than adaptation. Behavioural Processes 42:107-137.
DeWitt I, Weiss AP, Deckersbach T, Kunkel L, Goff D, Heckers S (2002) Assessing hippocampal volume in schizophrenia using a standardized MRI protocol. Biol Psychiatry 51:21S.
Dickins DW, Singh KD, Roberts N, Burns P, Downes JJ, Jimmieson P, Bentall RP (2001) An fMRI study of stimulus equivalence. Neuroreport 12:405-411.
Dusek JA, Eichenbaum H (1997) The hippocampus and memory for orderly stimulus relations. Proceedings of the National Academy of Sciences of the United States of America 94:7109-7114.
Eastwood SL, Harrison PJ (2005) Interstitial white matter neuron density in the dorsolateral prefrontal cortex and parahippocampal gyrus in schizophrenia. Schizophrenia Res 79:181-188.
Egeland J, Sundet K, Rund BR, Asbjornsen A, Hugdahl K, Landro NI, Lund A, Roness A, Stordal KI (2003) Sensitivity and Specificity of Memory Dysfunction in Schizophrenia: A Comparison with Major Depression. Journal of Clinical & Experimental Neuropsychology 25:79-93.
Eichenbaum H (1992) The hippocampus and declarative memory in animals. Journal of Cognitive Neuroscience 4:217-231.
Eichenbaum H (2000) A cortical-hippocampal system for declarative memory. Nature Reviews Neuroscience 1:41-50.

Eichenbaum H (2004) Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 44:109-120.
Eichenbaum H, Cohen NJ (2001) From conditioning to conscious recollection. Memory systems of the brain. Oxford: Oxford University Press.
Eichenbaum H, Otto T, Cohen NJ (1994) Two functional components of the hippocampal memory system. Brain and Behavioral Sciences 17:449-518.
Emilien G, Durlach C, Antoniadis E, Van Der Linden M, Maloteaux J-M (2004) Memory: neuropsychological, imaging and psychopharmacological perspectives. Hove and New York: Psychology Press.
Exner C, Weniger G, Schmidt-Samoa C, Irle E (2006) Reduced size of the pre-supplementary motor cortex and impaired motor sequence learning in first-episode schizophrenia. Schizophrenia Res.
Fannon D, Chinthis X, Doku VC, Tennakoon L, O'Ceallaigh S, Soni W, Sumich A, Lowe J, Santamaria M, Sharma T (2000) Features of Structural Brain Abnormality Detected in First-Episode Psychosis. Am J Psychiatry 157:1829-1834.
Faraone SV, Seidman LJ, Kremen WS, Kennedy D, Makris N, Caviness V, Goldstein J, Tsuang MT (2003) Structural brain abnormalities among relatives of patients with schizophrenia: implications for linkage studies. Schizophrenia Res 60:125-140.
Fias W, Lammertyn J, Reynvoet B, Dupont P, Orban GA (2003) Parietal Representation of Symbolic and Nonsymbolic Magnitude. Journal of Cognitive Neuroscience 15:47-56.
Fletcher PC, Zafiris O, Frith CD, Honey RAE, Corlett PR, Zilles K, Fink GR (2005) On the Benefits of not Trying: Brain Activity and Connectivity Reflecting the Interactions of Explicit and Implicit Sequence Learning. Cerebral Cortex 15:1002-1015.
Florencio PS, O'Driscoll GA (1999) The Medial Temporal Lobe and Schizophrenia. McGill Journal of Medicine 5:25-34.
Floresco SB, Blaha CD, Yang CR, Philips AG (2001) Modulation of hippocampal and amygdala-evoked activity of nucleus accumbens neurons by dopamine: cellular mechanisms of input selection. The Journal of Neuroscience 21:2851-2860.
Fortin NJ, Agster KL, Eichenbaum HB (2002) Critical role of the hippocampus in memory for sequences of events. Nature Neuroscience 5:458-462.
Frank MJ, Rudy JW, O'Reilly RC (2003) Transitivity, flexibility, conjunctive representations, and the hippocampus. II. A computational analysis. Hippocampus 13:299-312.
Frank MJ, Seeberger LC, O'Reilly RC (2004) By carrot or by stick: cognitive reinforcement learning in Parkinsonism. Science 306:1940-1943.
Frank MJ, Rudy JW, Levy WB, O'Reilly RC When logic fails: implicit transitive inference in humans. Memory & Cognition:In press.
Frank MJ, Rudy JW, Levy WB, O'Reilly RC (2005) When logic fails: Implicit transitive inference in humans. Memory & Cognition 33:742-750.
Geuze E, Vermetten E, Bremner AJ (2005) MR-based in vivo hippocampal volumetrics: 1. Review of methodologies currently employed. Molecular Psychiatry 10:147-159.

Gobel SM, Johansen-Berg H, Behrens T, Rushworth MF (2004) Response-Selection-Related Parietal Activation during Number Comparison. Journal of Cognitive Neuroscience 16:1536-1551.
Goel V, Dolan RJ (2001) Functional neuroanatomy of three-term relational reasoning. Neuropsychologia 39:901-909.
Goff DC, Heckers S, Freudenreich O (2001) Schizophrenia. In: The Medical Clinics of North America. Advances in the pathophysiology and treatment of psychiatric disorders: Implications for internal medicine (Nemeroff CB, ed), pp 663-689. Philadelphia: W.B. Saunders.
Gold JM, Weinberger DR (1995) Cognitive deficits and the neurobiology of schizophrenia. Curr Opin Neurobiol 5:225-230.
Gold JM, Randolph C, Carpenter CJ, Goldberg TE, Weinberger DR (1992) Forms of memory failure in schizophrenia. J Abnorm Psychol 101:487-494.
Gorno-Tempini ML, Hutton C, Josephs O, Deichmann R, Price C, Turner R (2002) Echo Time Dependence of BOLD Contrast and Susceptibility Artifacts. Neuroimage 15:136-142.
Gottesman II, Gould TD (2003) The Endophenotype Concept in Psychiatry: Etymology and Strategic Intentions. Am J Psychiatry 160:636-645.
Greene AJ, Spellman BA, Dusek JA, Eichenbaum HB, Levy WB (2001) Relational learning with and without awareness: transitive inference using nonverbal stimuli in humans. Memory & Cognition 29:893-902.
Greicius MD, Krasnow B, Boyett-Anderson JM, Eliez S, Schatzberg AF, Reiss AL, Menon V (2003) Regional analysis of hippocampal activation during memory encoding and retrieval: fMRI study. Hippocampus 13:164-174.
Hallmayer JF, Kalaydjieva L, Badcock J, Dragovic M, Howell S, Michie PT, Rock D, Vile D, Williams R, Corder EH, Hollingsworth K, Jablensky A (2005) Genetic Evidence for a Distinct Subtype of Schizophrenia Characterized by Pervasive Cognitive Deficit. American Journal of Human Genetics 77:468-476.
Harrison PJ (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122:593-624.
Harrison PJ (2004) The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. Psychopharmacology 174:151-162.
Harrison PJ, Roberts GW, eds (2000) The neuropathology of schizophrenia, 1. Edition. Oxford: Oxford University Press.
Harrison PJ, Owen MJ (2003) Genes for schizophrenia? Recent findings and their pathophysiological implications. Lancet 361:417-419.
Harrison PJ, Weinberger DR (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Molecular Psychiatry 10:40-68.
Heckers S (2001) Neuroimaging studies of the hippocampus in schizophrenia. Hippocampus 11:520-528.
Heckers S, Konradi C (2002) Hippocampal neurons in schizophrenia. Journal of Neural Transmission 109:891-905.
Heckers S, Zalesak M, Weiss AP, Ditman T, Titone D (2004a) Hippocampal activation during transitive inference in humans. Hippocampus 14:153-162.

Heckers S, Weiss AP, Deckersbach T, Goff D, Morecraft RJ, Bush G (2004b) Anterior cingulate cortex activation during cognitive interference in schizophrenia. Am J Psychiatry 161:707-715.

Heckers S, Rauch SL, Goff D, Savage CR, Schacter DL, Fischman AJ, Alpert NM (1998) Impaired recruitment of the hippocampus during conscious recollection in schizophrenia. Nature Neuroscience 1:318-323.

Heinrichs RW (2004) Meta-analysis and the science of schizophrenia: variant evidence or evidence of variants. Neuroscience and Biobehavioral Reviews 28:379-394.

Heinrichs RW (2005) The Primacy of Cognition in Schizophrenia. American Psychologist 60:229-242.

Hennig J, Speck O, Koch M, Weiller C (2003) Functional Magnetic Resonance Imaging: A Review of Methodological Aspects and Clinical Applications. Journal of Magnetic Resonance Imaging 18:1-15.

Heun R, Freymann N, Granath DO, Stracke CP, Jessen F, Barkow K, Reul J (2004) Differences of cerebral activation between superior and inferior learners during motor sequence encoding and retrieval. Psychiatry Research: Neuroimaging 132:19-32.

Hoff AL, Kremen WS (2002) Is there a cognitive phenotype for schizophrenia: the nature and course of the disturbance in cognition. Current Opinion in Psychiatry 15:43-48.

Hoff AL, Svetina C, Maurizio AM, Crow TJ, Spokes K, DeLisi LE (2005) Familial Cognitive Deficits in Schizophrenia. American Journal of Medical Genetics Part B 133B:43-49.

Honea R, Crow TJ, Passingham D, Mackay CE (2005) Regional Deficits in Brain Volume in Schizophrenia: A Meta-Analysis of Voxel-Based Morphometry Studies. Am J Psychiatry 162:2233-2245.

Hubbard EM, Piazza M, Pinel P, Dehaene S (2005) Interactions Between Number and Space in Parietal Cortex. Nature Reviews Neuroscience 6:435-448.

Huettel SA, Song AW, McCarthy G (2004) Functional magnetic resonance imaging, 1st Edition. Sunderland, MA, USA: Sinauer Associates, Inc.

Huettel SA, Song AW, McCarthy G (2005) Decisions under Uncertainty: Probabilistic Context Influences Activation of Prefrontal and Parietal Cortices. The Journal of Neuroscience 25:3304-3311.

Hutton C, Bork A, Josephs O, Deichmann R, Ashburner J, Turner RT (2002) Image distortion correction in fMRI: a quantiative evaluation. Neuroimage 16:217-240.

Innocenti GM, Ansermet F, Parnas J (2003) Schizophrenia, neurodevelopment and corpus callosum. Molecular Psychiatry 8:261-274.

Jablensky A (2000) Epidemiology of schizophrenia: the global burden of disease and disability. Eur Arch Psychiatry Clin Neurosci 250:274-285.

Jancke L, Himmelbach M, Shah NJ, Zilles K (2000) The Effect of Switching between Sequential and Repetitive Movements on Cortical Activation. Neuroimage 12:528-537.

Jezzard P, Clare S (1999) Sources of distortion in functional MRI data. Human Brain Mapping 8:80-85.

Job DE, Whalley HC, McConnell S, Glabus M, Johnstone EC, Lawrie SM (2002) Structural Gray Matter Differences between First-Episode Schizophrenics and Normal Controls Using Voxel-Based Morphometry. Neuroimage 17:880-889.

Kadosh RC, Henik A, Rubinstein O, Mohr H, Dori H, van de Ven V, Zorzi M, Hendler T, Goebel R, Linden DEJ (2005) Are numbers special? The comparison systems of the human brain investigated by fMRI. Neuropsychologia 43:1238-1248.

Keefe RSE, Eesley CE, Poe MP (2005) Defining a Cognitive Function Decrement in Schizophrenia. Biol Psychiatry 57:688-691.

Kelley AE (2004) Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 44:161-179.

Kerns JG, Cohen JD, MacDonald AW, 3rd, Johnson MK, Stenger VA, Aizenstein HJ, Carter CS (2005) Decreased Conflict- and Error-Related Activity in the Anterior Cingulate Cortex in Subjects with Schizophrenia. Am J Psychiatry 162:1833-1839.

Kraepelin E (1896) Psychiatrie. Ein Lehrbuch für Studirende und Aerzte. Fünfte, vollständig umgearbeitete Auflage., 5. vollständig umgearbeitete Auflage Edition. Leipzig: Johann Ambrosius Barth.

Krasnow B, Tamm L, Greicius MD, Yang TT, Glover GH, Reiss AL, Menon V (2003) Comparison of fMRI activation at 3 and 1.5 T during perceptual, cognitive, and affective processing. Neuroimage 18:813-826.

Kremen WS, Seidman LJ, Faraone SV, Tsuang MT (2001) Intelligence Quotient and Neuropsychological Profiles in Patients with Schizophrenia and in Normal Volunteers. Biol Psychiatry 50:453-462.

Kroger JK, Sabb FW, Fales CL, Bookheimer SY, Cohen MS, Holyoak KJ (2002) Recruitment of Anterior Dorsolateral Prefrontal Cortex in Human Reasoning: a Parametric Study of Relational Complexity. Cerebral Cortex 12:477-485.

Kruger G, Kastrup A, Glover GH (2001) Neuroimaging at 1.5T and 3.0T: comparison of oxygenation-sensitive magnetic resonance imaging. Magnetic Resonance in Medicine 45:495-604.

Kumaran D, Maguire EA (2006) The Dynamics of Hippocampal Activation during Encoding of Overlapping Sequences. Neuron 49:617-629.

Kuperberg G, Heckers S (2000) Schizophrenia and cognitive function. Current Opinion in Neurobiology 10:205-210.

Kuperberg GR, Broome MR, McGuire PK, David AS, Eddy M, Ozawa F, Goff D, West WC, Williams SCR, van der Kouwe AJW, Salat DH, Dale AM, Fischl B (2003) Regionally Localized Thinning of the Cerebral Cortex in Schizophrenia. Arch Gen Psychiatry 60:878-888.

Kurachi M (2003) Pathogenesis of schizophrenia: Part I. Symptomatology, cognitive characteristics and brain morphology. Psychiatry and Clinical Neurosciences 57:3-8.

Lawrie SM, Whalley HC, Job DE, Johnstone EC (2003) Structural and Functional Abnormalities of the Amygdala in Schizophrenia. Annals New York Academy of Sciences 985:445-460.

Lewis DA, Lieberman JA (2000) Catching up on schizophrenia: natural history and neurobiology. Neuron 28:325-334.

Lewis DA, Levitt P (2002) Schizophrenia As A Disorder of Neurodevelopment. Annu Rev Neurosci 25:409-432.
Lipschutz B, Friston KJ, Ashburner J, Turner R, Price CJ (2001) Assessing Study-Specific Regional Variations in fMRI Signal. Neuroimage 13:392-398.
Luo Q, Perry C, Peng D, Jin Z, Xu D, Ding G, Xu S (2003) The neural substrate of analogical reasoning: an fMRI study. Cognitive Brain Research 17:527-534.
Martin N, Alsop B (2004) Transitive inference and awareness in humans. Behavioral Processes 67:157-165.
McGonigle B, Chalmers M (1986) Representations and strategies during inference. In: Reasoning and discourse processes (Myers T, Brown K, McGonigle B, eds), pp 141-164. London: Academic Press.
McIntosh AM, Job DE, Moorhead WJ, Harrison LK, Forrester K, Lawrie SM, Johnstone EC (2004) Voxel-Based Morphometry of Patients with Schizophrenia or Bipolar Disorder and Their Unaffected Relatives. Biol Psychiatry 56:544-552.
Meeter M, Talamini LM, Murre JMJ (2002) A computational approach to memory deficits in schizophrenia. Neurocomputing 44:929-936.
Meredith GE, Totterdell S (1999) Microcircuits in nucleus accumbens' shell and core involved in cognition and reward. Psychobiology 27:165-186.
Miller MI, Beg MF, Ceritoglu C, Stark C (2005) Increasing the power of functional maps of the medial temporal lobe by using large deformation diffeomorphic metric mapping. Proc Natl Acad Sci U S A 102:9685-9690.
Mirenowicz J, Schultz W (1994) Importance of unpredictability for reward responses in primate dopamine neurons. Journal of Neurobiology 72:1024-1027.
Mitelman SA, Shihabuddin L, Brickman AM, Hazlett EA, Buchsbaum MS (2005) Volume of the cingulate and outcome in schizophrenia. Schizophrenia Res 72:91-108.
Miyamoto S, LaMantia AS, Duncan GE, Sullivan P, Gilmore JH, Lieberman JA (2003) Recent Advances in the Neurobiology of Schizophrenia. Molecular Interventions 3:27-39.
Moonen CTW, Bandettini PA, eds (2000) Functional MRI. Berlin: Springer-Verlag.
Muller BW, Sartory G, Bender S (2004) Neuropsychological Deficits and Concomitant Clinical Symptoms in Schizophrenia. European Psychologist 9:96-106.
Muller R-A, Kleinhans N, Pierce K, Kemmotsu N, Courchesne E (2002) Functional MRI of motor sequence acquisition: effects of learning stage and performance. Cognitive Brain Research 14:277-293.
Nagahama Y, Okada T, Katsumi Y, Hayashi T, Yamauchi H, Sawamoto N, Toma K, Nakamura K, Hanakawa T, Konishi J, Fukuyama H, Shibasaki H (1999) Transient Neural Activity in the Medial Superior Frontal Gyrus and Precuneus Time Locked with Attention Shift between Object Features. Neuroimage 10:193-199.
Nagode JC, Pardo JV (2002) Human hippocampal activation during transitive inference. Neuroreport 13:939-944.
Narr KL, van Erp TGM, Cannon TD, Woods RP, Thompson PM, Jang S, Blanton R, Poutanen VP, Huttunen M, Lonnqvist J, Standerksjold-Nordenstam CG, Kaprio J, Mazziotta J, Toga AW (2002) A Twin Study of Genetic Contributions to Hippocampal Morphology in Schizophrenia. Neurobiology of Disease 11:83-95.

Nuechterlein K, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK (2004) Identification of separable cognitive factors in schizophrenia. Schizophrenia Res 72:29-39.

Nyberg L, Eriksson J, Larsson A, Marklund P (2006) Learning by doing versus learning by thinking: An fMRI study of motor and mental training. Neuropsychologia 44:711-717.

O'Doherty JP (2004) Reward representations and reward-related learning in the human brain: insights from neuroimaging. Current Opinion in Neurobiology 14:769-776.

Ojeman JG, Akbudak E, Snyder AZ, McKinstry RC, Raichle ME, Conturo TE (1997) Anatomic localization and quantitative analysis of gradient refocused echo-planar fMRI susceptibility artifacts. Neuroimage 6:156-167.

Ongur D, Cullen TJ, Wolf DH, Rohan M, Barreira P, Zalesak M, Heckers S (2006) The Neural Basis of Relational Memory Deficits in Schizophrenia. Arch Gen Psychiatry 63:356-365.

O'Reilly RC, Rudy JW (2001) Conjunctive representations in learning and memory: principles of cortical and hippocampal function. Psychology Reviews 108:311-345.

Oshi K, Toma K, Bagarinao ET, Matsuo K, Nakai T, Chihara K, Fukuyama H (2005) Activation of the precuneus is related to reduced reaction time in serial reaction time tasks. Neuroscience Research 52:37-45.

Osterbauer RA, Wilson JL, Calvert GA, Jezzard P (2006) Physical and physiological consequences of passive intra-oral shimming. Neuroimage 29:245-253.

Owen MJ, Craddock N, O'Donovan MC (2005) Schizophrenia: genes at last? Trends in Genetics 21:518-525.

Pagnoni G, Zink CF, Montague PR, Berns GS (2002) Activity in human ventral striatum locked to errors of reward prediction. Nature Neuroscience 5:97-98.

Pantelis C, Yucel M, Wood SJ, Velakoulis D, Sun D, Berger G, Stuart GW, Yung A, Phillips L, McGorry PD (2005) Structural Brain Imaging Evidence for Multiple Pathological Processes at Different Stages of Brain Development in Schizophrenia. Schizophrenia Bull 31:672-696.

Paulus MP, Frank L, Brown GG, Braff DL (2003) Schizophrenia Subjects Show Intact Success-Related Neural Activation but Impaired Uncertainty Processing during Decision-Making. Neuropsychopharmacology 28:795-806.

Paulus MP, Hozack NE, Zauscher BE, Frank L, Grown GG, McDowell J, Braff DL (2002) Parietal dysfunction is associated with increased outcome-related decision making in schizophrenia patients. Biol Psychiatry 51:995-1004.

Pearlson GD (2000) Neurobiology of Schizophrenia. Ann Neurol 48:556-566.

Pearlson GD, Petty RG, Ross CA, Tien YA (1996) Schizophrenia: a disease of heteromodal association cortex? Neuropsychopharmacology 14:1-17.

Pelletier M, Achim AM, Montoya A, Lal S, Lepage M (2005) Cognitive and clinical moderators of recognition memory in schziophrenia: a meta-analysis. Schizophrenia Res 74:233-252.

Piaget J (1928) The Language and Thought of the Child. New York: Harcourt Brace.

Pinel P, Dehaene S, Riviere D, LeBihan D (2001) Modulation of parietal activation by semantic distance in a number comparison task. Neuroimage 14:1013-1026.

Pinel P, Piazza M, Le Bihan D, Dehaene S (2004) Distributed and Overlapping Cerebral Representations of Number, Size and Luminance during Comparative Judgments. Neuron 41:983-993.
Plum F (1972) Prospects for research on schizophrenia. 3. Neurophysiology. Neuropathological findings. Neurosci Res Program Bull 10:384-388.
Poldrack RA, Rodriguez P (2004) How do memory systems interact? Evidence from human classification learning. Neurobiology of Learning & Memory 82:324-332.
Popken GJ, Bunney Jr. W, Potkin SG, Jones EG (2000) Subnucleus-specific loss of neurons in medial thalamus of schizophrenics. Proc Natl Acad Sci U S A 97:9276-9280.
Powell HWR, Koepp MJ, Richardson MP, Symms MR, Thompson PJ, Duncan JS (2004) The Application of Functional MRI of Memory in Temporal Lobe Epilepsy: A Clinical Review. Epilepsia 45:855-863.
Preibisch C, Pilatus U, Bunke J, Hoogenraad F, Zanella F, Lanfermann H (2003) Functional MRI using sensitivity-encoded echo planar imaging (SENSE-EPI). Neuroimage 19:412-421.
Pruessner JC, Li LM, Serles W, Pruessner M, Collins DL, Kabani N, Lupien S, Evans AC (2000) Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. Cerebral Cortex 10:433-442.
Quintana J, Wong T, Ortiz-Portillo E, Marder S, Mazziotta JC (2004) Anterior cingulate dysfunction during choice anticipation in schizophrenia. Psychiatry Research: Neuroimaging 132:117-130.
Rapp PR (2004) Who's the fairest of them all? Role of the human hippocampus in the relational organization of memory. Hippocampus 14:141-142.
Rapp PR, Kansky MT, Eichenbaum H (1996) Learning and memory for hierarchical relationships in the monkey: effects of aging. Behavioral Neuroscience 110:887-897.
Redgrave P, Prescott TJ, Gurney K (1999) Is the short-latency dopamine response too short to signal reward error? Trends Neurosci 22:146-151.
Rhodes BJ, Bullock D, Verwey WB, Averbeck BB, Page MPA (2004) Learning and production of movement sequencs: Behavioral, neurophysiological, and modeling perspectives. Human Movement Science 23:699-746.
Ridderinkhof KR, van den Wildenberg WPM, Segalowitz SJ, Carter CS (2004) Neurocognitive mechanisms of cognitive control: The role of prefrontal cortex in action selection, response inhibition, performance monitoring, and reward-based learning. Brain and Cognition 56:129-140.
Rombouts SARB, Machielsen WCM, Witter MP, Barkhof F, Lindeboom J, Scheltens P (1997) Visual association encoding activates the medial temporal lobe: a functional magnetic resonance imaging study. Hippocampus 7:594-601.
Ross CA, Pearlson GD (1996) Schizophrenia, the heteromodal association neocortex and development: potential for a neurogenetic approach. Trends Neurosci 19:171-176.
Rushworth MF, Hadland KA, Paus T, Sipila PK (2002) Role of the human medial frontal cortex in task switching: a combined fMRI and TMS study. Journal of Neurophysiology 87:2577-2592.

Sakai K, Hikosaka O, Miyauchi S, Takino R, Sasaki Y, Putz B (1998) Transition of Brain Activation from Frontal to Parietal Areas in Visuomotor Sequence Learning. The Journal of Neuroscience 18:1827-1840.

Sandrini M, Rossini PM, Miniussi C (2004) The differential involvement of inferior parietal lobule in number comparison: a rTMS study. Neuropsychologia 42:1902-1909.

Sargolini F, Roullet P, Oliverio A, Mele A (1999) Effects of lesions to the glutamatergic afferents to the nucleus accumbens in the modulation of reactivity to spatial and non-spatial novelty in mice. Neuroscience 93:855-867.

Sawa A, Snyder SH (2002) Schizophrenia: Diverse Approaches to a Complex Disease. Science 296:692-695.

Schacter DL (1998) Memory and awareness. Science 280:59-60.

Schendan HE, Searl MM, Melrose RJ, Stern CE (2003) An fMRI Study of the Role of the Medial Temporal Lobe in Implicit and Explicit Sequence Learning. Neuron 37:1013-1025.

Schmidt CF, Degonda N, Luechinger R, Henke K, Boesiger P (2005) Sensitivity-encoded (SENSE) echo planar fMRI at 3T in the medial temporal lobe. Neuroimage 25:625-641.

Schultz W (1999) The reward signal of midbrain dopamine neurons. News Physiol Sci 14:249-255.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. Journal of Neurology, Neurosurgery & Psychiatry 20:11-21.

Seidman LJ, Stone WS, Jones R, Harrison RH, Mirsky AF (1998) Comparative effects of schizophrenia and temporal lobe epilepsy on memory. Journal of the International Neuropsychological Society 4:342-352.

Setlow B (1997) The nucleus accumbens and learning and memory. The Journal of Neuroscience Research 49:515-521.

Setlow B, Schoenbaum G, Gallagher M (2003) Neural encoding in ventral striatum during ofactory discrimination learning. Neuron 38:625-636.

Shenton ME, Dickey CC, Frumin M, McCarley RW (2001) A review of MRI findings in schizophrenia. Schizophrenia Research 49:1-52.

Shrout PE, Fleiss JL (1979) Intraclass correlations: uses in assessing rater reliability. Psychological Bulletin 86:420-428.

Sim K, DeWitt I, Ditman T, Zalesak M, Greenhouse I, Goff D, Weiss AP, Heckers S (2005) Hippocampal and Parahippocampal Volumes in Schizophrenia:A Structural MRI Study.

Simon O, Kherif F, Flandin G, Poline J-B, Riviere D, Mangin JF, Le Bihan D, Dehaene S (2004) Automatized clustering and functional geometry of human parietofrontal networks for language, space and number. Neuroimage 23:1192-1202.

Sperling RA, Bates JF, Cocchiarella AJ, Schacter DL, Rosen BR, Albert MS (2001) Encoding novel face-name associations: a functional MRI study. Human Brain Mapping 14:129-139.

Squire L (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychological Review 99:195-231.

Squire LR, Zola-Morgan S (1991) The medial temporal lobe memory system. Science 253:1380-1386.

Stark CE, Squire LR (2001) Simple and associative recognition memory in the hippocampal region. Learning & Memory 8:190-197.
Stocker T, Kellermann T, Schneider F, Habel U, Amunts K, Pieperhoff P, Zilles K, Shah NJ (2006) Dependence of amygdala activation on echo time: Results from olfactory fMRI experiments. Neuroimage 30:151-159.
Strange BA, Otten LJ, Josephs O, Rugg MD, Dolan RJ (2002) Dissociable human perirhinal, hippocampal, and parahippocampal roles during verbal encoding. Journal of Neuroscience 22:523-528.
Strasser R, Ehrlinger JM, Bingman VP (2004) Transitive Behavior in Hippocampal-Lesioned Pigeons. Brain, Behavior & Evolution 63:181-188.
Talairach J, Tournoux P (1988) Co-Planar Stereotaxic Atlas of the Human Brain. New York: Thieme Medical Publishers, Inc.
Tamminga CA, Holcomb HH (2005) Phenotype of schizophrenia: a review and formulation. Molecular Psychiatry 10:27-39.
Titone D, Warner JL, Levy DL, Holzman PS (2001) The hippocampal memory system in schizophrenia: evidence from an ordered transitivity task. Schizophrenia Res 49:S122-123.
Titone D, Ditman T, Holzman PS, Eichenbaum H, Levy DL (2004) Transitive inference in schizophrenia: impairments in relational memory organization. Schizophrenia Res 68 (2-3):235-247.
Todd CL, Grace AA (1999) Modulation of ventral tegmental area dopamine cell activity by the ventral subiculum and entorhinal cortex. Annals New York Academy of Sciences 877:688-690.
van der Graaf FHCE, de Jong BM, Maguire RP, Meiners LC, Leenders KL (2004) Cerebral activatio related to skills practice in a double serial reaction time task: striatal involvement in random-order sequence learning. Cognitive Brain Research 20:120-131.
Van Elzakker M, O'Reilly RC, Rudy JW (2003) Transitivity, flexibility, conjunctive representations, and the hippocampus. I. An empirical analysis. Hippocampus 13:334-340.
von Fersen L, Wynne CDL, Delius JD, Staddon JER (1991) Transitive inference formation in pigeons. Journal of Experimental Psychology: Animal Behavior Processes 17:334-341.
Ward HA, Riederer SJ, Jack CR (2002) Real-Time Autoshimming for Echo Planar Timecourse Imaging. Magnetic Resonance in Medicine 48:771-780.
Weiskopf N, Klose U, Birbaumer N, Mathiak K (2005) Single-shot compensation of image distortions and BOLD contrast optimization using multi-echo EPI for real-time fMRI. Neuroimage 24:1068-1079.
Weiss AP, DeWitt I, Goff D, Ditman T, Heckers S (2005) Anterior and posterior hippocampal volumes in schizophrenia. Schizophrenia Res 73:103-112.
Weiss AP, Zalesak M, DeWitt I, Goff D, Kunkel L, Heckers S (2004) Impaired hippocampal function during the detection of novel words in schizophrenia. Biol Psychiatry 55:668-675.
Weiss AP, Schacter DL, Goff DC, Rauch SL, Alpert NM, Fischman AJ, Heckers S (2003) Impaired hippocampal recruitment during normal modulation of memory performance in schizophrenia. Biol Psychiatry 53:48-55.

Wilk CM, Gold JM, McMahon RP, Humber K, Iannone VN, Buchanan RW (2005) No, It Is Not Possible to Be Schizophrenic Yet Neuropsychologically Normal. Neuropsychology 19:778-786.

Wilson JL (2002) Fast, Fully Automated Global and Local Magnetic Field Optimization for fMRI of the Human Brain. Neuroimage 17:967-976.

Wilson JL, Jenkinson M, Jezzard P (2002) Optimization of Static Field Homogeneity in Human Brain Using Diamagnetic Passive Shims. Magnetic Resonance in Medicine 48:906-914.

Windischberger C, Robinson S, Rauscher A, Barth M, Moser E (2004) Robust Field Map Generation Using a Triple-Echo Acquisition. Journal of Magnetic Resonance Imaging 20:730-734.

Wong AHC, Van Tol HHM (2003) Schizophrenia: from phenomenology to neurobiology. Neuroscience and Biobehavioral Reviews 27:269-306.

Wynne CDL (1998) A minimal model of transitive inference. In: Models of action (Wynne CDL, Staddon JER, eds), pp 269-307. Mahwah, N.J.: Lawrence Erlbaum Associates.

Yucel M, Pantelis C, Stuart GW, Wood SJ, Maruff P, Velakoulis D, Pipingas A, Crowe SF, Tochon-Danguy HJ, Egan GF (2002) Anterior cingulate activation during Stroop task performance: a PET to MRI coregistration study of individual patients with schizophrenia. Am J Psychiatry 159:251-254.

Zeineh MM, Engel SA, Bookheimer SY (2000) Application of cortical unfolding techniques to functional MRI of the human hippocampal region. NeuroImage 11:668-683.

Zeng H, Constable RT (2002) Image Distortion Correction in EPI: Comparison of Field Mapping With Point Spread Function Mapping. Magnetic Resonance in Medicine 48:137-146.

Zink CF, Pagnoni G, Martin ME, Dhamala M, Berns GS (2003) Human striatal responses to salient nonrewarding stimuli. The Journal of Neuroscience 23:8092-8097.

Zink CF, Pagnoni G, Martin-Skurski ME, Chappelow JC, Berns GS (2004) Human striatal responses to monetary reward depend on saliency. Neuron 42:509-517.

Chapter 3: Experiment 2 – Neural correlates of non end-item vs. end-item transitive inference, and of the symbolic distance effect

Introduction

Motivated by the deficit of transitive inference (TI) in schizophrenia, the dependence of TI performance on intact hippocampal function, and the known hippocampal abnormalities in schizophrenia, we sought a link between TI performance and hippocampal function in the experiment described in the previous chapter. Using a block-design functional magnetic resonance imaging (fMRI) experiment, we demonstrated a fundamental role for the human hippocampus in TI.

In the previous experiment, we employed the classical experiment, where participants learn the ordered sequence A>B>C>D>E and are then faced with the novel pairs A>E (non-transitive inference because both sequence end-items are included) and B>D (end-item TI because the items are embedded inside the sequence). Using this paradigm, the capacity for TI has been previously demonstrated in birds (Strasser et al., 2004), rodents (Davis, 1992; Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003), monkeys (McGonigle and Chalmers, 1986; Buckmaster et al., 2004), and in humans (Greene et al., 2001; Martin and Alsop, 2004). Similarities between animal and human performance have also been clearly demonstrated (Colombo and Frost, 2001).

As discussed previously, in rats (Dusek and Eichenbaum, 1997) and monkeys (Buckmaster et al., 2004), disconnection of the hippocampus from either its cortical or subcortical pathway results in the animals' inability to correctly chose B over D (non end-item TI), while their ability to pick A over E (non-transitive inference) is spared. This finding has been perceived as key evidence for the flexible relational memory

account for the role of the hippocampus in animals (McGonigle and Chalmers, 1986; Eichenbaum, 1992; Squire, 1992; Cohen and Eichenbaum, 1993; Dusek and Eichenbaum, 1997; Burgess et al., 2002). By this account, hippocampal function is closely related to all declarative memory, including all explicit memory, but is especially crucial for relational learning and flexible use of memory (Eichenbaum, 2004). In contrast to this theory, the excitatory strength/value transfer account posits that performance on TI is guided by the absolute excitatory strength that each stimulus acquires during training, rather than by flexible manipulation of the sequence representation (von Fersen et al., 1991; Wynne, 1998; Frank et al., 2003; Van Elzakker et al., 2003).

The functional magnetic resonance imaging (fMRI) study described in the previous chapter (Heckers et al., 2004a), which was the first human experiment to demonstrate hippocampal activation in TI, provided evidence for the unique role of the hippocampus in relational processing in humans (Eichenbaum, 2004; Rapp, 2004). The previous study, however, suffered from some limitations. Hippocampal recruitment was demonstrated in the contrast between inference on pairs drawn from a sequence (e.g., pairs A>C and B>D from the sequence A>B>C>D>E) and inference on pairs drawn from individual pairs (e.g., pairs a>d and c>b from the pairs a>b and c>d). Due to the block design nature of the experiment, TIs on end-item pairs (e.g., A>C or B>E) that could be guided by reference to a sequence end-item were grouped together TIs on the non end-item pair (i.e., B>D) that required full manipulation of the sequence. Moreover, the five-item sequence A through E allowed testing only on one non end-item TI pair (BD). According to the relational memory account of hippocampal function, only non end-item TIs require

hippocampal recruitment since end-item TIs can be solved by referring either to the always winning item A, or the never winning item E, without flexible use of the sequence representation. We were therefore unable to prove hippocampal specialization for non end-item TI trials despite demonstrating its role in TI in general.

In order to address these limitations, we designed an event-related fMRI experiment that allowed us to directly contrast hippocampal function in hard and easy inferences. We chose a six-item sequence A>B>C>D>E>F that allowed non end-item TI tests (B>D, B>E, C>E). We hypothesized that hard inferences would be associated with greater hippocampal activity than easy inferences. This design allowed a second test of hippocampal specificity for relational memory using the symbolic distance effect (SDE). SDE refers to the greater ease of inference decisions the further apart the two items are on the relational continuum (Rapp et al., 1996; Acuna et al., 2002a; Bond et al., 2003; Frank et al., 2005). For example, the decision on the novel pair BD (symbolic distance of one, i.e., one intervening item in the sequence) should require greater manipulation of the sequence than that on the novel pair BE (symbolic distance of two, i.e., two intervening items in the sequence). Inferences on BD should, therefore, elicit lower accuracies and/or longer latencies than inferences on BE. Under the relational memory account, more information has to be recalled about the sequence hierarchy in order to solve BD than BE. We, therefore, hypothesized that pairs with a symbolic distance of one would elicit greater hippocampal activity than pairs with a symbolic distance of two. To ensure the specificity of the TI effect (non end-item vs. end-item) and the symbolic distance effect for the hippocampus (especially given that we were moving from a 1.5T to a 3.0T

scanner where signal loss and geometric distortions within the MTL are more severe) we supplemented our whole-brain voxel-based analysis with an anatomically guided region-of-interest (ROI) analysis limited to individual participants' hippocampi. As a part of this effort, we also measured the goodness of overlap between individual participants' hippocampi normalized to standardized MNI space using regular whole-brain procedures.

In addition to the hippocampus, a number of cortical regions have been shown to be recruited in TI in previous studies: the pre-supplementary motor area (pre-SMA), parietal, prefrontal, and inferior temporal cortices (Heckers et al., 2004a), the insula, anterior cingulate cortex, and precuneus (Acuna et al., 2002b). Activation of the thalamus and caudate nucleus has also been reported (Acuna et al., 2002b). We hypothesized that this TI network would also be more active in our comparisons of non end-item and end-item TIs and of symbolic distances of one and two.

In addition, we wanted to evaluate the role of the dopaminergic midbrain - ventral striatal system in TI because increasing evidence shows that this system supports TI in addition to the hippocampus. Indirect evidence comes from deficits in TI performance seen in disease states associated with dopaminergic system dysfunction, namely schizophrenia (Titone et al., 2004) and parkinsonism (Frank et al., 2004). It is possible that this evidence points to the role of the interplay between the glutamatergic hippocampal and dopaminergic midbrain inputs in the ventral striatum, which has been implicated in various memory functions (Poldrack and Rodriguez, 2004). Specifically, the dopaminergic system may alert the hippocampal memory system for less predictable

(Pagnoni et al., 2002) or more salient stimuli (Zink et al., 2003). Because non end-item TI trials are inherently more salient and less predictable than end-item TI trials containing the end items A or F, we hypothesized greater ventral striatal activity for hard compared to easy inference trials.

Materials and methods

Participants

We studied 19 healthy participants (13 male and 6 female, aged 22 to 38, mean age = 26.6, mean estimated verbal IQ (Blair and Spreen, 1989) = 118.9), who gave informed consent in a manner approved by the IRB of the Massachusetts General Hospital. No participant had a history of significant medical, neurological, or psychiatric illness. Of these 19 participants, 13 (10 male and 3 female, aged 22 to 38, mean age = 26.9, mean estimated verbal IQ (Blair and Spreen, 1989) = 119.1) entered the final analysis based on behavioral exclusion criteria explained below.

Stimuli and paradigm

Stimuli

We selected 16 distinctive pattern fills from those provided by CorelDraw. Six of these pattern fills were used to create a set of overlapping pairs of either pentagon or ellipsoid shape for each participant as described in the previous chapter (Heckers et al., 2004a). To avoid bias associated with particular object shape and/or pattern, we rotated the position

of the fills within the set for each participant and the two shapes across all participants (10 participants saw pentagons and 9 saw ellipses). The 5 pairs created from the six stimuli, A through F, were denoted AB, BC, CD, DE, and EF.

Training prior to scanning

The training was divided into three stages. Participants were first informed that they would be trained on pairs of visual patterns and that one of the two patterns in the pair would be the "winner". In Stage 1, they saw a preview of the five pairs on a computer screen. They were told that the pattern associated with a smiley face (☺) on the screen was the superior one and were asked to remember the pairs and the winning pattern.

In Stage 2, they were trained on these pairs to reinforce their memory of the pairs and the winning pattern in each pair. They were informed that they would see the pairs of patterns on the computer screen and that one pattern in each pair would always hide a smiley face. The pattern hiding the smiley face would be the same one that was the superior one in each pair they saw in the preview. Left/right position of individual patterns for each pair was counterbalanced, and participants indicated their response by pressing "1" for the stimulus on the left and "2" for the stimulus on the right. When participants made a correct choice during training, the selected visual pattern would move to reveal the smiley face reinforcement. When participants made an incorrect choice, the selected visual pattern would move, but the smiley face would not appear. Stage 2 consisted of two training blocks. The first training block consisted of 90 trials that were front-loaded so that the first three pairs from the total of five pairs would occur more

often than the remaining two pairs. In this block, they saw 22 instances of the first three pairs (AB, BC, and CD) and 12 instances of the remaining two pairs (DE and EF). The second training block consisted of 80 trials that were back-loaded with the last two pairs. In this block, they saw 12 instances of the first three pairs and 22 instances of the remaining two pairs.

Stage 3 of the training period was a test session where they once again saw the pairs on the screen and had to indicate the winning pattern but received no feedback. In this training period, participants saw 30 randomly ordered pairs in total, each pair six times. They had 3 sec to respond to each pair and received no feedback. The main purpose of this test run was to help participants get accustomed to the random presentation of the pairs and the pace of the test in the scanner, where they would also only have 3 sec to respond to each pair.

Overall, this method of training ensured that all participants would not only be able to learn the correct response for each pairing but would also be likely to encode the overlapping stimulus set hierarchically. Our previous work suggested that the initial front loading of pairs was necessary for healthy participants to respond correctly during the TI test trials BD, CE, and BE (Heckers et al., 2004a; Titone et al., 2004).

Test during fMRI scan

Participants took part in in two fMRI runs, each comprising 96 randomly ordered pairs from the set of the overlapping stimuli. Of these, there were 30 instances of the old overlapping (S) pairs, 36 easy inference overlapping pairs (IS_E) and 30 hard inference pairs (IS_H). Within the old overlapping (S) pairs, they saw 6 instances of each of these pairs (AB, BC, CD, DE, and EF), 30 instances in total. Within the easy inference (IS_E) pairs, they saw 5 instances of each of these pairs (AD, AE, AC, BF, CF, and DF), 30 instances in total. Within the hard inference (IS_H) pairs, they saw 10 instances of each of these pairs (BD, CE, BE), 30 instances in total. The relative (left vs. right) position of the two patterns was counter-balanced for each pair.

For each trial, participants were instructed to indicate the pattern they considered superior based on the preview and the training sessions by pressing the appropriate button (left or right). Each pair remained on the screen for 3 sec before the screen was refreshed and a new pair came on. No feedback was supplied during the scanning. Our design (Table 1) was devised to enable us to test for the behavioral and neural correlates of the effect of inference (non end-item NETI vs. end-item ETI) and symbolic distance (one SDE1 vs. two SDE2).

Functional imaging

Participants were scanned in a Siemens 3.0 Tesla Trio high-speed echo-planar imaging device (Munich, Germany). Participants lay on a padded scanner bed in a dimly

illuminated room, wearing ear plugs. Foam padding was used to restrict head movement. Stimuli were generated using Presentation software (Neurobehavioral Systems) on a personal computer, projected onto a screen, and viewed by the participants via a tilted mirror placed in front of their eyes.

Functional scanning began with an initial sagittal localizer scan. A GRE scan with successive flip angles was then performed to allow for automatic alignment of anatomical images. A three-dimensional MPRAGE anatomical image was then obtained and automatically positioned within a common anatomical database based on the anterior commisure-posterior commisure (AC-PC) axis. The two functional series lasted 8 min and 44 sec each. The first 10 sec of each series were discarded to allow equilibration of the MRI signal. During the remaining time of each series, 257 BOLD gradient-echo EPI functional brain images were collected (TE/TR = 28/2000 ms; 34 axial slices, parallel to the AC-PC line and starting anterior at the frontal pole, interleaved order, 3 mm slice thickness, voxel size 3.1x3.1x3 mm, FOV = 200 mm, flip angle = 90 degrees), to capture 96 trials lasting 3 sec each. The intertrial period (3 sec) was different from the scanner repetition time (TR = 2 sec) to allow jitter in the design, and efficient sampling of the hemodynamic response curve for each stimulus type.

Data analysis

Participant exclusion

Behavioral data from the test runs in the scanner were first analyzed for evidence of understanding of the hierarchical sequence (A-F) present in the overlapping pairs. Participants displaying accuracy of 90% or higher on the three non end-item TI (NETI) pairs (BD, CE, BE) were deemed to have understood this sequence. Only data from those participants who understood the sequence inherent in the overlapping pairs were further analyzed. Of the 19 participants who performed the experiment, 13 satisfied this criterion. For the remaining 6 participants, the mean accuracy for non end-item and end-item TI trials was 89 ± 12% and 66 ± 25%, respectively. The difference showed a trend towards but no significance (paired sample t test, 5 df, p=0.072). For the other 13 participants, accuracy for all trial types exceeded 97% and was not analyzed further.

Behavioral data

The behavioral data for the novel pairs were analyzed to assess the effect of inference and symbolic distance. In both cases, we used a paired sample t test. In the first case, we compared reaction times for NETI (BD, BE, and CE) vs. ETI (AC, AD, AE, DF, CF, BF). In the second case, we compared reaction times for NETI trials with symbolic distance of one (BD and CE) and two (BE).

Whole-brain voxel-based analysis of the functional neuroimaging data

The fMRI data from each participant were analyzed using the Statistical Parametric Mapping SPM2 package (Wellcome Department of Imaging Neuroscience, London, UK). Pre-processing was carried out using this package. First, slice timing correction using sinc interpolation was applied to account for differences in acquisition time of the individual slices. Functional images were then realigned to correct for head motion during the scanning and a mean functional image was created. The anatomical image of each participant was then coregistered with the participant's mean functional image. The coregistered anatomical image was normalized to a T1-weighted anatomical template in the stereotactic MNI coordinates. The functional images were subsequently normalized using the identical parameters and smoothed with a three-dimensional 8-mm Gaussian kernel to eliminate spatial noise and to allow the application of the Gaussian Random Field Theory for statistical analysis.

A design matrix was then created to allow the application of the General Linear Model (GLM) to the functional data. Different instances of each pair were modeled together as a unique condition. The onset times of these conditions were entered into the GLM and convolved with the haemodynamic response function and its time and dispersion derivatives to model the hemodynamic response to each trial type. A high-pass filter of 128 sec was used to remove time-dependent drift in the functional data, and the data were corrected for serial correlations.

Functional neuroimaging data were analyzed with to assess the effects of inference (NETI vs. ETI) and symbolic distance (SDE1 vs. SDE2). The contrast images [NETI-ETI] and [SDE1-SDE2] were, therefore, formed for each participant. The individual contrast images were then entered as random effects into a one-sample t test to detect significant differences common to all participants. The reverse contrasts were also examined.

We used the threshold level of $p < 0.005$, uncorrected for multiple comparisons, to assess statistical significance in regions with *a priori* hypothesis: MTL, the TI network outside of the MTL (including preSMA, parietal and prefrontal cortices, insula and precuneus), midbrain and ventral striatum in TI. For other brain regions with no *a priori* hypothesis, we used the $p < 0.05$ threshold, corrected for multiple comparisons using the family-wise error control in SPM2. All of the resulting activation maps were examined for significant differences at a voxel extent threshold of five voxels. The resulting activation maps were overlaid on the mean anatomical image of the participants. The peak activations were identified and their MNI coordinates and peak z scores were noted.

Region-of-interest analysis of the functional neuroimaging data

The whole-brain voxel-based analysis described above was supplemented by an anatomically guided ROI analysis in individual participants' hippocampi. The hippocampus was outlined bilaterally on every participant's anatomical image in native space using guidelines described elsewhere (Pruessner et al., 2000; DeWitt et al., 2002). The reliability of the outlines was assessed using the intraclass correlation coefficient

(Shrout and Fleiss, 1979). In five randomly selected participants, the intra-rater reliability was 0.93 on the left and 0.94 on the right. The inter-rater reliability was 0.87 on the left and 0.88 on the right.

This anatomical ROI was then resampled to the same resolution as the native space BOLD functional images that had been previously realigned and coregistered with the native space anatomical image. The resampled anatomical ROI image was then used as a mask to create partial brain functional images where the signal intensity values were set to zero outside the hippocampal mask. These images were subsequently smoothed using a three-dimensional 3-mm Gaussian kernel to increase detection sensitivity.

The design matrix from the whole-brain analysis was used with these partial brain volumes to apply GLM and to extract parameter estimates for the conditions of interest as described above. As for the whole-brain analysis, the contrast images [NETI-ETI] and [SDE1-SDE2] were obtained for each participant bilaterally. Each hippocampus was then divided into nine segments longitudinally along the y-axis; average contrast values were computed for each of the nine segments bilaterally. Because of concerns about signal loss in the anterior portion of the MTL, we identified voxels within each hippocampus where the signal intensity was below 1/6 of the mean intensity within the whole hippocampus. These voxels exhibiting a pronounced signal loss were excluded from the analysis.

As in the group analysis for the whole-brain data, the contrast values from each segment were then entered as random effects into a one-sample t test to detect significant

activations common to all participants. In each case, a one-sided t test (based on our hypothesis that the hippocampus would be more active for NETI compared to ETI, and for NETI trials with symbolic distance of one compared with two) with a p value threshold of 0.05 was used. In addition, we performed a regression analysis to determine whether signal loss affected the estimated contrast values.

Overlap between individual participants' hippocampi normalized to standardized space

Our motivation for performing a ROI analysis limited to each participant's hippocampi in native space was the fact that the whole-brain normalization procedure that morphs each individual's brain into standardized space results in imperfect overlap between brain structures of different participants. This morphing is particularly problematic in the hippocampus given the high degree of its anatomical variability across the population (Pruessner et al., 2000; Miller et al., 2005).

We, therefore, set out to determine the degree of overlap between individual participants' hippocampi after the application of the 12-parameter affine normalization procedure. TO this end, we applied the whole-brain normalization parameters that had been used to morph each participant's brain into standardized space to each participant's hippocampi outlined on their native space brain. For each participant, the normalized hippocampus was then divided longitudinally (along the y-axis or the long axis of the hippocampus) into nine segments, as was originally done for the native space hippocampi. We found the limiting y-coordinates corresponding and computed the midpoint y-coordinate for each of the nine segments bilaterally. We then found the corresponding standard and maximum

deviation of the mid-points across participants. Note that this procedure only aimed to evaluate the degree of overlap between different participants' normalized hippocampi along a single direction, which is the long axis of the hippocampus. As a part of this effort, the locations of the activations detected using the whole-brain and ROI analysis were compared along the long axis of the hippocampus.

Results

Reaction times for non end-item and end-item TI pairs

The mean reaction times to the non end-item NETI (AC, AD, AE, BF, CF, and DF) and end-item ETI trials (BE, BD, and CE) were 1.01 ± 0.13s and 1.11 ± 0.16s, respectively (Figure 1). The mean reactions times to the NETI trials with symbolic distance of one SDE1 (BD and CE) and symbolic distance of two SDE2 (BE) were 1.13 ± 0.16 and 1.05 ± 0.16, respectively (Figure 1).

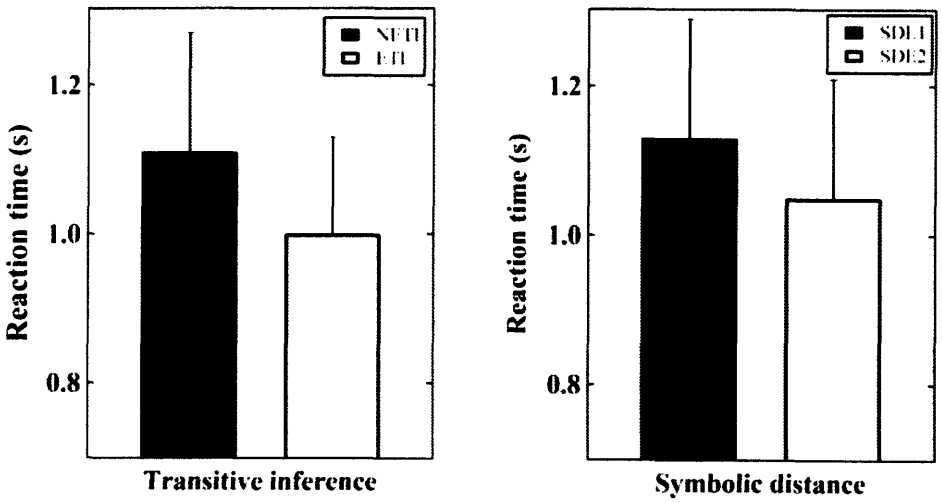

Figure 1: Average reaction times for non end-item (NETI) and end-item (ETI) pairs, and non end-item TI pairs with symbolic distance of one (SDE1) and two (SDE2) during the fMRI test (in seconds). The error bars indicate standard deviation of the mean.

A paired sample t test showed that reaction times were significantly longer for NETI than ETI ($t(12) = 3.6$, $p = 0.004$). Reaction times were also significantly longer for hard inference trials with symbolic distance of one than two (paired sample t test, $t(12) = 4.5$, $p = 0.001$).

Results for whole-brain analysis of the effect of inference and symbolic distance

We first tested for brain activation differences between NETI and ETI. This analysis allowed us to test the hypothesis that inference trials, which rely on the flexible representation of a sequence (i.e., NETI), are associated with greater hippocampal, TI network, midbrain and ventral striatal activation than those trials that include an end-item (i.e., ETI). We found two regions in the left hippocampus (MNI coordinates x, y, z = -24, -20, -10 mm, $z = 3.05$ and -38, -20, -16 mm, $z = 2.75$) that were more significantly ($p < 0.005$) activated during NETI than ETI within the MTL (Figure 2, left).

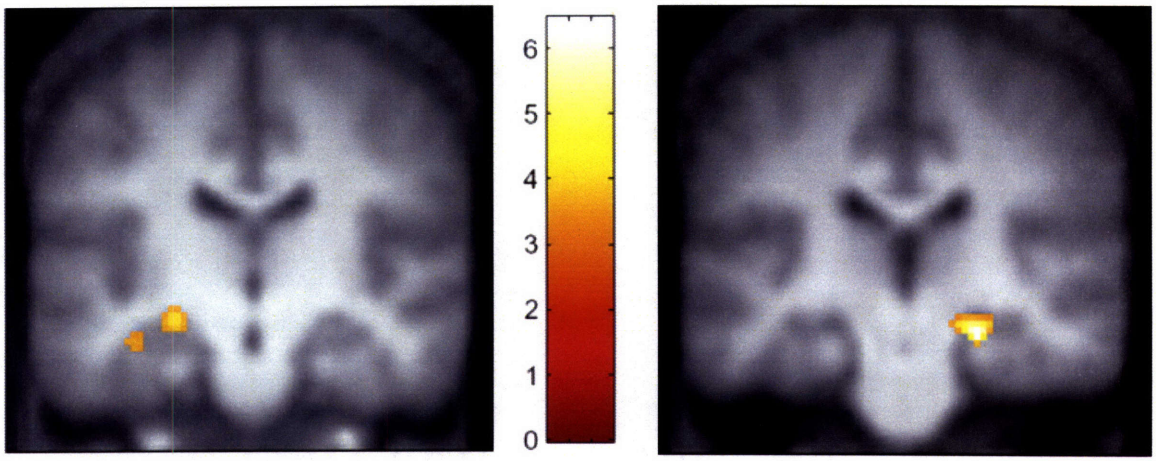

Figure 2: Medial temporal lobe activation in TI.
(Left) Medial temporal lobe activation in NETI vs. ETI. The focus of activation in the left hippocampus can be seen.
(Right) Medial temporal lobe activation in SDE1 vs. SDE2 for hard inference trials. The focus of activation in the right hippocampus can be seen.
Both statistical maps were thresholded at $p < 0.005$ and the voxel extent threshold was set to five. The maps are overlaid on the Talairach-normalized mean anatomical image of the participants used for the imaging analysis. The bar indicates the t values of activated voxels.

Within the broader TI network, we found activation in the right and left inferior frontal gyrus (x, y, z = -54, 18, 6 mm, $\underline{z}$ = 3.91, BA 45; x, y, z = 32, 28, -2 mm, $\underline{z}$ = 3.96 and x, y, z = 40, 30, 2 mm, $\underline{z}$ = 3.52, both BA 47), left insula (x, y, z = -36, 20, 4 mm, $\underline{z}$ = 2.86), right precuneus (x, y, z = 8, -44, 50 mm, $\underline{z}$ = 2.88 and 4, -46, 54 mm, $\underline{z}$ = 2.83) and right dorsomedial thalamus (x, y, z = 6, -2, 4 mm, $\underline{z}$ = 3.97) (Figure 3).

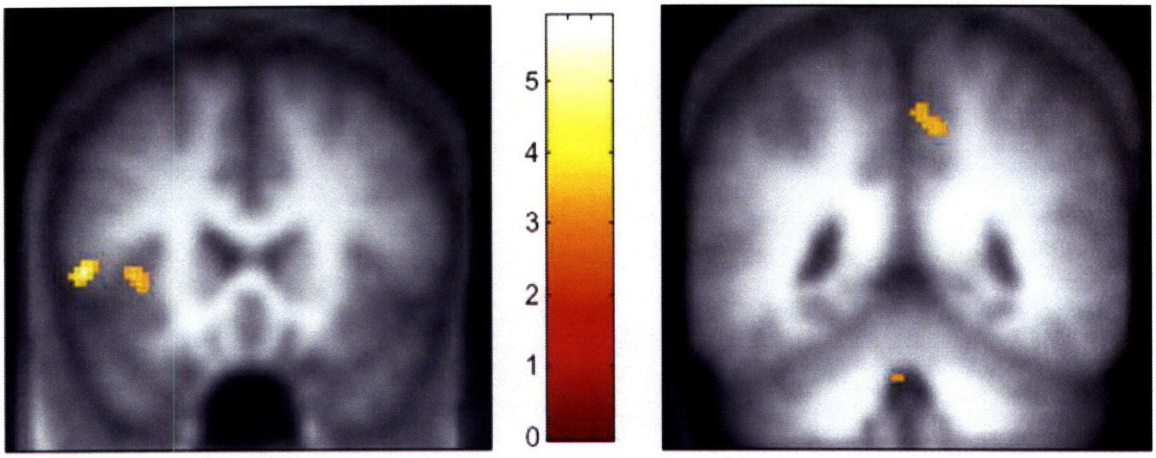

Figure 3: Cortical activation in TI.
Cortical activation in NETI vs. ETI. Three of the nodes of the TI network that was activated are shown. The left figure shows activations in the inferior frontal gyrus and the insula. The right figure shows activation in the parietal cortex. Both statistical maps were thresholded at $\underline{p} < 0.005$ and the voxel extent threshold was set to five. The maps are overlaid on the Talairach-normalized mean anatomical image of the participants used for the imaging analysis. The bar indicates the $\underline{t}$ values of activated voxels.

We also found one region in the right nucleus accumbens (x, y, z = 12, 10, -8 mm, $\underline{z}$ = 2.82) and one region in the left midbrain (x, y, z = -8, -26, -16 mm, $\underline{z}$ = 2.59) that were significantly more active during NETI than ETI (Figure 4). No significant activations were detected in the reverse contrast.

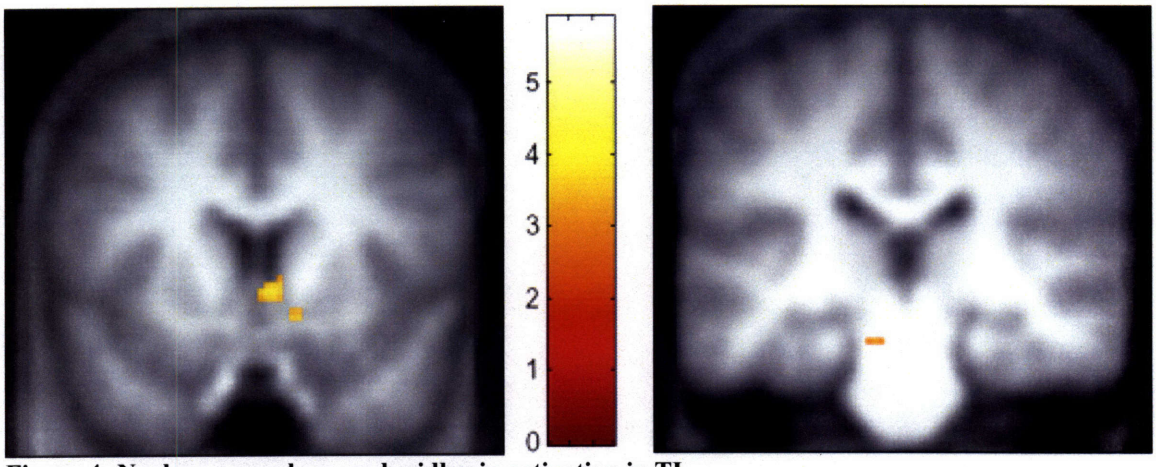

Figure 4: Nucleus accumbens and midbrain activation in TI.
Nucleus accumbens (left) and midbrain activation (right) in NETI vs. ETI. Both statistical maps were thresholded at $p < 0.005$ and the voxel extent threshold was set to five. The maps are overlaid on the Talairach-normalized mean anatomical image of the participants used for the imaging analysis. The bar indicates the t values of activated voxels.

Second, we tested for brain activation differences between the NETI trials with symbolic distance of one and symbolic distance of two. This analysis allowed us to test the hypothesis that hippocampal and TI network activation would be greater for those trials where the representation of the sequence has to be recalled in greater detail (symbolic distance of one compared with two). We found one region in the right hippocampus (MNI coordinates x, y, z = 24, -24, -12 mm, $z = 4.16$) that was more significantly ($p < 0.005$) activated during trials with symbolic distance of one compared with two within the MTL (Figure 2, right). Within the TI network, widespread activation occurred including the bilateral middle frontal gyrus (x, y, z = -52, 22, 32 mm, $z = 3.29$ and x, y, z = 54, 32, 26 mm, $z = 2.92$), left inferior parietal lobule (x, y, z = -38, -52, 40 mm, $z =$ 4.00 and x, y, z = -26, -64, 46 mm, $z = 3.39$, right superior parietal lobule (x, y, z = 32, -74, 28 mm, $z = 3.32$ and x, y, z = 14, -68, 56 mm, $z = 2.98$), left angular gyrus in the parietal lobe (x, y, z = 30, -58, 36 mm, $z = 3.48$ and x, y, z = 34, -68, 24 mm, $z = 3.50$), and left inferior (x, y, z = -44, -50, -6 mm, $z = 3.68$), right middle (x, y, z = 60, -36, 0

mm, $\underline{z}$ = 3.48), and left superior (x, y, z = -48, 14, -4 mm, $\underline{z}$ = 2.94 and x, y, z = -46, -2, -10 mm, $\underline{z}$ = 2.83) temporal gyri (Figure 5). In addition, multiple foci of activation were observed in the occipital cortex (x, y, z = -14, -70, -12 mm, $\underline{z}$ = 3.59, x, y, z = 20, -98, 0 mm, $\underline{z}$ = 3.38 and x, y, z = 38, -88, 4 mm, $\underline{z}$ = 3.1). No significant activations were detected in the reverse contrast.

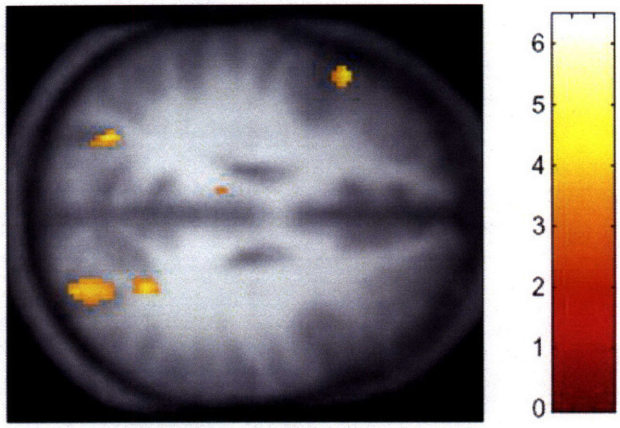

Figure 5: Cortical activation related to symbolic distance.
TI network activation in symbolic distance of one vs. two for NETI trials. Amongst the nodes of the TI network related to symbolic distance, those at the level of z = 32 mm are shown and include the left middle frontal gyrus, bilateral superior parietal lobule and right angular gyrus.
The statistical map was thresholded at $\underline{p} < 0.005$ and the voxel extent threshold was set to five. The maps are overlaid on the Talairach-normalized mean anatomical image of the participants used for the imaging analysis. The color bar indicates the $\underline{t}$ values of active voxels.

Results of the region-of-interest analysis of the effect of inference and symbolic distance

Using the anatomically guided ROI analysis, we found one segment in the left hippocampus where activity was consistently higher for NETI than ETI across the thirteen participants ($\underline{t}(12) = 3.04$, one-tailed $\underline{p}$ value = 0.005) (Figure 6). This was the fifth segment out of nine along the long axis of the left hippocampus, corresponding approximately to the midpoint of the hippocampus in each of the 13 participants. No consistent activation was detected in the right hippocampus. Regression analysis revealed

no correlation between the degree of signal loss and the estimated contrast values in any of the 13 participants.

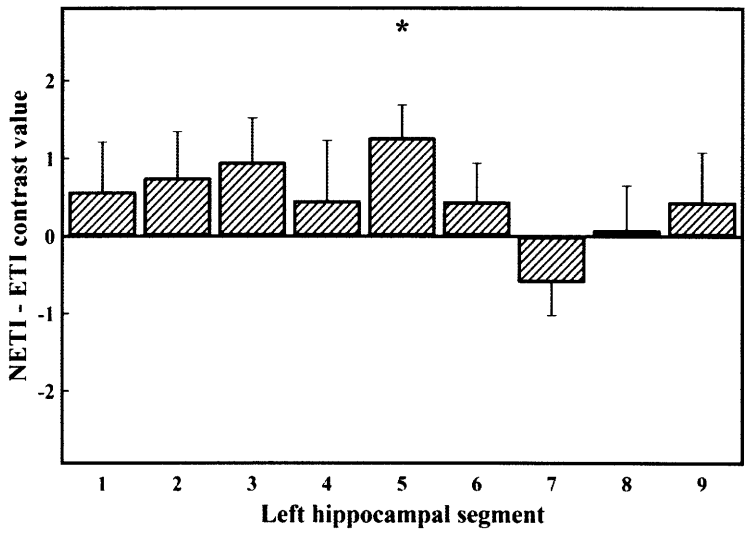

Figure 6: Contrast of parameter estimates values corresponding toNETI vs. ETI in each of the nine slices along the long axis of the left hippocampus. The star indicates a significant (one-tailed p value < 0.05) activation. Error bars represent the standard error of the mean.

We found two segments in the right hippocampus where activity was consistently higher for NETI trials with a symbolic distance of one compared to two across the 13 participants. These were the third ($t(12)=2.15$, one-tailed p value = 0.027) and the fifth ($t(12)=2.04$, one-tailed p value = 0.032) segments out of nine along the long axis of the right hippocampus, corresponding approximately to a location one-third of the way from the anterior tip and a location in the midpoint of the hippocampus (Figure 7). No consistent activation was detected in the left hippocampus. Regression analysis revealed no correlation between the degree of signal loss and the estimated contrast values in any of the 13 participants.

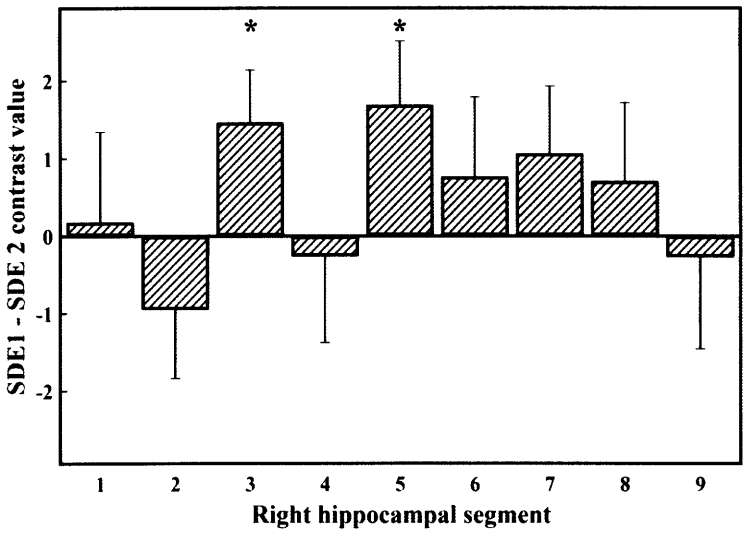

Figure 7: Contrast of parameter estimates values corresponding to symbolic distance of one vs. two for NETI trials in each of the nine slices along the long axis of the right hippocampus. The stars indicate a significant (one-tailed p value < 0.05) activation. Error bars represent the standard error of the mean.

Overlap between individual participants' hippocampi normalized to standardized space

In the standardized MNI space, the mean left hippocampal length was 30.9 ± 3.0 mm; the average segment length across the 13 participants was 3.6 ± 0.4 mm. The mean right hippocampal length was 31.4 ± 2.0 mm; the average segment length across the 9 participants was 3.7 ± 0.2 mm. Therefore, some participants' hippocampi were a full segment length shorter or longer than other participants' hippocampi.

Across the 13 participants, the SD of the midpoint of each segment along the y-axis ranged from 2.7 mm (anterior) to 2.0 mm (posterior) on the left (Figure 8). The maximum deviation was 7.1 mm, which is approximately twice the average segment length across participants. On the right, the standard deviation of the mid-point of each segment ranged

from 1.8 mm (anterior) to 0.9 mm (posterior) (Figure 8). The maximum deviation was 4.5mm, which is still larger than the mean segment length across the participants.

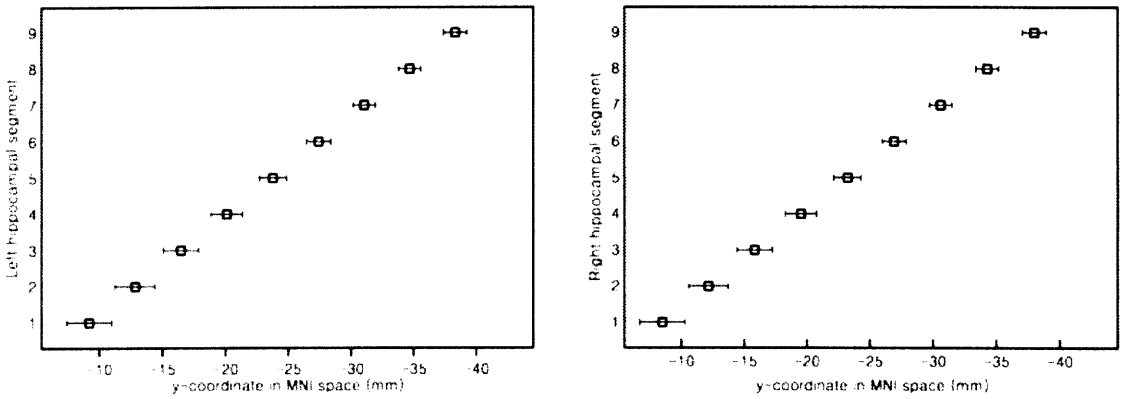

Figure 8: Mean locations of the midpoints of each of the nine hippocampal segments normalized to the standard MNI space. The mean y-coordinate in MNI space for midpoints of each of the nine hippocampal midpoints is shown. The error bars indicate the SD of the midpoint location across the 13 participants, and thus provide a measure of inter-subject variability.

When normalized to standardized MNI space using regular whole-brain normalization, we found a considerable degree of inter-subject variability in the size and position of the hippocampus along the longitudinal axis. Identical locations along the longitudinal axis of the hippocampus in different participants' native space could end up as far as 7 mm apart in standardized MNI space when regular whole-brain normalization procedure is used. This variability could potentially create problems in detecting and localizing activation using whole-brain voxel-based methods.

To evaluate this possibility, we compared the locus of activation determined using a whole-brain voxel-based analysis with that based on the anatomically guided ROI analysis. For the NETI vs. ETI contrast, the peak activation occurred at the y-coordinate of y = -20 mm on the left in whole-brain voxel-based analysis. In the ROI analysis,

activation was detected in the fifth segment on the left for this contrast, corresponding to the range of y = <-21.9, -25.6 mm> with a SD of 1.9 mm in the standardized MNI space.

For the contrast of symbolic distance of one vs. two, the peak activation occurred at the y-coordinate y = -24 mm on the right in whole-brain voxel-based analysis. In the ROI analysis, activation was detected in the fifth segment on the right for this contrast, corresponding to the range of y = <-21.4, -25.1 mm> with a SD of 1.1 mm in the standardized MNI space. Additional activation was detected in the third segment on the right, corresponding to the range of y = <-14.0, -17.7 mm> with a SD of 1.4 mm in the standardized MNI space.

Overall, we found for both contrasts a reasonable overlap between activations detected using the two methodss. For the contrast of symbolic distance of one vs. two, activation in the third longitudinal segment of the right hippocampus was detected using the anatomically guided ROI analysis that was not found in the whole-brain voxel-based analysis.

Discussion

In the previous study (Chapter 1), we showed hippocampal recruitment in TI in humans, and confirmed the existence of a TI network. In the present experiment, we investigated the specific role of these brain areas in TI. I will first discuss implications of the results for the specific role of the hippocampus with respect to two prominent accounts of

hippocampal function in TI – the relational flexibility account and the value transfer account. I will then turn to the role of the extra-hippocampal TI network. Then, I will consider the contribution of the midbrain-ventral striatum (MB-VS) dopaminergic (DA) system.

Contribution of the hippocampus to TI

The primary goal of this study was to examine the role of the hippocampus in TI. In this experiment, participants learned the overlapping sequence A>B>C>D>E>F and were then tested on TI. We hypothesized that non end-item inferences (NETI, i.e., inferences on pairs devoid of the end items A and F, e.g. B>D and C>E) would lead to greater hippocampal activity than end-item inferences (ETI, i.e., inferences on pairs including the end item A or F, which can be solved by referring to the always reinforced A or the never reinforced E). Second, we conjectured that inference pairs with a symbolic distance of one (SDE1, i.e., one intervening item between the items in the sequence, such as BD) would lead to higher hippocampal activity than inference pairs with a symbolic distance of two (SDE2, i.e., two intervening items between the items in the sequence, such as BE).

Our previous study (Heckers et al., 2004a), which demonstrated hippocampal activation in TI in humans and was described in the previous chapter, employed a block design where NETI and ETI, and inferences with different symbolic distances were grouped together. The study was, therefore, limited in terms of investigating the specificity of the hippocampal contribution to TI. In the present study, we extend our previous finding of hippocampal involvement in TI and demonstrate its specificity for NETI compared with

ETI using both whole-brain voxel-based analysis and anatomically guided ROI analysis limited to individual participants' hippocampi. Using both methods, we also showed greater hippocampal activity for hard NETI pairs with symbolic distance of one than two. In both situations, the hippocampus exhibits greater activity in situations requiring greater degree of flexible manipulation of the sequence consisting of overlapping stimulus pairs. Whereas both situations require greater degree of flexible sequence manipulation, this increased manipulation demand is not simply due to difference in difficulty or task load. First, the load is equal for NETI and ETI trials, and SDE1 and SDE2 trials, because the number of items presented (i.e., two) is the same across all trials. All items came from a single sequence that had been previously studied and there was no reason why any item from the sequence would be remembered more easily than the other items because all items were seen an equal number of times. Second, our group of participants for the imaging analysis was selected so as to equalize accuracy across the different trials to eliminate the potential confound of differences in difficulty. The accuracy did not differ between NETI and ETI, and SDE1 and SDE2, suggesting that the tasks did not present varying degrees of difficulty in this group. Different levels of difficulty could be also reflected by reaction time differences. While response times were significantly longer for NETI than ETI, and SDE1 than SDE2, the difference was only 100 msec. One might expect this difference to be larger if the difficulty markedly differed between the different trials. Different levels of task load or difficulty alone therefore cannot explain the observed differences in hippocampal and extra-hippocampal activation.

Our finding contributes to the debate between the two prominent views on how TIs are

solved. In the relational flexibility account, the hippocampus links related memories according to their common features, and this linkage results in a logical network that can support inferences between items in memory that are only indirectly related (McGonigle and Chalmers, 1986; Eichenbaum, 1992; Squire, 1992; Cohen and Eichenbaum, 1993; Dusek and Eichenbaum, 1997; Burgess et al., 2002; Eichenbaum, 2004). In contrast, in the excitatory strength/value transfer account, each item from the sequence acquires a distinct associative strength through training. Performance on novel pairs is then guided by the absolute difference between the associative strengths of the items composing the novel pairs. In this account, the hippocampus plays only a minor role in TI, which is limited to the early phases of learning of the associative strengths and does not play an active role during behavior on the test pairs (Frank et al., ; von Fersen et al., 1991; Wynne, 1998; Frank et al., 2003; Van Elzakker et al., 2003; Frank et al., 2005). The imaging results presented here point clearly to the relational flexibility account of hippocampal function as documented by its greater activity for NETI than for ETI, and for smaller symbolic distances.

In addition to making different predictions about hippocampal contribution to TI, the two views also predict different behavioral outcomes. According to the value transfer theory, the associative strengths of B and D are too similar in the six item sequence A>B>C>D>E>F, resulting in poor performance on the BD but not the BE pairing (Frank et al., 2003; Van Elzakker et al., 2003). In our study, all participants entering the imaging analysis demonstrated greater than 95% accuracy on the BD trial, in direct contradiction to prediction of the value transfer theory. The proponents of this theory do acknowledge

that their account may not be valid when the participants have awareness of the sequence hierarchy (Frank et al., 2005). All the participants entering the imaging analysis indeed demonstrated post-experimental awareness of the hierarchy. Further, the six participants who displayed poor TI performance and were, therefore, excluded from the imaging analysis, BD and BE accuracies were poor and similar. These participants demonstrated a complete lack of awareness of the sequence hierarchy, yet their performance was not in accordance with the value transfer account. On the whole, our behavioral results therefore also support the relational flexibility account for TI.

We have, therefore, confirmed our previous finding of hippocampal recruitment in TI, and demonstrated its specificity for situations where previously learned sequence of overlapping pairs has to be manipulated flexibly to solve novel pairings (hard inference). Our results also suggest that the mechanism of TI lies in the flexible manipulation of the underlying sequence hierarchy (the relational flexibility account) rather than the different stimuli in the sequence's acquiring distinct associative strengths (the associative strength/value transfer account). More importantly, the results point to a specific role of the hippocampus in declarative memory. In our view, our results underscore the theory of the special role of the hippocampus within the MTL memory system in that it is selectively activated in the flexible manipulation of previously learned relational information (Eichenbaum, 2004).

Contribution of cortical areas to TI

Several cortical areas were more active for NETI than for ETI, including the inferior frontal gyrus (BA 45 and 47), left insula, right precuneus and right dorsomedial thalamus. Except for the area 45 in the inferior frontal gyrus, these areas are in close agreement with previous reports of cortical networks activated in TI (Acuna et al., 2002b; Heckers et al., 2004a). Whereas the area 45 is classically known as part of the Broca's area involved in speech production, it has also been shown to be activated in verbal analogy tasks compared with a semantic judgment task (Luo et al., 2003). It is also known to support mental arithmetic and fact retrieval (Simon et al., 2004). Moreover, the adjacent area BA 44 was shown to be activated in complex reasoning task when a 2-relational problem was compared with a 1-relational problem (Christoff et al., 2001). The activation of BA 45 in NETI compared with ETI could, therefore, reflect the higher relational complexity of the hard inference trials and the greater need for retrieval of the sequence and for analogical reasoning required for the hard inference trials. Similarly, area BA 47 and the precuneus increase their activity in proportion to the relational complexity of the Raven's Progressive Matrices to be solved (Kroger et al., 2002). It is in this context that we interpret their activation in NETI in the present study, and previous studies (Acuna et al., 2002b). The thalamus and the insula, on the other hand, show increased activity in proportion to uncertainty (Huettel et al., 2005). Their greater activation for NETI trials may, therefore, reflect the more ambiguous nature of these trials, given that both items in the pair have been previously rewarded, depending on the other item they had been paired with during training.

In the comparison of NETI trials with symbolic distance of one and two, widespread cortical activation was also detected, including bilateral PFC, left inferior (IPL) and right superior parietal lobules (SPL), left angular gyrus, left and right lateral temporal lobe, and areas in the occipital lobe. Insight into the role of these cortical areas in the symbolic distance effect comes from investigations of number processing. The numerical continuum is known to have a spatial representation in humans (Hubbard et al., 2005) similar to the ordered sequence on which TIs are made, which is also thought to be spatially represented (McGonigle and Chalmers, 1986; Davis, 1992). Analogously to the symbolic distance effect in TI where reaction times increase as the distance between two non-adjacent stimuli in the sequence decreases, reaction times increase in number comparisons as the difference between two numbers decreases (Pinel et al., 2001; Fias et al., 2003; Ansari et al., 2005). This effect extends to comparisons of many physical magnitudes, including length, size and luminance (Pinel et al., 2004; Kadosh et al., 2005). This spatial-numerical interaction has been shown to be subserved by posterior parietal areas. In particular, the numerical distance effect has been related to IPL function (Ansari et al., 2005). Left IPL has also been shown to be particularly relevant for number comparisons (Gobel et al., 2004; Sandrini et al., 2004; Kadosh et al., 2005). More broadly, both IPL and SPL are recruited in quantitave comparisons in general, irrespective of the type of stimulus (Fias et al., 2003; Pinel et al., 2004). In addition, SPL also shows increased activity with relational complexity and manipulation of spatial relations (Kroger et al., 2002). IPL activation, in contrast, scales with uncertainty (Huettel et al., 2005). Lateral temporal and occipital activation has also been reported in comparisons (Hubbard et al., 2005; Kadosh et al., 2005). Together, these two areas may

be part of the occipito-temporal pathway of visual processing that assists in comparisons (Hubbard et al., 2005). In addition, area BA 22 in the postero-superior temporal cortex, which was found to be related to the symbolic distance effect here, may play a special role in integrating and mapping different attributes of relationships (Luo et al., 2003).

The parietal activation reported here for the symbolic distance effect is, therefore, most likely to reflect the greater difficulty in comparing items that are closer together on a spatial continuum that represents the sequence. Although the left IPL has previously been thought to subserve number comparisons exclusively (Sandrini et al., 2004), the present results suggest that it may also underlie comparisons within a non-numerical sequence. The occipito-temporal activation may represent greater activation of a visual processing pathway that assists in comparisons of items with smaller symbolic distances. The angular gyrus has been previously shown to support mental arithmetic (Simon et al., 2004), which could be implicitly involved in the comparisons made in hard inferences. The caudate nucleus, which was also activated here, is activated in planning, organizing and sequencing (Christoff et al., 2001) and number comparisons (Gobel et al., 2004). It is, therefore, likely to be a part of the comparison network in TI. The dorsolateral PFC activation related to symbolic distance probably reflects its role in manipulating the sequence information (Acuna et al., 2002b). Together, these areas in the TI network may assist the hippocampus in flexibly expressing the previously acquired sequence information.

Contribution of the ventral striatal dopaminergic system to TI

Another goal of our study was to elucidate the contribution of the midbrain - ventral striatal DA system to TI. We show greater MB and VS activity in NETI than ETI. Increasing indirect evidence favors the role of the MB – VS system in TI. Deficits in TI performance have been demonstrated in schizophrenia (Titone et al., 2004) and parkinsonism (Frank et al., 2004), both of which are disease states associated with dysfunction of the dopaminergic system. Our results confirm the activation of the dopaminergic MB –VS system and suggest its interplay with the glutamatergic hippocampus – VS system. Having considered the role of the hippocampus in TI, we will now turn to the roles of the VS and MB in turn, and then discuss the different ways that these areas might interplay.

The ventral striatum has been classically perceived as the neural substrate for limbic-motor interactions implicated in converting environmental information into an appropriate behavioral response (Alvarez-Jaimes et al., 2004). More relevant to TI, lesions in this area cause deficits in spatial learning (Meredith and Totterdell, 1999) and on spatial tasks (Setlow, 1997; Atallah et al., 2004). Given that many participants report learning the sequence order in TI as a spatial order, this ability could be one way in which VS contributes to TI performance. In addition, VS has been hypothesized to use information acquired through associations (Setlow et al., 2003), which is the case in the acquisition of the sequence hierarchy.

In concert with MB DA neurons, VS also responds more to unpredictable compared to predictable stimuli (Berns et al., 2001), and more salient compared to less salient stimuli (Schultz, 1999; Zink et al., 2003; O'Doherty, 2004). Greater of VS and MB would then be expected to NETI than ETI because NETI is more salient (two stimuli previously not seen together without any end item whose reinforcement history is unambiguous) and less predictable due to the absence of any end items aiding in the decision. DA neurons of MB have also been implicated in reward-driven (Mirenowicz and Schultz, 1994) and associative (Redgrave et al., 1999) learning. Of particular relevance to TI performance, MB DA neurons are modulated by uncertainty in the stimulus-outcome association in associative learning tasks (Aron et al., 2004). Uncertainty is greater for hard inference trials where neither of the items has an unambiguous reinforcement history compared to easy inference trials where one of the items was either always rewarded (A) or never rewarded (F).

Having explored multiple possibilities for the recruitment of VS and MB in TI, we will now turn to the possible rationale for their interplay for both hard and easy inferences. As discussed above, MB and VS cooperate in signaling predictability and salience. In addition, the MB - VS system plays an important role in response selection and behavioral switching in animals (Floresco et al., 2001). It is proposed that DA transmission from MB could prepare the participant to deal with the unexpected by promoting the switch of attentional and behavioral resources toward biologically significant stimuli (Redgrave et al., 1999). It is plausible that when participants are faced

with novel pairs (hard and easy inference pairs), behavioral and attentional switching is elicited in a way that activates this functional connection.

Other reasons exist for the interplay between the hippocampus and VS in NETI. In general, the hippocampus – VS connection is deemed important for memory tasks, particularly those with context-dependent behavior (Casey et al., 2002). It has been suggested that the hippocampus and VS work in concert. Whereas the hippocampus can rapidly bind information into conjunctive representations, VS can help modulate responding based on these spatial inputs, as informed by prior reward-based learning history (Atallah et al., 2004). In both NETI and ETI inferences, reward-based learning history is flexibly used in making decisions. Synchronous activity of hippocampal and VS neurons has been previously reported in animals (Kelley, 2004). Direct connection between the hippocampus and VS has also been reported in an imaging study of probabilistic classification learning, a task not completely dissimilar from TI (Poldrack and Rodriguez, 2004).

Further, the activation of the hippocampus, VS and MB points to the possibility of an interaction between them, as suggested in recent literature. In particular, interaction between MTL and VS through the action of the mesencephalic DA system has been proposed, where MB neurons release DA in response to unpredicted rewards, cues that predict subsequent rewards, and other salient events (Poldrack and Rodriguez, 2004). On a primitive level, the hippocampal glutamatergic and MB DA neural systems interact with VS to interface biologically relevant information with motor output (Sargolini et al.,

1999). In another view, DA induced synaptic plasticity in VS would serve to enhance behavioral flexibility (Todd and Grace, 1999). Of particular relevance to TI, dopaminergic inputs, which respond to salient events, may interact with convergent glutamatergic inputs in VS by amplifying strong (salient-related) inputs and dampening weak (nonsalient-related) inputs (Zink et al., 2004). Our data suggest that the hippocampus may supply flexibly manipulated sequence information, whereas the midbrain codes the salience and/or predictability of the winning choice from the novel pair of items. This information may be integrated in the VS to guide the appropriate choice between the two stimuli.

Variability in hippocampal location and size when normalized into a standardized space

As a part of this effort, we were also able to compare the result of whole-brain voxel-based analysis in standardized MNI space and anatomically guided ROI analysis in the native space of each participant. We demonstrated a substantial degree of variability in the size and location of individual participants' hippocampi upon normalization to standard MNI space. Despite this variability, however, the activations detected for the hard inference and symbolic distance effects using the two different methods are in good agreement, except for one additional activation detected for the symbolic distance effect using the ROI analysis that was not detected by whole-brain voxel-based methods. Based on our results, the variability in hippocampal size and position when normalized into standard MNI space may not always be as severe an impediment in group analysis as one might have anticipated.

Variability in hippocampal size and position is, however, not the only issue that affects fMRI of this structure. The medial temporal lobe is one of the areas most severely affected by susceptibility artifacts, which are inherent in the nature of echo-planar imaging (EPI) used for fMRI. Such artifacts can result in imperfect coregistration between the structural and functional images, an effect that becomes more pronounced at higher field strengths. The imaging for the block-design experiment described in Chapter 1 was carried out in a 1.5T magnet, where these effects are not as important. For the event-related experiment described in this chapter, we moved to a higher field strength (3.0T) where susceptibility artifacts became more pronounced, and it thus became necessary to evaluate their impact on the data. The origin of the mismatch between anatomical and functional MRI images and the degree to which it is present in our data will be examined in the next chapter.

References

Acuna BD, Sanes JN, Donoghue JP (2002a) Cognitive mechanisms of transitive inference. Experimental Brain Research 146:1-10.

Acuna BD, Eliassen JC, Donoghue JP, Sanes JN (2002b) Frontal and parietal lobe activation during transitive inference in humans. Cerebral Cortex 12:1312-1321.

Alvarez-Jaimes L, Betancourt E, Centeno-Gonzalez M, Feliciano-Rivera MZ, Rodriguez D, Pena de Ortiz S, Maldonado-Vlaar CS (2004) Spatial learning in rats is impaired by microinfusions of protein kinase C-γ antisense oligodeoxynucleotide within the nucleus accumbens. Neurobiology of Learning & Memory 81:120-136.

Ansari D, Garcia N, Lucas E, Hamon K, Dhital B (2005) Neural correlates of symbolic number processing in children and adults. Neuroreport 16:1769-1773.

Aron AR, Shohamy D, Clark J, Myers C, Gluck MA, Poldrack RA (2004) Human midbrain sensitivity to cognitive feedback and uncertainty during classification learning. Journal of Neurophysiology 92:1144-1152.

Atallah HE, Frank MJ, O'Reilly RC (2004) Hippocampus, cortex, and basal ganglia: insights from computational models of complementary learning systems. Neurobiology of Learning & Memory 82:253-267.

Berns GS, McClure SM, Pagnoni G, Montague PR (2001) Predictability Modulates Human Brain Response to Reward. The Journal of Neuroscience 21:2793-2798.

Blair J, Spreen O (1989) Predicting premorbid IQ: a revision of the national adult reading test. The Clinical Neuropsychologist 3:129-136.

Bond AB, Kamil AC, Balda RP (2003) Social complexity and transitive inference in corvids. Animal Behaviour 65:479-487.

Buckmaster CA, Eichenbaum H, Amaral DG, Suzuki WA, Rapp PR (2004) Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. The Journal of Neuroscience 24:9811-9825.

Burgess N, Maguire EA, O'Keefe J (2002) The human hippocampus and spatial and episodic memory. Neuron 35:625-641.

Casey BJ, Thomas KM, Davidson MC, Kunz K, Franzen PL (2002) Dissociating striatal and hippocampal function developmentally with a stimulus-response compatibility task. The Journal of Neuroscience 22:8647-8652.

Christoff K, Prabhakaran V, Dorfman J, Zhao Z, Kroger JK, Holyoak KJ, Gabrieli JD (2001) Rostrolateral Prefrontal Cortex Involvement in Relational Integration during Reasoning. Neuroimage 14:1136-1149.

Cohen NJ, Eichenbaum H (1993) Memory, amnesia, and the hippocampal system. Cambridge, MA: MIT Press.

Colombo M, Frost N (2001) Representation of serial order in humans: a comparison to the findings with monkeys (*Cebus apella*). Psychonomic Bulletin & Review 8:262-269.

Davis H (1992) Transitive inference in rats (*Rattus norvegicus*). Journal of Comparative Psychology 106:342-349.

DeWitt I, Weiss AP, Deckersbach T, Kunkel L, Goff D, Heckers S (2002) Assessing hippocampal volume in schizophrenia using a standardized MRI protocol. Biol Psychiatry 51:21S.

Dusek JA, Eichenbaum H (1997) The hippocampus and memory for orderly stimulus relations. Proceedings of the National Academy of Sciences of the United States of America 94:7109-7114.

Eichenbaum H (1992) The hippocampus and declarative memory in animals. Journal of Cognitive Neuroscience 4:217-231.

Eichenbaum H (2004) Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 44:109-120.

Fias W, Lammertyn J, Reynvoet B, Dupont P, Orban GA (2003) Parietal Representation of Symbolic and Nonsymbolic Magnitude. Journal of Cognitive Neuroscience 15:47-56.

Floresco SB, Blaha CD, Yang CR, Philips AG (2001) Modulation of hippocampal and amygdala-evoked activity of nucleus accumbens neurons by dopamine: cellular mechanisms of input selection. The Journal of Neuroscience 21:2851-2860.

Frank MJ, Rudy JW, O'Reilly RC (2003) Transitivity, flexibility, conjunctive representations, and the hippocampus. II. A computational analysis. Hippocampus 13:299-312.

Frank MJ, Seeberger LC, O'Reilly RC (2004) By carrot or by stick: cognitive reinforcement learning in Parkinsonism. Science 306:1940-1943.

Frank MJ, Rudy JW, Levy WB, O'Reilly RC When logic fails: implicit transitive inference in humans. Memory & Cognition:In press.

Frank MJ, Rudy JW, Levy WB, O'Reilly RC (2005) When logic fails: Implicit transitive inference in humans. Memory & Cognition 33:742-750.

Gobel SM, Johansen-Berg H, Behrens T, Rushworth MF (2004) Response-Selection-Related Parietal Activation during Number Comparison. Journal of Cognitive Neuroscience 16:1536-1551.

Greene AJ, Spellman BA, Dusek JA, Eichenbaum HB, Levy WB (2001) Relational learning with and without awareness: transitive inference using nonverbal stimuli in humans. Memory & Cognition 29:893-902.

Heckers S, Zalesak M, Weiss AP, Ditman T, Titone D (2004) Hippocampal activation during transitive inference in humans. Hippocampus 14:153-162.

Hubbard EM, Piazza M, Pinel P, Dehaene S (2005) Interactions Between Number and Space in Parietal Cortex. Nature Reviews Neuroscience 6:435-448.

Huettel SA, Song AW, McCarthy G (2005) Decisions under Uncertainty: Probabilistic Context Influences Activation of Prefrontal and Parietal Cortices. The Journal of Neuroscience 25:3304-3311.

Kadosh RC, Henik A, Rubinstein O, Mohr H, Dori H, van de Ven V, Zorzi M, Hendler T, Goebel R, Linden DEJ (2005) Are numbers special? The comparison systems of the human brain investigated by fMRI. Neuropsychologia 43:1238-1248.

Kelley AE (2004) Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 44:161-179.

Kroger JK, Sabb FW, Fales CL, Bookheimer SY, Cohen MS, Holyoak KJ (2002) Recruitment of Anterior Dorsolateral Prefrontal Cortex in Human Reasoning: a Parametric Study of Relational Complexity. Cerebral Cortex 12:477-485.

Luo Q, Perry C, Peng D, Jin Z, Xu D, Ding G, Xu S (2003) The neural substrate of analogical reasoning: an fMRI study. Cognitive Brain Research 17:527-534.

Martin N, Alsop B (2004) Transitive inference and awareness in humans. Behavioral Processes 67:157-165.
McGonigle B, Chalmers M (1986) Representations and strategies during inference. In: Reasoning and discourse processes (Myers T, Brown K, McGonigle B, eds), pp 141-164. London: Academic Press.
Meredith GE, Totterdell S (1999) Microcircuits in nucleus accumbens' shell and core involved in cognition and reward. Psychobiology 27:165-186.
Miller MI, Beg MF, Ceritoglu C, Stark C (2005) Increasing the power of functional maps of the medial temporal lobe by using large deformation diffeomorphic metric mapping. Proc Natl Acad Sci U S A 102:9685-9690.
Mirenowicz J, Schultz W (1994) Importance of unpredictability for reward responses in primate dopamine neurons. Journal of Neurobiology 72:1024-1027.
O'Doherty JP (2004) Reward representations and reward-related learning in the human brain: insights from neuroimaging. Current Opinion in Neurobiology 14:769-776.
Pagnoni G, Zink CF, Montague PR, Berns GS (2002) Activity in human ventral striatum locked to errors of reward prediction. Nature Neuroscience 5:97-98.
Pinel P, Dehaene S, Riviere D, LeBihan D (2001) Modulation of parietal activation by semantic distance in a number comparison task. Neuroimage 14:1013-1026.
Pinel P, Piazza M, Le Bihan D, Dehaene S (2004) Distributed and Overlapping Cerebral Representations of Number, Size and Luminance during Comparative Judgments. Neuron 41:983-993.
Poldrack RA, Rodriguez P (2004) How do memory systems interact? Evidence from human classification learning. Neurobiology of Learning & Memory 82:324-332.
Pruessner JC, Li LM, Serles W, Pruessner M, Collins DL, Kabani N, Lupien S, Evans AC (2000) Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. Cerebral Cortex 10:433-442.
Rapp PR (2004) Who's the fairest of them all? Role of the human hippocampus in the relational organization of memory. Hippocampus 14:141-142.
Rapp PR, Kansky MT, Eichenbaum H (1996) Learning and memory for hierarchical relationships in the monkey: effects of aging. Behavioral Neuroscience 110:887-897.
Redgrave P, Prescott TJ, Gurney K (1999) Is the short-latency dopamine response too short to signal reward error? Trends Neurosci 22:146-151.
Sandrini M, Rossini PM, Miniussi C (2004) The differential involvement of inferior parietal lobule in number comparison: a rTMS study. Neuropsychologia 42:1902-1909.
Sargolini F, Roullet P, Oliverio A, Mele A (1999) Effects of lesions to the glutamatergic afferents to the nucleus accumbens in the modulation of reactivity to spatial and non-spatial novelty in mice. Neuroscience 93:855-867.
Schultz W (1999) The reward signal of midbrain dopamine neurons. News Physiol Sci 14:249-255.
Setlow B (1997) The nucleus accumbens and learning and memory. The Journal of Neuroscience Research 49:515-521.
Setlow B, Schoenbaum G, Gallagher M (2003) Neural encoding in ventral striatum during ofactory discrimination learning. Neuron 38:625-636.

Shrout PE, Fleiss JL (1979) Intraclass correlations: uses in assessing rater reliability. Psychological Bulletin 86:420-428.

Simon O, Kherif F, Flandin G, Poline J-B, Riviere D, Mangin JF, Le Bihan D, Dehaene S (2004) Automatized clustering and functional geometry of human parietofrontal networks for language, space and number. Neuroimage 23:1192-1202.

Squire L (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychological Review 99:195-231.

Strasser R, Ehrlinger JM, Bingman VP (2004) Transitive Behavior in Hippocampal-Lesioned Pigeons. Brain, Behavior & Evolution 63:181-188.

Titone D, Ditman T, Holzman PS, Eichenbaum H, Levy DL (2004) Transitive inference in schizophrenia: impairments in relational memory organization. Schizophrenia Res 68 (2-3):235-247.

Todd CL, Grace AA (1999) Modulation of ventral tegmental area dopamine cell activity by the ventral subiculum and entorhinal cortex. Annals New York Academy of Sciences 877:688-690.

Van Elzakker M, O'Reilly RC, Rudy JW (2003) Transitivity, flexibility, conjunctive representations, and the hippocampus. I. An empirical analysis. Hippocampus 13:334-340.

von Fersen L, Wynne CDL, Delius JD, Staddon JER (1991) Transitive inference formation in pigeons. Journal of Experimental Psychology: Animal Behavior Processes 17:334-341.

Wynne CDL (1998) A minimal model of transitive inference. In: Models of action (Wynne CDL, Staddon JER, eds), pp 269-307. Mahwah, N.J.: Lawrence Erlbaum Associates.

Zink CF, Pagnoni G, Martin ME, Dhamala M, Berns GS (2003) Human striatal responses to salient nonrewarding stimuli. The Journal of Neuroscience 23:8092-8097.

Zink CF, Pagnoni G, Martin-Skurski ME, Chappelow JC, Berns GS (2004) Human striatal responses to monetary reward depend on saliency. Neuron 42:509-517.

Chapter 4: Challenges in imaging medial temporal lobe function with fMRI

Introduction

In the previous chapter, I described the event-related experiment that examined the neural correlates of TI. For this and all subsequent experiments, we used a 3.0T scanner instead of the 1.5T scanner that had been used for the block-design TI study described in Chapter 1.

Higher field strengths confer several important advantages. As static field strength increases linearly, raw signal increases quadratically. Thermal noise, in contrast, scales linearly with the field strength. The raw signal-to-noise ratio (SNR), therefore, increases linearly with the field strength (Kruger et al., 2001; Huettel et al., 2004). In addition, the BOLD (blood-oxygenation level dependent) effect, which refers to the change in signal intensity based on blood oxygenation levels, which in turn depends on local neuronal activity, increases linearly with the static magnetic field strength in large vessels, but quadratically in small vessels and capillaries. Thus, higher field strengths provide a significant improvement in sensitivity to oxygenation changes in small vessels and capillaries, which are located closer to the origin of neuronal activity than larger vessels (Krasnow et al., 2003). This advantage has been confirmed by findings of superlinear increase in BOLD sensitivity with increasing field strength (Kruger et al., 2001).

Increasing field strength, however, also leads to susceptibility artifacts, namely geometric distortions and signal losses, in the functional images. Because of susceptibility artifacts, signal from parts of the brain may be shifted to neighboring voxels or may be lost entirely. This problem also leads to a mismatch between the participant's distorted

functional image and the corresponding structural image, which is used to localize activations detected in the functional images.

In this chapter, I will first describe the origin of these artifacts and the brain areas most affected by them, one of which is the medial temporal lobe (MTL) region, an area of primary interest for TI. I will then turn to evaluating the impact of these artifacts in the images that were acquired for the experiments described in this thesis. Next, I will discuss strategies that have been suggested for reduction and elimination of these artifacts. I will then describe the effect of using one of these strategies, field map-based correction, on the images acquired in the TI experiments.

Susceptibility artifacts

Functional magnetic resonance imaging (fMRI) is based on the sensitivity of the MR signal to changes in blood oxygenation levels. Blood oxygenation levels, in turn, depend on the metabolic demand of active neurons. When neurons in a particular location in the brain become more active and extract more oxygen, the local ratio of oxygenated/deoxygenated hemoglobin (oHb/dHb) transiently decreases. The local vasculature compensates by increasing the blood flow, which raises the level of oxygenated hemoglobin. Because this change is an overcompensation, the oHb/dHb ratio goes up within a few seconds of the original neuronal event. As oHb is diamagnetic and dHb is paramagnetic, the increase in oHb/dHb ratio leads to reduced local magnetic susceptibility, which in turn leads to an increase in the MR signal that can be measured (Huettel et al., 2004).

Local magnetic susceptibility determines how quickly the signal decays in transverse orientation following its disturbance from its original alignment along the main magnetic field of the scanner. The speed of the decay is governed by the transverse relaxation constant T_2^*, which reflects both spin-spin interaction and changes in spin precession frequency due to local magnetic susceptibilities.

Signal intensity (SI) has the following dependence on T_2^*:

$$SI \propto \rho \cdot \exp\left(\frac{-TE}{T_2^*}\right) \quad [1]$$

Here, ρ is the spin (proton) density and TE is the echo time, which is set by the scanner operator. It can be shown that signal is maximized when TE is equal to T_2^* (Deichmann et al., 2002). The local magnetic susceptibility, in part, scales with the local oHb/dHb ratio; increasing this ratio leads to prolonged T_2^*, which in turn leads to increased signal intensity as given by the equation above. This increased signal intensity is the basis of the BOLD effect.

Using a first-order Taylor expansion of the equation above, it can be shown that BOLD sensitivity (BS) is proportional to TE and SI as follows (Deichmann et al., 2002):

$$BS = TE \cdot SI \propto TE \cdot \exp\left(\frac{-TE}{T_2^*}\right) \quad [2]$$

This equation assumes a homogenous magnetic field throughout the brain, where BOLD sensitivity is dependent only on local susceptibility changes due to the altered oHB/dHb ratio. Moreover, assuming that the magnetic field throughout the brain is homogeneous,

this signal change can be localized precisely using the three magnetic field gradients used in MRI: slice-selection, frequency readout, and phase-encoding gradients (Moonen and Bandettini, 2000).

Unfortunately, the magnetic field throughout the brain is inherently inhomogeneous leading to difficulty in both signal detection and accurate signal localization. This difficulty is especially prominent in those parts of the brain where the inhomogeneities are most severe (Ojeman et al., 1997; Jezzard and Clare, 1999; Cordes et al., 2000; Hutton et al., 2002). Magnetic field inhomogeneities arise in brain tissue that neighbors sharp interfaces, thick bones, and air-filled cavities. The most problematic areas in this respect are the inferior frontal region and the inferior temporal lobe, especially in its lateral aspect (Ojeman et al., 1997). In the inferior frontal region, the magnetic field inhomogeneity is induced by the large air-filled cavities of nasal sinuses. In the temporal region, it is caused by the dense petrous bone, the auditory canals, and mastoid air cells (Cordes et al., 2000). The inhomogeneity arises due to different magnetic properties of the materials present in the image field. Because of its high water content, brain tissue acts as a diamagnetic material developing small magnetizations counteracting the applied field in the scanner. Thus, brain tissue behaves as a material with a weak negative susceptibility. Bone and air have essentially zero susceptibility. When these different materials are in close proximity to each other, especially with abrupt and structurally complicated interfaces, the magnetic field becomes inhomogeneous. The inhomogeneity, in turn, causes macroscopic susceptibility variations within the scanner field (Cusack and Osswald, 2003).

Magnetic field inhomogeneities have a profound effect on the echo-planar imaging (EPI) technique, which is typically used for fMRI. EPI gained its popularity due to its ability to rapidly and sensitively detect signal changes associated with brain activation. EPI uses fast-switching gradients that enable acquisition of a whole slice of raw data after a single excitation pulse. The cost of this ability to acquire a whole slice at a time is the relatively long readout time in this type of imaging. The long readout time and the use of weak phase-encoding gradients render EPI especially sensitive to the effects of magnetic field inhomogeneities (Lipschutz et al., 2001; Hutton et al., 2002).

In MRI, the phase evolution of a voxel in the image depends on the local magnetic field it experiences during the excitation pulse. Macroscopic susceptibility variation across a voxel due to the local magnetic field inhomogeneity causes intravoxel dephasing. Intravoxel dephasing can lead to either a complete loss of signal or a mis-localization of the signal to a different voxel than the one in which the signal originated. Intravoxel dephasing happens as a result of the different magnetic fields experienced by spins in different locations within the voxel. These spins then precess at slightly different rates so that they go out of phase from one another over time. If the spins go completely out of phase, complete signal loss ensues. If, on the other hand, local susceptibility effects add to or subtract from the encoded phase of a particular voxel, the signal will be assigned to a different voxel than the one from which the signal originated. These effects are proportional to the overall strength of the overall magnetic field of the scanner, the local field inhomogeneity, and the data acquisition time. Because the total readout time is

relatively long in EPI, this type of imaging is particularly sensitive to intravoxel dephasing resulting in "susceptibility artifacts" (Ojeman et al., 1997; Hutton et al., 2002).

Susceptibility artifacts are particularly prominent in the phase-encoding direction in EPI because of two effects. First, the weaker the imaging gradient across a plane, the more it is affected by field inhomogeneities and gradients caused by macroscopic susceptibility effects. The gradients used for phase-encoding are much weaker than those used for slice selection and frequency encoding, rendering the phase-encoding direction more prone to susceptibility artifacts (Ojeman et al., 1997). Second, the time between the acquisitions of adjacent *k* space points can differ substantially between two dimensions because a whole slice of raw data is acquired in a single EPI image. As a result, distortions arise in the direction where the acquisition time between adjacent points is the greatest, the phase-encoding direction (Hutton et al., 2002). Susceptibility artifacts also depend on basic imaging parameters. As explained above, they are caused by spin dephasing due to macroscopic susceptibility effects. Spin dephasing increases with TE and voxel volume. The longer the TE, the greater the opportunity for individual spins within a voxel to go out of phase. The larger the voxel, the larger the variations in Larmor frequencies across the voxel and the greater the dephasing. Reducing the TE and the voxel size, however, comes at a cost. A relatively long TE is beneficial for BOLD sensitivity, and larger voxels enable coverage of a larger area at a given time resolution.

The impact of magnetic field inhomogeneities on BS can be quantified by the following equation, which takes into account both through-plane (along the slice direction) and in-plane (perpendicular to the slice direction) effects (Stocker et al., 2006):

$$BS \propto I_P(TE) \cdot \left(\frac{TE}{Q^2}\right) \cdot \exp\left(\frac{-TE}{Q \cdot T_2^*}\right) \text{ [3]}$$

In the above expression, I_p quantifies signal loss due to dephasing induced by through-place susceptibility gradient $\mathbf{G_s}$. It is given by the following equation:

$$I_P(TE) = \int_{\Delta z} P(z) \cdot \exp\left(I \cdot \gamma \cdot TE \cdot z \cdot \left(\vec{G}_S \cdot \hat{h}_z\right)\right) dz \text{ [4]}$$

In the equation above, Δz is the slice thickness, P(z) is the slice profile of the RF pulse, γ is the gyromagnetic ratio, and $\mathbf{n_z}$ is the normal vector (parallel to the slice direction).

In equation [3] above, the BOLD sensitivity changes that result from in-plane susceptibility gradients are given by the following dimensionless parameter:

$$Q = 1 + \frac{\gamma \cdot \tau}{2 \cdot \pi} \cdot FoV \cdot \left(\vec{G}_S \cdot \hat{h}_P\right) \text{ [5]}$$

In the expression above, τ is the EPI echo spacing, FoV is the field-of-view and $\mathbf{n_p}$ is the unit vector pointing in the phase-encode direction.

It can be seen from equation [3] above that BOLD sensitivity changes not only in magnitude but also in its maximum (which is the optimal TE) shifting to $TE = Q \cdot T_2^*$, even for the case when $\vec{G}_S \cdot \hat{h}_z$. If through-plane gradients (i.e. this dot product of the vectors $\mathbf{G_s}$ and $\mathbf{n_z}$ is non-zero) are present, I_p(TE) decays with increasing TE which causes an additional shift of the optimal TE to lower values. An important case differentiation is given by the sign of $\vec{G}_S \cdot \hat{h}_P$. First, the sign is positive if the susceptibility gradient component in the phase encode direction is parallel to the direction of the blipped phase-encode gradient (the gradient pulse between readout gradients).

Thus, Q>1, and, therefore, BOLD sensitivity decreases, and the optimal TE shifts to a higher value. Second, anti-parallel susceptibility gradient and blip gradient components yield Q<1 (i.e., the sensitivity increases, and the maximum shifts to lower TE values). The shift of the maximum, however, is due to a shifted echo caused by susceptibility gradients. If the echo formation is shifted outside the acquisition window, TA, the signal is lost completely (Stocker et al., 2006):

$$Q<1 \text{ and } \left|TE - \frac{TE}{Q}\right| > \frac{TA}{2} \Rightarrow BS = 0 \quad [6]$$

In summary, the component of the local susceptibility gradient along the slice direction causes a through-plane spin dephasing and thus a loss in image sensitivity. Its extent depends on the magnitude of the local susceptibility gradient along that direction, the slice thickness, the slice profile, and the TE (equation [4] above). The component of the local susceptibility gradient in the in-plane direction causes a distortion of the data in *k* space. This effect occurs mainly in the phase encode direction because of the lower data sampling rate. The three major in-plane effects are the following: the center of the region is shifted in the image, and the size of the region is changed by the factor Q, the apparent spin density and thus the image intensity is changed by the factor 1/Q, and TE is changed by the factor 1/Q. Depending on the direction of the local susceptibility gradient (parallel or anti-parallel), the shift in TE can result either in signal intensity reduction or complete signal loss. Because of the factor (TE/Q^2) in equation [3] above, BOLD sensitivity is reduced by Q^2, and is thus more affected by in-plane susceptibility gradients than the image intensity alone, which scales with 1/Q (Deichmann et al., 2002).

As mentioned before and pertinent to TI experiments described in this work, two of the areas that are most severely affected by susceptibility artifacts are the inferior temporal lobe and MTL. Susceptibility artifacts in this area may cause signal dropout and geometric distortions or mis-localization of the signal. The sections of the inferior temporal lobe and MTL most affected by these artifacts are the anterior hippocampal region, fusiform gyrus, perirhinal cortex and some parts of the parahippocampal cortex (Cordes et al., 2000; Zeineh et al., 2000; Lipschutz et al., 2001; Greicius et al., 2003). In these areas, significant signal dropout occurs. Quantitatively, the medial inferior temporal area was found to have the most inhomogeneity of up to 0.8 ppm in the axial plane, while the coronal and sagittal planes were not affected as severely (0.2 and 0.3 ppm, respectively). Using a field of view (FoV) of 24x24 cm at 1.5T, 1 ppm inhomogeneity resulted in a geometric distortion of approximately 2 voxels, corresponding to approximately 8 mm in the phase-encoding direction (Cordes et al., 2000). Given that the structures relevant for memory processing in MTL are relatively small (the hippocampus, for example, only extends 40 mm along its long axis), this inhomogeneity can cause profound error in the location of the signal (Greicius et al., 2003).

One further consequence of the geometric distortions caused by the macroscopic susceptibility effect is the difficulty in achieving accurate registration between a functional activation map calculated from EPI time series and a high-resolution anatomical image, whichis not subject to the distortions. In regions with susceptibility artifacts, voxels are shifted from their true positions and may be stretched or compressed, thus distorting the intensity map of the activation. Even one or two voxel mismatches can

be important because the dimension of a single voxel can extend several millimeters. In the next section, I will describe a quantitative evaluation of misregistration in hippocampal position between functional and structural images acquired for the study described in Chapter 4.

Accuracy of registration between functional and structural images

The geometric distortions and signal loss due to magnetic field inhomogeneities render accurate registration between the distorted functional EPI images and the undistorted structural images difficult. In this section, I will examine the degree of mismatch in the hippocampus between these two types of images present in the event-related TI study described in Chapter 4.

In this study, participants were scanned in a Siemens 3.0 Tesla Trio high-speed echo-planar imaging device. The structural image was a three-dimensional MP-RAGE (TR = 2100 ms, TE = 2.74 ms, 128 slices, FoV = 256 mm, 1.0 x 1.0 x 1.3 mm resolution). The EPI functional images were acquired in transversal acquisition with orientation perpendicular to the long axis of the hippocampus (TR = 2000 ms, TE = 30 ms, 34 slices, FoV = 200 mm, 3.1 x 3.1 x 3.0 mm resolution, anterior-posterior phase encode direction).

The functional images were coregistered to the structural image using the coregistration utility in SPM2 (Wellcome Department of Imaging Neuroscience, London, UK) (Figure 1). SPM2 uses multimodal coregistration that first performs intermediate within modality registration to two template images that are already in register. A least-squares

minimization is then carried out to determine affine transformation that maps between the templates and the images. By incorporating suitable constraints, a rigid body transformation that maps directly between the two images is then extracted from these more general affine transformations (Ashburner and Friston, 1997).

To determine the degree of misregistration of the hippocampus in two types of images, I chose two landmarks near the hippocampus that could be reliably identified in structural and functional image in five participants. Because the hippocampus cannot be discerned on the functional EPI images, we chose landmarks at the border of posterior aspect of lateral ventricles and brain tissue near the hippocampus (Figure 2).

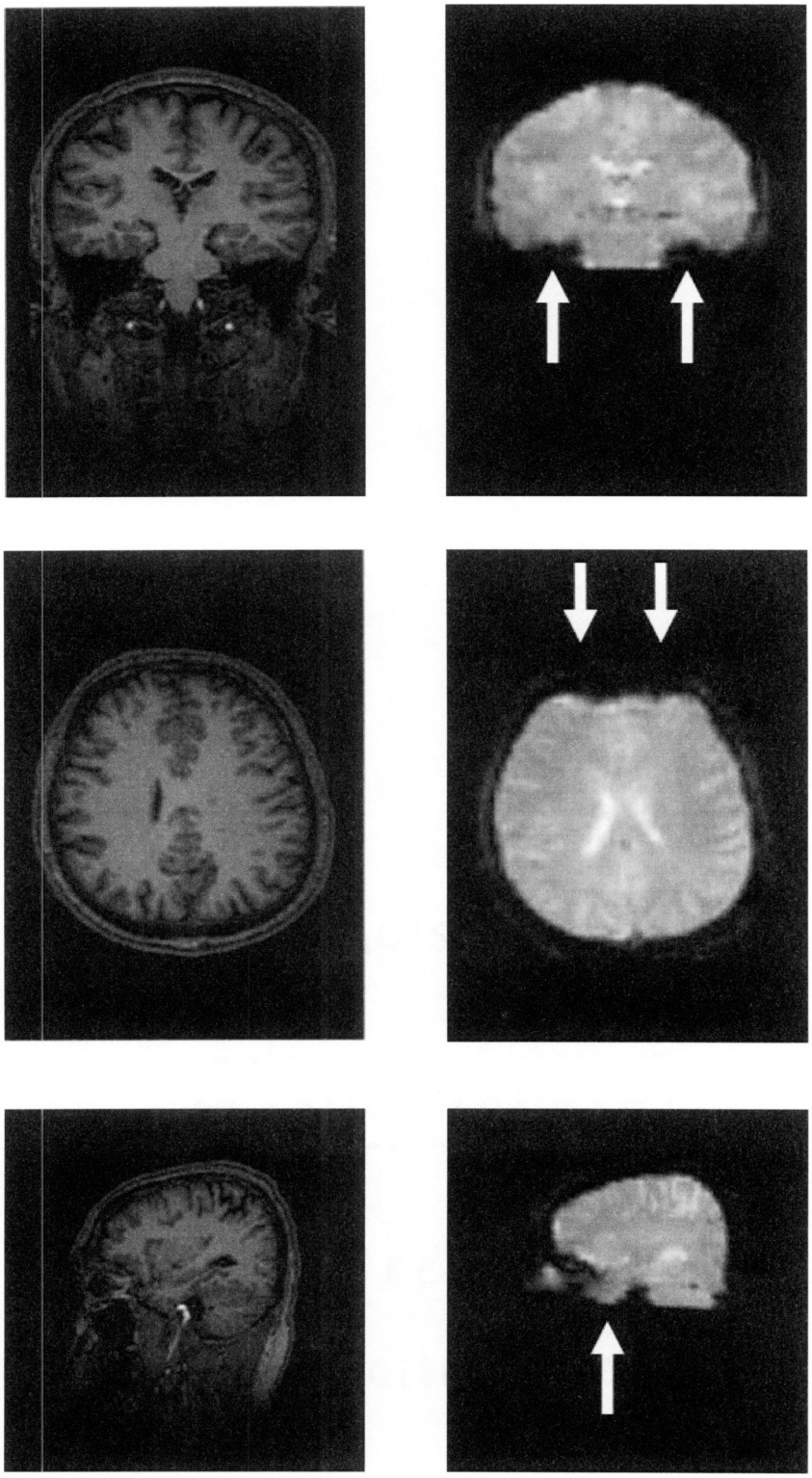

Figure 1. Coregistered functional and structural images. These images were coregistered using SPM2. Note the signal loss in the parahippocampal gyrus and prefrontal regions and distortion in the inferior temporal lobe (arrows).

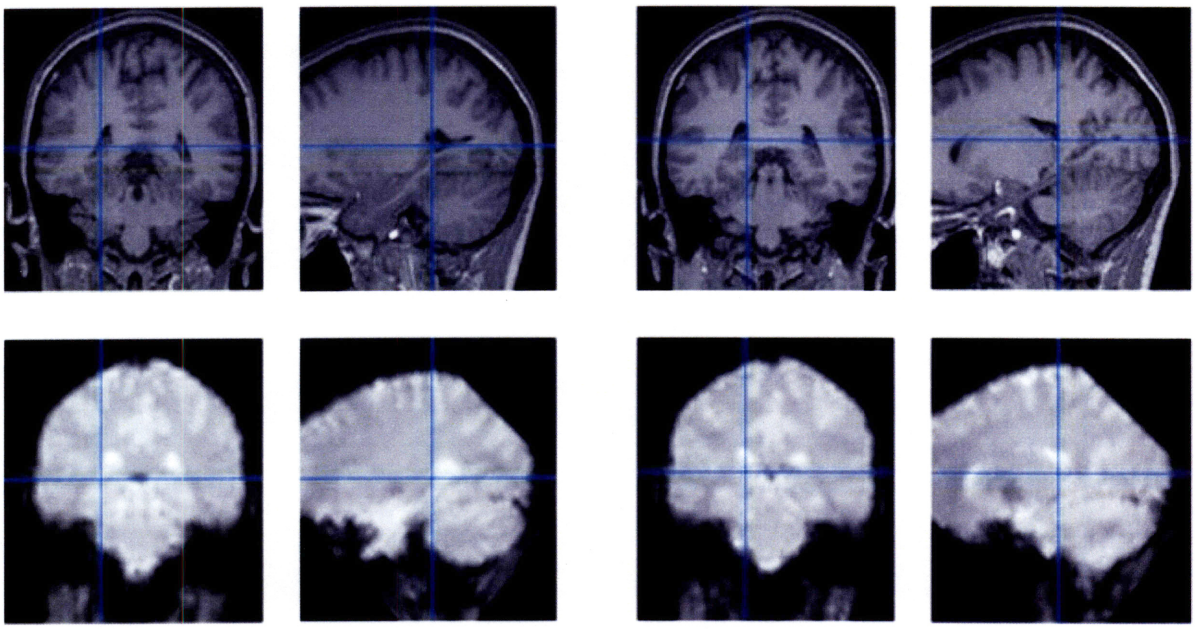

Figure 2. Anatomical landmarks used for assessing coregistration mismatch between structural and functional images.

Because of the lower resolution of the functional images compared with the structural image (3.1 mm vs. 1.3 mm), the landmarks could be identified only approximately in the functional images within an error margin of one voxel. The average deviations in landmark locations between the two types of images across the five participants examined were 2.9 ± 0.7 and 3.2 ± 1.3 mm in the left and right hemisphere, respectively. These values are only an estimate of the registration mismatch because the hippocampus cannot be reliably identified on functional images. They suggest, however, that the mismatch is on the order of one voxel size, and thus does not exceed the resolution of the images.

Having examined the degree of mismatch between coregistered functional and structural images due to susceptibility artifacts, I will now turn to the methods that have been proposed for correcting EPI images for these artifacts.

Correcting for susceptibility artifacts

Given the prominence of susceptibility artifacts in fMRI, it is not surprising that a multitude of approaches have been suggested to correct for them at different stages of data acquisition and processing steps. First, the basic imaging parameters chosen for the experiment (field strength, geometry including voxel size and orientation, and TE) all affect the magnitude of susceptibility artifacts. Susceptibility artifacts become more severe as field strength increases, but high magnetic field strengths are desirable to maximize signal-to-noise ratios. The larger the voxel size, the larger the dephasing gradient within a voxel and the larger the signal loss and distortion (Hennig et al., 2003). A recent study showed that it was beneficial to reduce the in-plane voxel dimension to 1.7 mm while maintaining slice thickness of 2.0-2.5 mm for functional imaging of the amygdala, a brain structure notoriously plagued by susceptibility artifacts (Chen et al., 2003).

Because the phase-encode direction is the one most affected by magnetic field inhomogeneities, it is desirable to set this direction along the smallest susceptibility gradient. It is, therefore, beneficial to set the slice direction along the largest susceptibility gradient. So, if the susceptibility gradient is largest in the inferior-superior

direction such as in MTL, it is desirable to use coronal image orientation. Because of current gradient coil designs, however, if one uses the same imaging parameters as one would for imaging using axial orientation, participants may experience peripheral neural stimulation when the EPI frequency encoding is along the inferior-superior direction (Chen et al., 2003). One way to avoid this problem is to use axial orientation with anterior-posterior phase encoding, but to tip the image orientation by nearly 45 degrees (i.e. perpendicular to the long axis of the hippocampus). I chose this approach for Experiment 3 described in the next chapter.

Another imaging parameter that affects the magnitude of susceptibility artifacts is TE. First, the longer the TE, the more opportunity for spins to dephase in the presence of a local susceptibility gradient. Second, susceptibility gradients most often tend to decrease the effective TE. Thus, the optimal TE in areas affected by susceptibility gradients will be lower than in other brain areas. One can, in fact, obtain a T_2^* map throughout the brain using a multiecho gradient-echo scan prior to functional EPI imaging and determine the appropriate TE for each brain region. Using this approach, it has been shown that the optimal TE at 1.5T is 40 ms for the amygdala but 60 ms for the rest of the brain. One can then use slice-dependent TE to reduce artifacts in the amygdala (Stocker et al., 2006). Even though there may be less signal loss at lower TE, BOLD sensitivity still suffers as predicted by equation [3] above and confirmed by recent studies (Gorno-Tempini et al., 2002). This effect is also important for structures where the baseline BOLD signal differs due to different degrees of signal intensity loss. One such structure is the hippocampus, where the anterior portion is more affected by magnetic inhomogeneities and, thus, has a

lower baseline signal than the posterior portion. Because of the lower baseline signal, BOLD sensitivity will be lower in the anterior portion (Powell et al., 2004). In the study described in the previous chapter, however, activation was detected only in the anterior portion of the left hippocampus for non end-item vs. end-item TI contrast.

Second, one can use shimming to achieve global and local field optimization. Shimming adjusts the scanner field gradients to minimize field inhomogeneity within the brain. Shimming can be performed during pre-scan calibration; automated shimming methods that first perform brain segmentation to remove non-brain tissue from the optimization routine have already been suggested (Wilson, 2002). Alternatively, real-time autoshimming methods arenow available that detect linear shim changes due to motion during scanning and use a shim-compensated EPI pulse sequence for dynamic correction of linear shim changes (Ward et al., 2002). One can also use "passive shims", which are strongly diamagnetic materials (e.g., continuously nucleated pyrolytic graphite) placed in the roof of the oral cavity to reduce susceptibility gradients in the orbital frontal cortex. While these devices can lead to detection of previously unseen activation in the orbital frontal cortex, they tend to induce increased swallowing and thereby cause increased head motion (Osterbauer et al., 2006). Moreover, they do not tend to reduce susceptibility artifacts in temporal cortex (Wilson et al., 2002).

It has been noted above in connection with equation [4] that through-plane gradients (gradients along the slice direction) also cause signal loss. Unlike susceptibility gradients in the phase-encode direction, however, gradients along the slice direction can be

compensated for. One can counteract the effect of the susceptibility gradient $\mathbf{G_{ss}}$ by a preparation gradient $\mathbf{G_{ps}}$ applied for a duration τ that satisfies the following equation:

$$G_{PS} \cdot \tau + G_{ss} \cdot TE = 0 \quad [7]$$

The magnitude of the preparation gradient can be determined by incrementing the amplitude of the preparation gradient and collecting a field inhomogeneity map of the whole brain. Because this method corrects only for inhomogeneities along the slice direction, one has to choose the slice direction so that it is aligned with the largest inhomogeneity gradient. Further, this method merely accounts for signal loss and does not correct for slice warping (Cordes et al., 2000), and it can can reduce BOLD sensitivity in areas unaffected by susceptibility gradients (Deichmann et al., 2002).

Besides shimming, another intervention on the hardware end is the use of parallel imaging. Parallel imaging exploits the spatial inhomogeneity in the sensitivity of receiver coil arrays to reduce the number of conventional (cycling of gradient pulses) phase encoding steps. The upper limit of this reduction is determined by the number of coils used, but in practice a reduction factor (R) of 2 is commonly used. One such parallel technique is SENSE (sensitivity encoding), which requires a full FoV reference scan and accomplishes the unfolding of aliased single-coil images in image space. The reconstruction process that consists of non-unitary operations leads to spatially varying noise amplification, however (Schmidt et al., 2005). Because of the faster *k* space traversal, parallel imaging can reduce typical EPI susceptibility artifacts. It has been demonstrated that with R set to 2, distortions and blurring were substantially reduced,

while the time-course signal-to-noise and statistical power were hardly affected (Preibisch et al., 2003; Schmidt et al., 2005). In addition, instead of using the classical right-angled traversal of *k* space in EPI, one can cover *k* space by spiraling in or out. This technique is exploited by spiral imaging. Here, susceptibility gradients lead to blurring rather than warping, but the raw data can be corrected prior to reconstruction to account for this blurring (Hennig et al., 2003).

Third, instead of shimming techniques or hardware adjustments, one can also acquire additional images and use them for correction. Because the distortions reverse direction as the direction of the phase encode gradient is reversed, one can acquire images using phase encoding gradients of alternating polarity and then perform a weighted average of the resulting images in real time (Weiskopf et al., 2005).

Another kind of extra images is phase field maps, which can be obtained by acquiring either a resting symmetric/asymmetric spin-echo (Wilson, 2002) or a pair of EPI images with two different TEs (Hutton et al., 2002). The latter approach is based on the computation of a phase map from gradient-recalled EPIs with two different TEs. Because the range of phase values is limited to $[-\pi,\pi]$, phase wrapping may occur depending on how far apart the two TEs are. Unwrapping the phase map has proven to be one of the main challenges for widespread application of phase maps (Windischberger et al., 2004). Once the phase map is unwrapped and properly scaled, one can calculate the change in local magnetic field B_0 due to local inhomogeneities using the following equation:

$$\Delta B_0(x,y,z) = \frac{\Delta\Theta(x,y,z)}{2\pi\gamma\Delta TE} \quad [8]$$

Here, $\Delta B_0(x,y,z)$ is measured in Hz, $\Delta\Theta(x,y,z)$ is the phase evolution over time ΔTE and γ is the gyromagnetic ratio. One can then use this field map to compute a map of one-dimensional voxel shifts due to susceptibility gradients as follows:

$$\Delta y(x,y,z) = \gamma \cdot \Delta B_0(x,y,z) \cdot T_{acq} \quad [9]$$

In the above expression, T_{acq} is the readout time for a slice of raw data and Δy is the map of one-dimensional voxel shifts in the y-axis. This technique can account for compression or stretching of voxels due to geometric distortions, but not for signal loss (Hutton et al., 2002). Further, it is possible that signal from two discrete voxels in real space may be mapped to a single voxel in distorted space. Field maps do not provide a way to discern the contribution of signal from two different voxels that are mapped onto a single voxel in distorted space. Also, the appropriate voxel brightness in the undistorted space cannot be determined exactly and must, therefore, be approximated. Field maps are notoriously difficult to obtain near edges or in regions with high inhomogeneity. In addition, in the presence of large field inhomogeneity, the phase offset may not scale linearly with TE and equation [8] may no longer apply (Zeng and Constable, 2002).

As an alternative, the use of point spread function (PSF) mapping has been suggested. This method requires additional acquisitions with phase-encoding gradients applied in the x, y and/or z directions to map the 1D, 2D or 3D PSF for each voxel. These PSFs encode spatial information about the distortion and overall distribution of intensities from a

single voxel. The measured image is the convolution of the undistorted density and the PSF. Measuring the PSF allows distortions in geometry and intensity to be corrected. The major advantage of PSF over field mapping is that it can assign the correct voxel intensity when unwarping the image even in the case of highly overlapping voxels (Zeng and Constable, 2002).

To date, the PSF method has not found widespread application compared with field mapping techniques, which have now been implemented in several analysis packaged including SPM2 and FSL. I will evaluate the utility of field mapping on the Siemens Trio 3.0T scanner, the machine used for two of the three studies described in this thesis.

Field map correction in TI datasets

Of the methods that can be used to correct for susceptibility artifacts in EPI images, the one that was available to us was the field map method. This method was evaluated on the TI dataset that has already been used in this chapter for evaluation of registration mismatch between functional and structural images. This dataset will be described further in Chapter 4.

The strategy used here was to outline the hippocampi bilaterally on the undistorted 3-D MP-RAGE and then forward distort them using a reversed field map to determine the extent to which application of field maps alters hippocampal size and position. The main structural image, the functional images, and the field map images were first coregistered

using SPM2. Anatomical regions-of-interest (ROIs) were then created for the hippocampus bilaterally on the main structural image as described in Chapters 2 and 4. A phase difference map was obtained from the two phase images. It was then scaled to the range of [-π, π] and converted to a reversed field map using a modified equation [8] from above:

$$\Delta\theta_{rev}(x,y,z) = \frac{-\Delta\Theta(x,y,z)}{2\pi\Delta TE} \quad [10]$$

Using the FieldMap toolbox in SPM2 (Hutton and Anderson, 2004), the reversed field map was first applied to the GRE image to determine the degree of distortion that could be corrected using the field map (Figure 3).

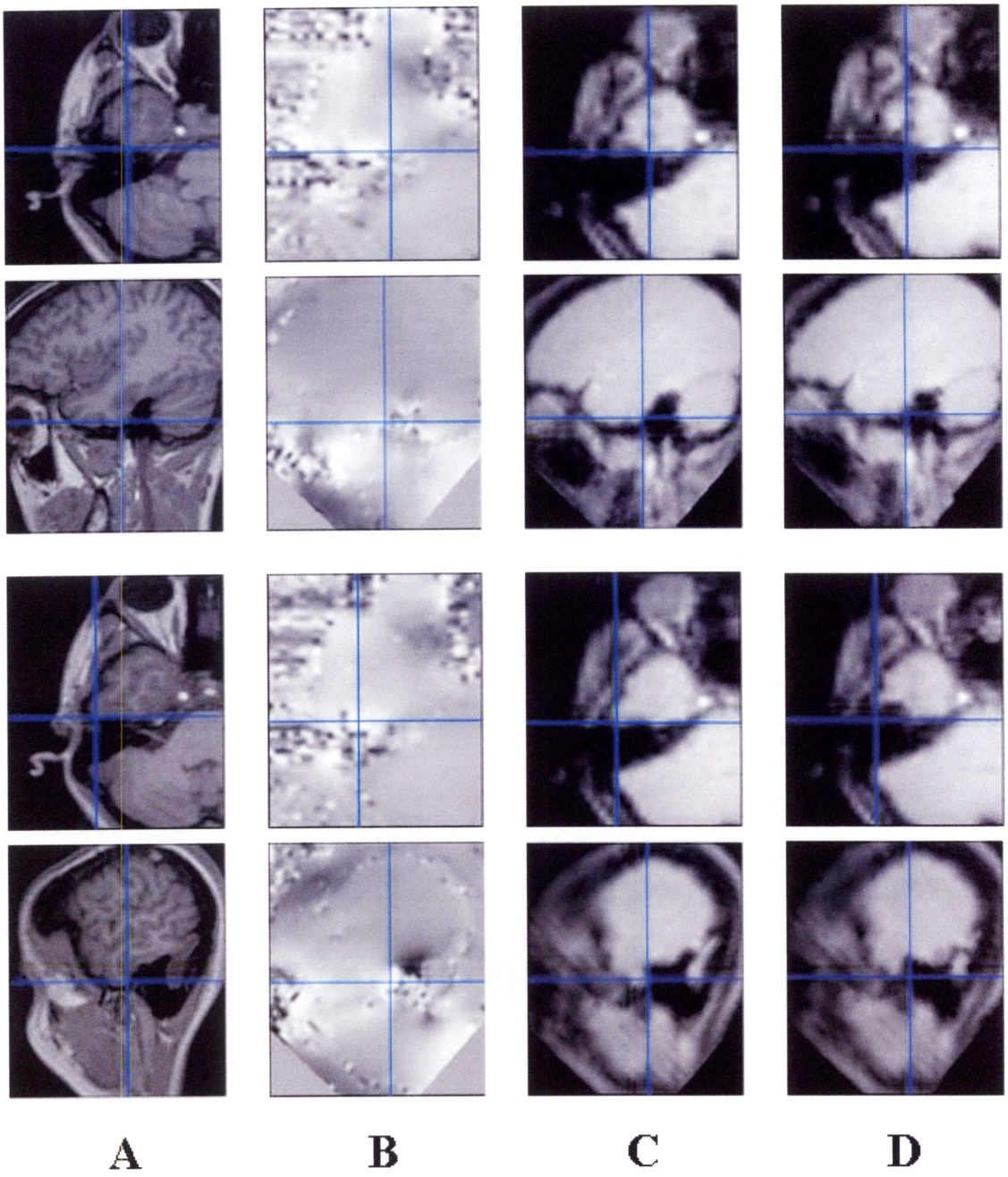

Figure 3. Application of field maps to distort undistorted anatomical images. Reverse fieldmap in Hz (B) was applied to an undistorted GRE image (C) to evaluate the degree of distortion that can be shifted by application of the reverse fieldmap in the resulting distorted GRE image (D). The distorted GRE image clearly deviates from the undistorted 3-D structural image (A).

Qualitatively, applying the reverse field map to an undistorted image results in a mismatch between the main structural and the distorted structural image, mainly in the lateral aspect of the inferior portion of the temporal lobe. In the experiments described in this thesis, the area of concern is the medial aspect of the temporal lobe, especially the hippocampus. Specifically, the crucial question is the accuracy of registration between the anatomical ROIs of the hippocampus and the position of the hippocampus in functional images. The reverse field map was applied to the hippocampal ROIs to assess the effect of using field maps on the position of the hippocampus (Figure 4).

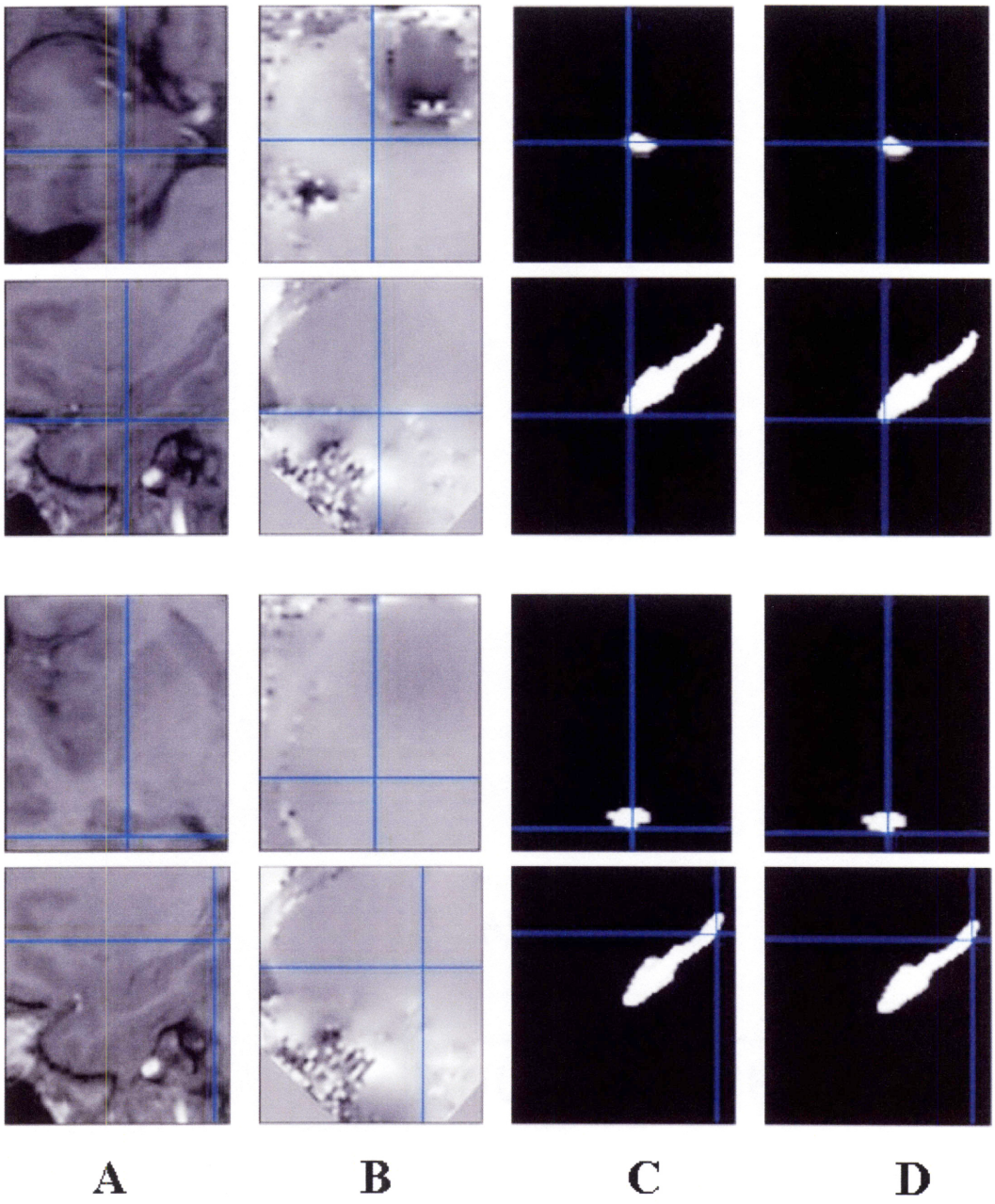

Figure 4. Application of field maps to distort undistorted hippocampal region-of-interest images. Reverse fieldmap in Hz (B) was applied to hippocampal ROI images (C) to evaluate the degree of distortion that can be shifted by application of the reverse fieldmap application in resulting the distorted ROI images (D). The distorted ROI images (D) show little deviation from the original ROI images (C) and the 3-D structural image (A).

The inferior, superior, anterior, and posterior borders were identified on both the original and distorted images and used as landmarks. The average deviation between the original and distorted images was 1.2 ± 0.2 mm on the left and 1.3 ± 0.5 mm on the right. The magnitude of the deviation is, therefore, on the order of one voxel size and, therefore, the resolution of the structural images. Application of the field map changed the position of the hippocampus by less than the limit of the resolution of the functional images. The effect of field map application is, therefore, negligible.

Conclusion

Higher magnetic field strengths afford improved SNR at the cost of larger susceptibility artifacts, which are signal losses and geometric distortions. Two of the three experiments described in this thesis were carried out at high field strength (3.0T). Because the MTL including the hippocampus, is particularly affected by susceptibility artifacts, and because the hippocampus is the structure of major interest in this work, we set out to determine the magnitude of distortions and the degree to which they could be corrected using field maps. We found that the magnitude of distortions in the hippocampus is on the order of the resolution of the functional images. Further, we demonstrated that application of field maps led to negligible changes in the position of the hippocampus. We, therefore, omitted the computationally intensive application of field maps to the functional images in Experiments 2 and 3. We conclude that susceptibility artifacts do not affect the hippocampal data in these experiments severely. The agreement between the position of the hippocampus in structural and functional images is on the order of the resolution limit

of the latter. Hippocampal activations found in the ROI analysis in Experiments 2 and 3 can thus be reliably attributed to that structure.

References

Ashburner J, Friston K (1997) Multimodal Image Coregistration and Partitioning - A Unified Framework. Neuroimage 6:209-217.

Chen N-K, Dickey CC, Yoo SS, Guttmann CRG, Panych LP (2003) Selection of voxel size and slice orientation for fMRI in the presence of susceptibility field gradients: application to imagin of amygdala. Neuroimage 19:817-825.

Cordes D, Turski PA, Sorenson JA (2000) Compensation of susceptibility-induced signal loss in echo-planar imaging for functional applications. Magnetic Resonance Imaging 18:1055-1068.

Cusack RB, Osswald K (2003) An evaluation of the use of magnetic field maps to undistort echo-planar images. Neuroimage 18:127-142.

Deichmann R, Josephs O, Hutton C, Corfield DR, Turner R (2002) Compensation of Susceptibility-Induced BOLD Sensitivity Losses in Echo-Planar fMRI Imaging. Neuroimage 15:120-135.

Gorno-Tempini ML, Hutton C, Josephs O, Deichmann R, Price C, Turner R (2002) Echo Time Dependence of BOLD Contrast and Susceptibility Artifacts. Neuroimage 15:136-142.

Greicius MD, Krasnow B, Boyett-Anderson JM, Eliez S, Schatzberg AF, Reiss AL, Menon V (2003) Regional analysis of hippocampal activation during memory encoding and retrieval: fMRI study. Hippocampus 13:164-174.

Hennig J, Speck O, Koch M, Weiller C (2003) Functional Magnetic Resonance Imaging: A Review of Methodological Aspects and Clinical Applications. Journal of Magnetic Resonance Imaging 18:1-15.

Huettel SA, Song AW, McCarthy G (2004) Functional magnetic resonance imaging, 1st Edition. Sunderland, MA, USA: Sinauer Associates, Inc.

Hutton C, Bork A, Josephs O, Deichmann R, Ashburner J, Turner RT (2002) Image distortion correction in fMRI: a quantiative evaluation. Neuroimage 16:217-240.

Jezzard P, Clare S (1999) Sources of distortion in functional MRI data. Human Brain Mapping 8:80-85.

Krasnow B, Tamm L, Greicius MD, Yang TT, Glover GH, Reiss AL, Menon V (2003) Comparison of fMRI activation at 3 and 1.5 T during perceptual, cognitive, and affective processing. Neuroimage 18:813-826.

Kruger G, Kastrup A, Glover GH (2001) Neuroimaging at 1.5T and 3.0T: comparison of oxygenation-sensitive magnetic resonance imaging. Magnetic Resonance in Medicine 45:495-604.

Lipschutz B, Friston KJ, Ashburner J, Turner R, Price CJ (2001) Assessing Study-Specific Regional Variations in fMRI Signal. Neuroimage 13:392-398.

Moonen CTW, Bandettini PA, eds (2000) Functional MRI. Berlin: Springer-Verlag.

Ojeman JG, Akbudak E, Snyder AZ, McKinstry RC, Raichle ME, Conturo TE (1997) Anatomic localization and quantitative analysis of gradient refocused echo-planar fMRI susceptibility artifacts. Neuroimage 6:156-167.

Ongur D, Cullen TJ, Wolf DH, Rohan M, Barreira P, Zalesak M, Heckers S (2006) The Neural Basis of Relational Memory Deficits in Schizophrenia. Arch Gen Psychiatry 63:356-365.

Osterbauer RA, Wilson JL, Calvert GA, Jezzard P (2006) Physical and physiological consequences of passive intra-oral shimming. Neuroimage 29:245-253.

Powell HWR, Koepp MJ, Richardson MP, Symms MR, Thompson PJ, Duncan JS (2004) The Application of Functional MRI of Memory in Temporal Lobe Epilepsy: A Clinical Review. Epilepsia 45:855-863.

Preibisch C, Pilatus U, Bunke J, Hoogenraad F, Zanella F, Lanfermann H (2003) Functional MRI using sensitivity-encoded echo planar imaging (SENSE-EPI). Neuroimage 19:412-421.

Schmidt CF, Degonda N, Luechinger R, Henke K, Boesiger P (2005) Sensitivity-encoded (SENSE) echo planar fMRI at 3T in the medial temporal lobe. Neuroimage 25:625-641.

Stocker T, Kellermann T, Schneider F, Habel U, Amunts K, Pieperhoff P, Zilles K, Shah NJ (2006) Dependence of amygdala activation on echo time: Results from olfactory fMRI experiments. Neuroimage 30:151-159.

Ward HA, Riederer SJ, Jack CR (2002) Real-Time Autoshimming for Echo Planar Timecourse Imaging. Magnetic Resonance in Medicine 48:771-780.

Weiskopf N, Klose U, Birbaumer N, Mathiak K (2005) Single-shot compensation of image distortions and BOLD contrast optimization using multi-echo EPI for real-time fMRI. Neuroimage 24:1068-1079.

Wilson JL (2002) Fast, Fully Automated Global and Local Magnetic Field Optimization for fMRI of the Human Brain. Neuroimage 17:967-976.

Wilson JL, Jenkinson M, Jezzard P (2002) Optimization of Static Field Homogeneity in Human Brain Using Diamagnetic Passive Shims. Magnetic Resonance in Medicine 48:906-914.

Windischberger C, Robinson S, Rauscher A, Barth M, Moser E (2004) Robust Field Map Generation Using a Triple-Echo Acquisition. Journal of Magnetic Resonance Imaging 20:730-734.

Zeineh MM, Engel SA, Bookheimer SY (2000) Application of cortical unfolding techniques to functional MRI of the human hippocampal region. NeuroImage 11:668-683.

Zeng H, Constable RT (2002) Image Distortion Correction in EPI: Comparison of Field Mapping With Point Spread Function Mapping. Magnetic Resonance in Medicine 48:137-146.

Chapter 5: Neural correlates of ordered sequence learning

Introduction

The two experiments described earlier in this thesis examined the neural correlates of transitive inference (TI) in humans. In the first experiment (Chapter 1), which was a block-design fMRI experiment (Heckers et al., 2004a), hippocampal activation was detected in the contrast between TIs on pairs drawn from a sequence (e.g. pairs A>C and B>D from the sequence A>B>C>D>E) and non-TI on pairs drawn from individual pairs (e.g. pairs a>d and c>b from the pairs a>b and c>d). We thus confirmed an important role for the hippocampus in TI in humans, consistent with previous studies in rodents (Dusek and Eichenbaum, 1997) and monkeys (Buckmaster et al., 2004). In the second experiment (Chapter 2), an event-related fMRI paradigm, we used a six-item sequence (A>B>C>D>E>F) that allowed us to test for a differential role of hippocampus in non end-item TI (NETI, e.g., B>D) compared with end-item TI (ETI, e.g., A>C) conditions. We demonstrated the specificity of hippocampal activation to NETI. We also showed greater hippocampal activation for NETI pairs with a symbolic distance of one (B>D) than two (B>E). In both cases, the hippocampus exhibited greater activity in situations requiring a greater degree of flexible manipulation of the sequences that consisted of overlapping stimulus pairs. Our results thus supported the relational flexibility account of hippocampal function, which posits that the hippocampus links memories in support of their flexible expression (Eichenbaum, 2004).

In the experiment described here, we investigated whether hippocamus also supported the learning of the ordered sequence in addition to playing a key role in the flexible manipulation for TIs. To address this question, we compared brain activation between

learning overlapping pairs from the ordered sequence (A>B, B>C, C>D and D>E) and non-overlapping individual pairs (a>b, c>d, e>f and g>h), as used in the paradigm for Experiment 1. Hippocampal lesions in rats (Dusek and Eichenbaum, 1997) and monkeys (Buckmaster et al., 2004) did not impair learning of the ordered sequence. This result, however, could be due to the specifics of the training paradigm: The animals were trained on each pair from the sequence in turn. In a previous positron emission tomography (PET) TI experiment in humans, right hippocampal activation was demonstrated in learning of "bridging" pairs (B>C, D>E and F>G) compared with learning of "independent" pairs (A>B, C>D, E>F and G>H) from the ordered sequence A-H (Nagode and Pardo, 2002). Their participants were scanned only during training, however. It was thus unclear whether this training scheme would result in hippocampal activation during TIs. In the study described here, we use a different approach. We used the paradigm from Experiment 1 and scanned participants while they learned the sequence A-E by random presentation of the overlapping pairs (A>B, B>C, C>D, D>E). The difference between the training in Experiment 1 and here is that participants were informed about the underlying hierarchy they needed to uncover in the present study. This information was provided to encourage organization of the pairs within the ordered sequence. We hypothesized that hippocampal activation would be detected in the comparison between learning the overlapping pairs, when participants constructed the ordered sequence, and learning the non-overlapping pairs.

Our previous experiments also detected a network of cortical and subcortical regions that support TI judgments, namely prefrontal cortex (PFC), insula, precuneus, posterior

parietal cortex, thalamus, and ventral striatum. Given that these areas have also been previously implicated in verbal (Bor et al., 2004), visual (Kumaran and Maguire, 2006) and motor (Muller et al., 2002; Daselaar et al., 2003; Schendan et al., 2003; Aizenstein et al., 2004; Bischoff-Grethe et al., 2004) sequence learning, we hypothesized that they would be activated for learning the overlapping pairs compared with learning the non-overlapping pairs.

Materials and methods

Participants

We studied 23 healthy participants (9 male and 14 female, aged 22 to 36, mean age = 26.8, mean estimated verbal IQ (Blair and Spreen, 1989) = 116.9), who gave informed consent in a manner approved by the IRB of the McLean Hospital. No participant had a history of significant medical, neurological, or psychiatric illness. Of these 23 participants, 17 (8 male and 9 female, aged 22 to 36, mean age = 26.5, mean estimated verbal IQ (Blair and Spreen, 1989) = 117.9) entered the final analysis based on behavioral exclusion criteria explained below.

Stimuli and paradigm

Stimuli

Identically to Experiment 1 (Heckers et al., 2004a), we selected 13 visually distinctive exemplars from pattern fills provided by CorelDraw. Two sets of pattern fills (8 for the

non-overlapping pairs, and 5 for the overlapping pairs) were randomly assigned to pentagon and ellipsoid shapes for each participant. The four non-overlapping pairs were denoted ab, cd, ef, and gh. The four overlapping pairs were denoted AB, BC, CD, and DE.

Training during scanning

Training took place in the scanner and was divided into two blocks. Before entering the scanner, participants were informed that they would be trained on two sets of four pairs of images. They were told that one set consisted of unrelated individual pairs (the non-overlapping pairs) and that the other set consisted of pairs that form a hierarchical sequence (the overlapping pairs). They were instructed that, in the scanner, they would see one pair at a time and that they would need to determine the winning item in each pair. If they picked the correct item, they viewed a smiley face. They were told that each pair would be shown for 2.5 sec during which they would have to indicate by button press the item they considered superior. Next, 1.5 sec of feedback occurred, when they would see either the smiley face or not, depending on whether they made a correct choice.

In each of the two imaging blocks, participants saw a total of 64 pairs of which 32 were the overlapping pairs (S) and 32 were the non-overlapping pairs (P). The pairs were presented in a pseudo-blocked fashion. Thus, they first saw the S pairs followed by the P pairs in both blocks or vice versa. The design was optimized for efficiency of estimation of the fMRI response by inserting periods of blank screen with a cross-hair (fixation)

from 2 to 12 sec in duration between consecutive presentations of the pairs. A fixation period of 10 sec was inserted in the beginning and the end of each block to allow estimation of baseline in the fMRI design. The total length of each run was approximately 470 sec.

Testing of old pairs

The training period was followed by a test in which participants saw the original S and P pairs on which had been trained. Participants saw 16 instances of each pair. Each pair was shown for 2 sec during which participants had to indicate by button press the item they considered superior based on the training. No feedback was provided. The presentation of the pairs was completely randomized. This test was administered to determine how well participants learned the original pairs.

Testing of novel pairs

Next, participants were tested on novel pairs drawn from the overlapping sequence (IS: pairs AC, AD, BD, BE, and CE) and novel pairs drawn from the individual pairs where a previous "winner" was paired up with a previous "loser" (IP: pairs ad, af, cf, ch, and eh). Participants would see a total of 14 instances of each of the 10 pairs. Each pair was shown for 2 sec during which participants had to indicate by button press the item they considered superior based on the training. The presentation of the pairs was completely randomized. This test was administered to determine whether participants understood the

ordered sequence of the overlapping pairs, and to allow exclusion from the analysis of those participants who did not.

Functional imaging

Participants were scanned in a Siemens 3.0 Tesla Trio high-speed echo-planar imaging device (Munich, Germany). Participants wore ear plugs and laid on a padded scanner bed in a dimly illuminated room. Foam padding restricted head movement. Stimuli were generated using Presentation software (Neurobehavioral Systems) on a personal computer, projected onto a screen, and viewed by the participants via a tilted mirror placed in front of their eyes.

Functional scanning began with an initial sagittal localizer scan. Then, a high-resolution anatomical MP-RAGE image was obtained for each participant (TR = 2100 ms, TE = 2.74 ms, 128 slices, FoV = 256 mm, 1.0 x 1.0 x 1.3 mm resolution). The two functional series that followed lasted 8 min and 24 sec each. The first 14 sec of each series were discarded to allow for T_1 equilibration. During the remaining time of each series, 250 BOLD gradient-echo EPI functional brain images were collected (TR = 2000 ms, TE = 30 ms, 34 slices, FoV = 200 mm, transversal acquisition, orientation perpendicular to the long axis of the hippocampus, 3.1 x 3.1 x 3.0 mm resolution, anterior-posterior phase encode direction). To evaluate the effect of magnetic field inhomogeneities, fieldmaps were also acquired. They consisted of a gradient-recalled echo (GRE) magnitude and

phase scans (TR = 555 ms, TE_1 = 4.99 ms, TE_2 = 7.45 ms, ΔTE = 2.46 ms, FoV = 200 mm, 35 slices, 3.1 x 3.1 x 5.0 mm).

Data analysis

Participant exclusion

Behavioral data from the final test were first analyzed for evidence of understanding of the hierarchical sequence (A-E) present in the overlapping pairs. Participants displaying accuracy of 60% or lower on any of the novel pairs drawn from the sequence (IS pairs) were deemed not to have understood this sequence. Only data from the participants who understood the sequence inherent in the overlapping pairs were further analyzed. Of the 23 participants initially entering the analysis, 17 passed this criterion. For the remaining 6 participants, the mean accuracy for the IS and IP trials was 67.1 ± 11.2% and 74.1 ± 12.0%, respectively. The difference showed a trend but no significance (paired sample t test, 5 df, $p = 0.072$). For the other 17 participants, accuracy for the IS and IP trials was 97.6 ± 4.4% and 98.8 ± 1.9%, respectively. A repeated-measures ANOVA with group (included and excluded participants) and novel pair type (IS and IP) revealed a significant effect of group ($F(1,5) = 49.8$, $p = 0.001$). The novel pair type and the group-by-type interaction were not significant.

Thus, the excluded participants were poor performers in general whether it was on inference on novel pairs from the sequence or on novel pairs constituted from individual pairs. Further, there was no difference between accuracy on the ETI trials (non-BD IS

trials including either the end-item A or E) and the NETI trial BD (the IS trial devoid of any end-item) (paired-samples t test, $t(5) = 1.9$, $p = 0.1$) for the excluded participants. This finding further confirmed that these participants performed poorly overall and that the deficit on the NETI pair BD (accuracy 35.2 ± 41.8 %), which required flexible manipulation of the sequence hierarchy, was not selective.

Behavioral data

First, accuracy data from the two training blocks were analyzed using a 2 x 2 repeated-measures ANOVA with pair type (S or P) and block (1 and 2) as the main effects. The latency of responses was also analyzed using a 2 x 2 repeated-measures ANOVA. Second, accuracy data from the test on the old pairs were examined for evidence of learning of both the overlapping (S) and non-overlapping (P) pairs. Accuracy was compared with that in the second training block to determine whether the two-block training scheme was sufficient for good performance on the test. Also, accuracy and response latency data from the test on the novel pairs were analyzed to determine whether the expected pattern would be observed. This result would confirm that the participants learned the ordered sequence of the overlapping pairs in the same way as in Experiments 1 and 2. We expected the accuracy to be lower for IS compared to IP pairs and reaction times to be longer for IS compared to IP pairs, which was assessed using paired-samples t test. Further, we expected the NETI trial BD that required flexible manipulation of the sequence hierarchy to elicit lower accuracy and longer reaction times than the other IS trials, which could all be solved by referring either to the end-item A or E. This prediction was also assessed using a paired-samples t test.

Whole-brain voxel-based analysis of the functional neuroimaging data

The fMRI data from each participant were analyzed using the Statistical Parametric Mapping SPM2 package (Wellcome Department of Imaging Neuroscience, London, UK). Pre-processing was carried out using this package. First, slice timing correction using sinc interpolation was applied to account for differences in acquisition time of the individual slices. Functional images were then realigned to correct for head motion during scanning, and a mean functional image was created. The anatomical image of each participant was then coregistered with the participant's mean functional image. The coregistered anatomical image was normalized to a T_1-weighted anatomical template in the stereotactic MNI coordinates. The functional images were subsequently normalized using the identical parameters and smoothed with a three-dimensional 8-mm Gaussian kernel to eliminate spatial noise, and to allow the application of the Gaussian Random Field Theory for statistical analysis.

A design matrix was then created to allow the application of the General Linear Model (GLM) to the functional data. Different instances of each pair were modeled together as a unique condition. Each of the eight pairs (S: AB, BC, CD, and DE and P: ab, cd, ef, and gh) was modeled separately. The onset times of these conditions were entered into the GLM and convolved with the hemodynamic response function and its time and dispersion derivatives to model the hemodynamic response to each trial type. A high-pass filter of 128 sec was used to remove time-dependent drift in the functional data, and the data were corrected for serial correlations.

Functional neuroimaging data were analyzed with the aim to assess the difference between training on pairs from the sequence (S) and individual pairs (P) over the two training blocks. The contrast image [S-P] was, therefore, formed, and the individual contrast images were entered as random effects into a one-sample t test to detect significant differences common to all participants. Because reaction times differed significantly between the S and P conditions, the reaction time differences were entered as covariates in the above analysis. We also examined the reverse contrast.

A significance level of $p < 0.0005$, uncorrected for multiple comparisons, assessed the statistical significance of this effect. At this threshold, we examined brain regions that have been implicated previously in TI and sequence learning, namely pre-supplementary (preSMA) and supplementary motor (SMA) areas, prefrontal and parietal cortices, insula, precuneus, ventral striatum, and cerebellum (Acuna et al., 2002b; Heckers et al., 2004a). For other brain regions with no *a priori* hypothesis, we used the $p < 0.05$ threshold, corrected for multiple comparisons using the family-wise error control in SPM2. All of the resulting activation maps were examined for significant differences at a voxel extent threshold of 3 voxels. The resulting activation maps were overlaid on the mean anatomical image of the participants whose contrast images were used for imaging analysis. The peak activations were identified and their MNI coordinates and peak z scores were noted.

Region-of-interest analysis of the functional neuroimaging data

The whole-brain voxel-based analysis described above was supplemented by an anatomically guided ROI analysis in individual participants' hippocampi. The hippocampus was outlined bilaterally on every participant's anatomical image in native space using guidelines described elsewhere (Pruessner et al., 2000; DeWitt et al., 2002). The reliability of the outlines was assessed using the intraclass correlation coefficient (Shrout and Fleiss, 1979). In five randomly selected participants, the intra-rater reliability was 0.93 on the left and 0.94 on the right. The inter-rater reliability was 0.87 on the left and 0.88 on the right.

This anatomical ROI was then resampled to the same resolution as the native space BOLD functional images that had been previously realigned and coregistered with the native space anatomical image. The resampled anatomical ROI image was then used as a mask to create partial brain functional images where the signal intensity values were set to zero outside the hippocampal mask. These images were subsequently smoothed using a three-dimensional 3-mm Gaussian kernel to increase detection sensitivity.

The design matrix from the whole-brain analysis was used with these partial brain volumes to apply GLM and to extract parameter estimates for the conditions of interest as described above. As for the whole-brain analysis, the contrast images [S-P] were obtained for each participant bilaterally. Each hippocampus was then divided into nine segments longitudinally along the y-axis, and average contrast values were computed for each of the nine segments bilaterally. Because of concerns about signal loss in the anterior

portion of the MTL, we identified voxels within each hippocampus where the signal intensity was below 1/6 of the mean intensity within the whole hippocampus. These voxels exhibiting a pronounced signal loss were excluded from the analysis.

Analogously to the group analysis for the whole-brain data, the contrast values from each segment were then entered as random effects into a one-sample t-test to detect significant activations common to all participants. In each case, a one-sided t test (based on our hypothesis that the hippocampus would be more active for training on overlapping than on non-overlapping pairs) with a p value threshold of 0.05 was used. Finally, we performed a regression analysis to determine whether signal loss affected the estimated contrast values.

Results

Behavioral data

A 2 x2 repeated measures ANOVA revealed that accuracy was significantly lower in the first training block (S: 70.8 ± 11.6%, P: 84.6 ± 10.0%) compared with the second training block (S: 93.5 ± 6.6%, P: 99.6 ± 1.5%) (main effect of block, $F(1,16) = 69.9$, $p < 0.001$) and significantly lower for S compared with P trials (main effect of type, $F(1,16) = 49.3$, $p < 0.001$). There was a significant block-by-type interaction ($F(1,16) = 12.6$, $p = 0.003$), indicating that the increase in accuracy from block 1 to block 2 was greater for S trials than for the P trials (Figure 1).

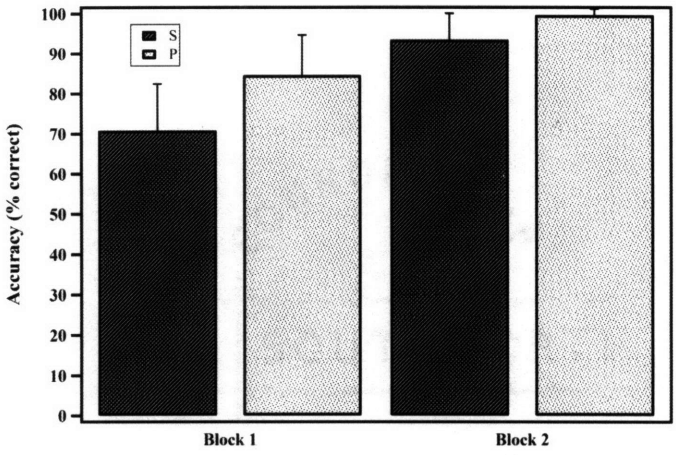

Figure 1. Accuracy on S and P in training blocks 1 and 2. Accuracy was significantly lower in training block 1 and for S trials; it increased from block 1 to block 2, and was more pronounced for S trials.

Another 2x2 repeated measures ANOVA revealed that reaction times were significantly longer in the first training block (S: 1.44 ± 0.19 s, P: 1.24 ± 0.15 s) than in the second training block (S: 1.30 ± 0.15 s, P: 0.95 ± 0.15 s) (main effect of block, $F(1,16) = 58.6$, $p < 0.001$), and significantly longer for S than P trials (main effect of type, $F(1,16)=75.5$, $p < 0.001$). A significant block-by-type interaction ($F(1,16) = 10.1$, $p = 0.006$) indicated that reaction times decreased more for the P trials than for S trials between blocks 1 and 2 (Figure 2).

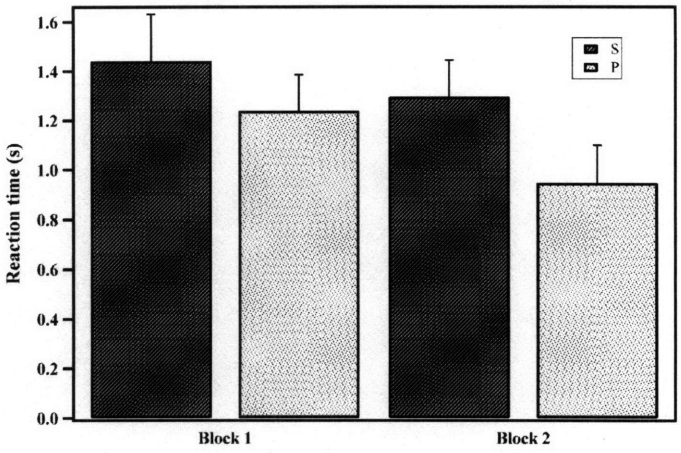

Figure 2. Reaction times for S and P in training blocks 1 and 2. Reaction times were significantly longer in training block 1 and for S trials and their decrease from block 1 to 2 was more pronounced for P trials.

Accuracy on the S pairs in the test phase did not differ significantly from that in the training block 2 (95.2 ± 5.8 vs. 93.5 ± 6.6 %). Accuracy on the P pairs in the test phase decreased slightly but significantly (98.1 ± 1.2 vs. 99.6 ± 1.5 %, paired-samples t test, $t(16)=2.531$, $p = 0.02$). Overall, the results indicate that the two training blocks provided sufficient learning experience for participants to learn the overlapping (S) and non-overlapping (P) pairs.

In the test of novel pairs, accuracy did not differ significantly between IS and IP pairs. Reaction times were significantly longer for IS (0.93 ± 0.16 s) than IP (0.81 ± 0.12 s) pairs (paired-samples t test, $t(16) = 4.59$, $p < 0.001$). Accuracy did not differ significantly between the NETI BD trials and the remaining IS (ETI) trials. Reaction times were significantly longer for the BD trial (1.00 ± 0.19 s) than for the remaining IS trials (0.91 ± 0.16 s) (paired-samples t test, $t(16) = 4.58$, $p < 0.001$).

Results for whole-brain analysis

We first tested for brain activation differences between training on overlapping (S) and non-overlapping pairs (P). Significant activations for the S condition compared with the P condition [S-P] were detected in PFC, preSMA, SMA, anterior cingulate cortex, insula, postcentral gyrus, superior and inferior parietal lobules, precuneus, middle temporal gyrus, thalamus, and cerebellum (Table 1 and Figure 3). No activation was detected in the reverse contrast [P-S].

Table 1. Significant activations for training on overlapping (S) compared with training on non-overlapping (P) pairs. H denotes hemisphere. Z denotes the z score. All coordinates are given in MNI space in mm.

Brain Region	H	S > P (z>3.5)			
		Z	MNI (x, y, z)		
Frontal lobe					
IFG (BA 47)	R	3.7	38	22	-14
MFG (BA 8)	R	4.1	36	14	38
	L	3.8	-24	24	50
MFG (BA 9)	R	3.8	44	24	38
	R	3.7	30	42	38
	R	3.5	4	40	30
Pre-SMA (BA 6)	L	3.5	12	2	66
SMA (BA 6)	R	3.7	38	0	32
	L	3.5	-10	-20	74
Anterior cingulate (BA 32)	L	3.7	-8	40	26
Insula	R	4.1	40	16	18
	R	3.5	40	12	-8
Postcentral gyrus (BA 2)	L	3.7	-50	-22	46
Parietal lobe					
Superior parietal lobule (BA 7)	R	4.1	30	-58	40
Inferior parietal lobule (BA 40)	R	3.8	54	-60	38
Precuneus (BA 19)	L	4.4	-44	-74	40
Precuneus (BA 7)	B	4.1	0	-66	30
	L	4.1	-16	-50	58
	L	3.9	-20	-54	52
	L	3.8	-2	-48	60
	L	3.6	-2	-48	60
	L	3.6	-4	-58	46
Posterior cingulate (BA 31)	L	4.4	-20	-32	40
Temporal lobe					
Middle temporal gyrus (BA 21)	R	4.3	54	-20	-6
Middle temporal gyrus (BA 39)	L	3.7	-56	-60	10
	L	3.6	-48	-66	20
Subcortical nuclei					
Thalamus (pulvinar)	L	4.0	-10	-28	8
Thalamus (vl)	R	3.9	14	-14	14
	L	3.8	-10	-10	4
Cerebellum	L	3.7	-36	-74	-40

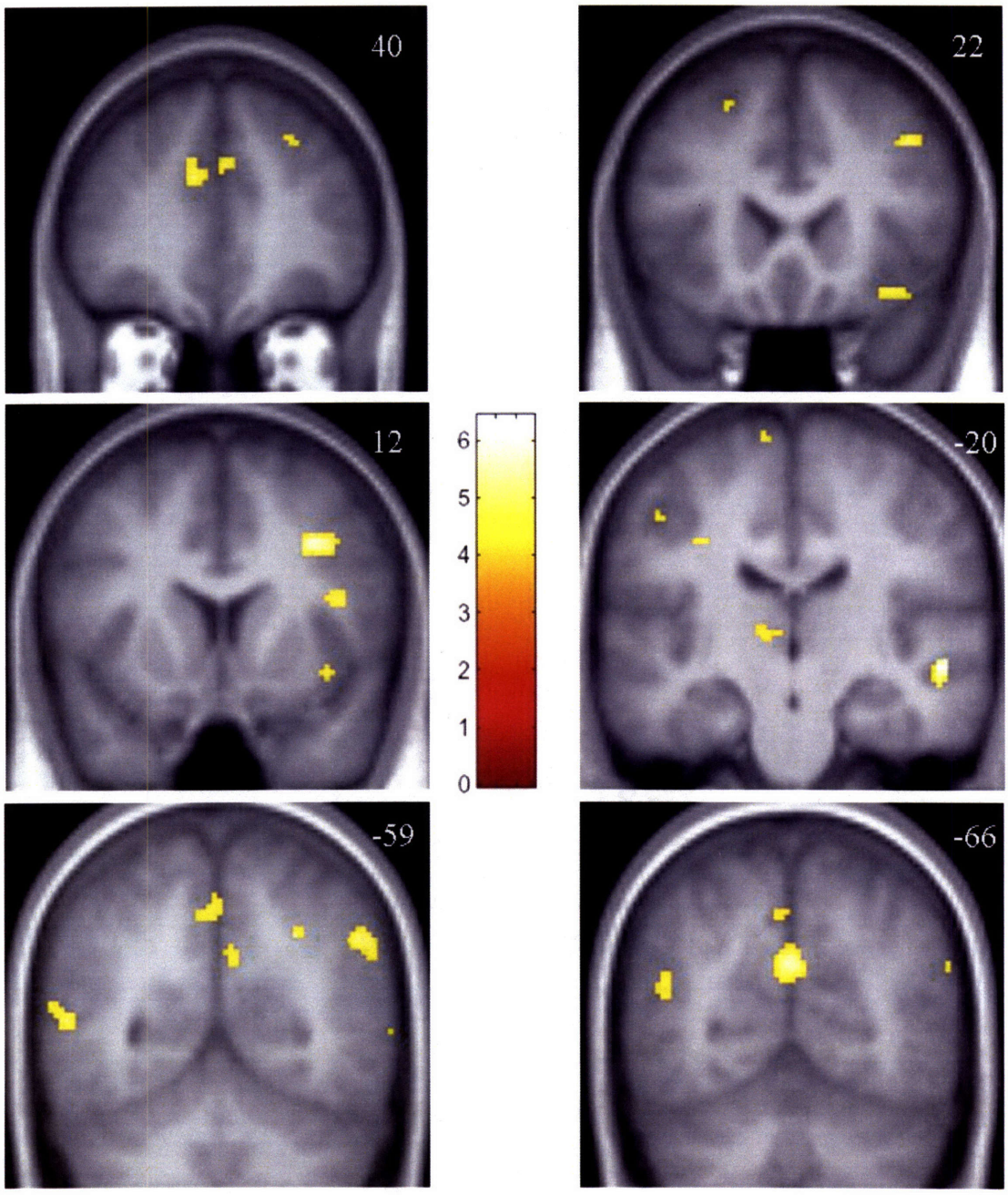

Figure 3. Significant activations for training on overlapping (S) compared with training on non-overlapping (P) pairs. Six coronal views are provided along with their position along the anterior-posterior axis in MNI space (mm). The panels show the following activations: (y = 40 mm) anterior cingulate cortex and middle frontal gyrus (BA 9); (y = 22 mm) middle frontal gyri (BA 8 and BA 9 and, inferior frontal gyrus (BA 47); (y = 12 mm) middle frontal gyrus (BA 8) and insula; (y = -20 mm) supplementary motor area (BA 6), precentral gyrus (BA 2), pulvinar and middle temporal gyrus; (y = -59 mm) superior (BA 7) and inferior (BA 40) parietal lobules, precuneus (BA 7) and middle temporal gyrus (BA 39); (y = -66 mm) precuneus (BA 7 and BA 19). The bar indicates the t value of activated voxels.

Results of the region-of-interest analysis

Using an anatomically guided ROI analysis, we found two segments in the right hippocampus where activity was consistently higher for training on overlapping (S) compared with non-overlapping (P) pairs. These were the third ($t(16) = 1.98$, one-tailed p value = 0.033) and the fifth ($t(16) = 2.05$, one-tailed p value = 0.028) segments out of nine along the long axis of the right hippocampus, corresponding approximately to a location one-third of the way from the anterior tip and a location in the midpoint of the hippocampus. No consistent activation was detected in the left hippocampus (Figure 6). Regression analysis revealed no correlation between the degree of signal loss and the estimated contrast values in any of the 17 participants.

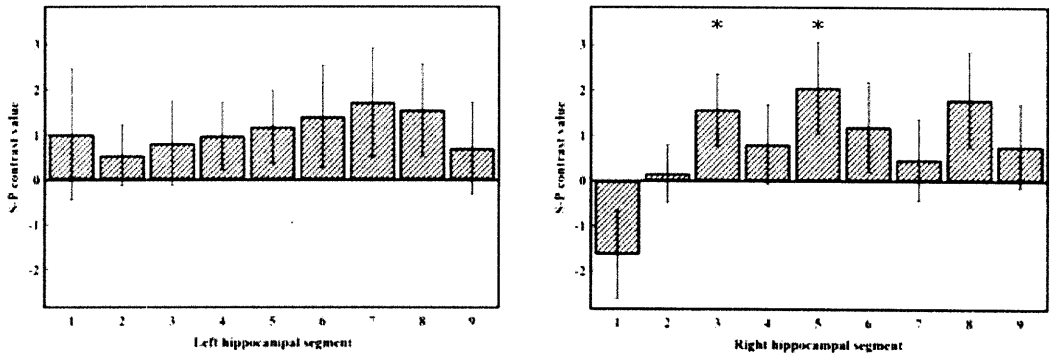

Figure 6: Contrast of parameter estimates values corresponding to S vs. P in each of the nine segments along the long axis of the hippocampus bilaterally. The star indicates significant (one-tailed p value < 0.05) activation. Error bars represent the standard error of the mean.

Discussion

We previously demonstrated that TIs on ordered sequences (Experiment 1), especially on NETI pairs devoid of end-items (Experiment 2), elicited hippocampal activation. We also confirmed the existence of a previously reported (Acuna et al., 2002b) TI network that

includes PFC and parietal cortex, insula, precuneus, thalamus, and ventral striatum. In this experiment, we investigated whether hippocampus and the TI network also supports learning of the overlapping pairs that constitute the ordered sequence. We used the paradigm employed in Experiment 1 where participants learned overlapping (S) and non-overlapping (P) pairs. Here, we tested the hypothesis that the hippocampus and the TI network would be differentially activated during learning of the S compared with the P pairs in the paradigm that is known to show hippocampal activation during TI on the ordered sequence composed of the S pairs.

An important difference occurred between the training employed in the original experiment (Experiment 1) and the present experiment. In Experiment 1, participants were not explicitly informed that the S pairs constitute a sequence, whereas participants in the present experiment received this information prior to training in order to facilitate learning on the timescale of an fMRI experiment. Nevertheless, the behavioral results suggested that the participants in the present study encoded the sequence in the same way as the uninformed participants in Experiment 1, as seen in their ability to perform TI on the ordered sequence and the longer reaction times on the NETI pair BD compared with the ETI non-BD trials.

Using a paradigm that is thus similar to the one that demonstrated hippocampal activation in TIs on the ordered sequence of overlapping pairs compared with inferences on non-overlapping pairs in Experiment 1, we showed right hippocampal activation during learning of the overlapping pairs compared with learning of the non-overlapping pairs.

Further, we showed activation of the previously identified cortical and subcortical TI network that includes PFC, preSMA, SMA, anterior and posterior cingulate, insula, posterior parietal cortex, precuneus, thalamus, lateral temporal lobe, and cerebellum. In the next section, I will discuss potential contributions of the hippocampus and the TI network to learning of the overlapping pairs.

Contribution of the hippocampus to ordered sequence learning

Hippocampal recruitment in TI judgments has now been firmly established in animals (von Fersen et al., 1991; Dusek and Eichenbaum, 1997; Buckmaster et al., 2004) and in humans in the experiments described in Chapter 1 and Chapter 2. Its role in TI can be interpreted in accordance with the relational memory account of hippocampal function. In this account, the hippocampus plays a critical role in linking related memories according to their common features, which results in a network that can support inferences between items in memory that are only indirectly related (Eichenbaum, 2004).

In this study, we show right hippocampal activation in the comparison of training on overlapping pairs that constitute the ordered sequence with training on non-overlapping individual pairs. What is the role of the hippocampus in learning of the overlapping pairs and of the sequence? In both animal studies (Dusek and Eichenbaum, 1997; Buckmaster et al., 2004) that demonstrated hippocampal role in TI using the 5-item ordered sequence (A>B>C>D>E) employed here, hippocampally lesioned animals were able to learn the individual premise pairs (A>B, B>C, C>D, and D>E) correctly. Based on this observation, one may conclude that the hippocampus is not required for encoding of the

original premise pairs, making its activation in this study surprising. The discrepancy can be explained by the nature of the training, however. In both of the animal studies, participants were trained on each pair separately in a repeated sequence. They were first trained on the pair A>B followed by B>C and so on. The flexible expression and manipulation of the sequence by the hippocampus needs to be implemented only during TI testing. In contrast, human participants can be informed, as in this study, that the S pairs constitute an ordered sequence that they need to determine, whereas the P pairs are unrelated pairs. Thus, when trained on the overlapping pairs that were randomly presented, the participants were encouraged to manipulate the individual pairs into the ordered sequence representation. We interpret the hippocampal activation associated with training on the overlapping pairs as consistent with the relational account under which the hippocampus acts to rapidly bind common features into a unified representation that supports flexible inferential memory expression (Eichenbaum, 2004).

This interpretation is supported by the findings of the one previous study of sequence learning for TI (Nagode and Pardo, 2002). In this study, right hippocampal activation was demonstrated in learning of "bridging" pairs (B>C, D>E and F>G) compared with previous learning of "independent" pairs (A>B, C>D, E>F, and G>H) from the ordered sequence A-H (Nagode and Pardo, 2002). Their contrast of interest was analogous to the contrast between learning of the S compared with P pairs that showed right hippocampal activation here. However, they did not scan during TI testing and were, therefore, unable to link hippocampal activation in training on the ordered sequence with subsequent hippocampal activation during TI. Our study used an ordered sequence paradigm that had

previously elicited right hippocampal activation in TI (Heckers et al., 2004a). Together, these two experiments (Experiments 1 and 3) suggest that the hippocampus is recruited for both the learning and organizing of sequence information as well as for the flexible expression and manipulation of the sequence as required for TIs.

Contribution of cortical and subcortical areas to ordered sequence learning

In addition to the hippocampus, a network of brain regions including PFC, preSMA, SMA, postcentral gyrus, anterior and posterior cingulate cortex, insula, posterior parietal cortex, precuneus, lateral temporal lobe, thalamus, and cerebellum was also activated in learning of the overlapping pairs contrasted with non-overlapping pairs. Except for the cerebellum and postcentral gyrus, all of these areas have been reported to be activated in TI either in previous studies described in this thesis (Experiments 1 and 2) or elsewhere (Acuna et al., 2002b). The TI network that supports TI judgments thus seems to be active also in the learning and organizing of the overlapping pairs into a sequence representation. Contrary to our expectation, we did not detect ventral striatal (VS) activation. VS has been associated with spatial learning (Meredith and Totterdell, 1999) and stimulus-association learning (Schendan et al., 2003) among other functions. In motor sequence learning, VS is activated when participants do not have explicit awareness of the presence of a sequence (Schendan et al., 2003). Its lack of activation in this study may, therefore, be attributed to the fact that participants are explicitly informed that the non-overlapping pairs constitute an ordered sequence which they need to determine.

In a previous fMRI study of TI, PFC activation was interpreted in terms of its role in manipulating and integrating information (Acuna et al., 2002b). Parietal activation was thought to be related to the spatial-like operations used during TI (Acuna et al., 2002b) because the ordered sequence on which TIs are made is believed to be spatially represented (McGonigle and Chalmers, 1986; Davis, 1992). The activation of preSMA was not interpreted beyond its role in reasoning (Acuna et al., 2002b).

Here, I will attempt a more thorough interpretation of the activation of cortical and subcortical regions in the learning of overlapping pairs constituting the ordered sequence. Beyond a role in reasoning, PFC activation has been reported in the learning of visual (Kumaran and Maguire, 2006), verbal (Bor et al., 2004) and motor (Muller et al., 2002; Schendan et al., 2003; Aizenstein et al., 2004; Oshi et al., 2005) sequence learning. The insula has also been shown to be activated for visual (Kumaran and Maguire, 2006) and motor (Aizenstein et al., 2004) sequence learning. In addition, its activation increases with uncertainty associated with decisions (Huettel et al., 2005). During learning, responses to the overlapping pairs are likely to be associated with greater uncertainty than the non-overlapping pairs because the same stimulus can appear in two different overlapping pairs, and presence of uncertainty may provide a partial explain the recruitment of PFC and the insula here. The preSMA has also been implicated in motor (Muller et al., 2002; Bischoff-Grethe et al., 2004; Heun et al., 2004; van der Graaf et al., 2004) and visual (Kumaran and Maguire, 2006) sequence learning. Its activation in motor sequence learning scales with the complexity of the sequence (Boecker et al., 1998). The preSMA has also been implicated in task (Rushworth et al., 2002), attention set

(Nagahama et al., 1999) and movement sequence (Jancke et al., 2000) switching. Interestingly, the preSMA activated for discovery of new motor sequences (Rhodes et al., 2004). Like preSMA, SMA was active during visual (Kumaran and Maguire, 2006) and motor (Oshi et al., 2005) sequence learning. Both anterior and posterior cingulate cortices mediate explicit motor learning (Aizenstein et al., 2004). Anterior cingulate cortex, in addition, also monitors response errors and decision uncertainty (Ridderinkhof et al., 2004). Its activation in ordered sequence learning may reflect uncertainty as participants learn to choose the correct response from two stimuli in a pair, given that the same stimulus may be the correct response for one pair but not for another pair (e.g. C for the pair CD and BC). The precentral gyrus, which also activated here, also supports motor sequence learning (Aizenstein et al., 2004). The PFC and cingulate cortex as well as preSMA, SMA, and the precentral gyrus are thus likely to be recruited during learning of the sequence of overlapping pairs.

Posterior parietal cortex is activated during the learning of verbal (Bor et al., 2004), visual (Kumaran and Maguire, 2006) and visuomotor (Sakai et al., 1998; Schendan et al., 2003; Heun et al., 2004) sequences. It may also play a role in learning a motor sequence as a visuospatial pattern (Jancke et al., 2000). In addition, the superior parietal lobule is activated in manipulation of spatial relations, and its activation scales with relational complexity (Kroger et al., 2002). The precuneus has been shown to be important for all of the above functions (Kroger et al., 2002; Bor et al., 2004; Oshi et al., 2005), and also in switching attentional sets (Nagahama et al., 1999) and switching between movement sequences (Jancke et al., 2000). In the present experiment, parietal cortex also likely

contributed to learning of the sequence of overlapping pairs, and this area may play a special role in the manipulation of the overlapping pairs to create a spatial representation of the ordered sequence.

Lateral temporal cortex has been previously implicated in learning verbal (Bor et al., 2004) and motor (Bischoff-Grethe et al., 2004) sequences. The thalamus is activated by motor sequence learning (Muller et al., 2002; Fletcher et al., 2005; Oshi et al., 2005) and its activation scales with uncertainty on decisions (Huettel et al., 2005). In addition, the cerebellum mediates visual (Kumaran and Maguire, 2006) and motor (van der Graaf et al., 2004; Oshi et al., 2005; Nyberg et al., 2006) sequence learning.

Thus, nearly all of the extra-hippocampal brain areas that activated during learning of overlapping pairs and their sequence, compared with non-overlapping pairs, play a role in motor sequence learning. Interestingly, the hippocampus was recently activated in such learning (Schendan et al., 2003). Our results, therefore, suggest a generalization of the network supporting motor sequence learning into learning of other ordered sequences that takes place by motor response to visual stimuli. Whereas these areas contribute to the learning and organization of the sequence within a spatial structure, the hippocampus plays a special role in binding common features of the overlapping pairs into a unified representation of the ordered sequence that supports flexible inferential memory expression.

References

Acuna BD, Eliassen JC, Donoghue JP, Sanes JN (2002) Frontal and parietal lobe activation during transitive inference in humans. Cerebral Cortex 12:1312-1321.

Aizenstein HJ, Stenger VA, Cochran J, Clark K, Johnson M, Nebes RD, Carter CS (2004) Regional Brain Activation during Concurrent Implicit and Explicit Sequence Learning. Cerebral Cortex 14:199-208.

Bischoff-Grethe A, Goedert KM, Willingham DT, Grafton ST (2004) Neural Substrates of Response-based Sequence Learning using fMRI. Journal of Cognitive Neuroscience 16:127-138.

Blair J, Spreen O (1989) Predicting premorbid IQ: a revision of the national adult reading test. The Clinical Neuropsychologist 3:129-136.

Boecker H, Dagher A, Ceballos-Baumann AO, Passingham RE, Samuel M, Friston KJ, Poline J-B, Dettmers C, Conrad B, Brooks DJ (1998) Role of the Human Rostral Supplementary Motor Area and the Basal Ganglia in Motor Sequence Control: Investigations With H_2O^{15} PET. Journal of Neurophysiology 79:1070-1080.

Bor D, Cumming N, Scott CEL, Owen AM (2004) Prefrontal cortical involvement in verbal encoding strategies. European Journal of Neuroscience 19:3365-3370.

Buckmaster CA, Eichenbaum H, Amaral DG, Suzuki WA, Rapp PR (2004) Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. The Journal of Neuroscience 24:9811-9825.

Daselaar SM, Rombouts SARB, Veltman DJ, Raaijmakers JGW, Jonker C (2003) Similar network activated by young and old adults during the acquisition of a motor sequence. Neurobiology of Aging 24:1013-1019.

Davis H (1992) Transitive inference in rats (*Rattus norvegicus*). Journal of Comparative Psychology 106:342-349.

DeWitt I, Weiss AP, Deckersbach T, Kunkel L, Goff D, Heckers S (2002) Assessing hippocampal volume in schizophrenia using a standardized MRI protocol. Biol Psychiatry 51:21S.

Dusek JA, Eichenbaum H (1997) The hippocampus and memory for orderly stimulus relations. Proceedings of the National Academy of Sciences of the United States of America 94:7109-7114.

Eichenbaum H (2004) Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 44:109-120.

Fletcher PC, Zafiris O, Frith CD, Honey RAE, Corlett PR, Zilles K, Fink GR (2005) On the Benefits of not Trying: Brain Activity and Connectivity Reflecting the Interactions of Explicit and Implicit Sequence Learning. Cerebral Cortex 15:1002-1015.

Heckers S, Zalesak M, Weiss AP, Ditman T, Titone D (2004) Hippocampal activation during transitive inference in humans. Hippocampus 14:153-162.

Heun R, Freymann N, Granath DO, Stracke CP, Jessen F, Barkow K, Reul J (2004) Differences of cerebral activation between superior and inferior learners during motor sequence encoding and retrieval. Psychiatry Research: Neuroimaging 132:19-32.

Huettel SA, Song AW, McCarthy G (2005) Decisions under Uncertainty: Probabilistic Context Influences Activation of Prefrontal and Parietal Cortices. The Journal of Neuroscience 25:3304-3311.

Jancke L, Himmelbach M, Shah NJ, Zilles K (2000) The Effect of Switching between Sequential and Repetitive Movements on Cortical Activation. Neuroimage 12:528-537.

Kroger JK, Sabb FW, Fales CL, Bookheimer SY, Cohen MS, Holyoak KJ (2002) Recruitment of Anterior Dorsolateral Prefrontal Cortex in Human Reasoning: a Parametric Study of Relational Complexity. Cerebral Cortex 12:477-485.

Kumaran D, Maguire EA (2006) The Dynamics of Hippocampal Activation during Encoding of Overlapping Sequences. Neuron 49:617-629.

McGonigle B, Chalmers M (1986) Representations and strategies during inference. In: Reasoning and discourse processes (Myers T, Brown K, McGonigle B, eds), pp 141-164. London: Academic Press.

Meredith GE, Totterdell S (1999) Microcircuits in nucleus accumbens' shell and core involved in cognition and reward. Psychobiology 27:165-186.

Muller R-A, Kleinhans N, Pierce K, Kemmotsu N, Courchesne E (2002) Functional MRI of motor sequence acquisition: effects of learning stage and performance. Cognitive Brain Research 14:277-293.

Nagahama Y, Okada T, Katsumi Y, Hayashi T, Yamauchi H, Sawamoto N, Toma K, Nakamura K, Hanakawa T, Konishi J, Fukuyama H, Shibasaki H (1999) Transient Neural Activity in the Medial Superior Frontal Gyrus and Precuneus Time Locked with Attention Shift between Object Features. Neuroimage 10:193-199.

Nagode JC, Pardo JV (2002) Human hippocampal activation during transitive inference. Neuroreport 13:939-944.

Nyberg L, Eriksson J, Larsson A, Marklund P (2006) Learning by doing versus learning by thinking: An fMRI study of motor and mental training. Neuropsychologia 44:711-717.

Oshi K, Toma K, Bagarinao ET, Matsuo K, Nakai T, Chihara K, Fukuyama H (2005) Activation of the precuneus is related to reduced reaction time in serial reaction time tasks. Neuroscience Research 52:37-45.

Pruessner JC, Li LM, Serles W, Pruessner M, Collins DL, Kabani N, Lupien S, Evans AC (2000) Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. Cerebral Cortex 10:433-442.

Rhodes BJ, Bullock D, Verwey WB, Averbeck BB, Page MPA (2004) Learning and production of movement sequencs: Behavioral, neurophysiological, and modeling perspectives. Human Movement Science 23:699-746.

Ridderinkhof KR, van den Wildenberg WPM, Segalowitz SJ, Carter CS (2004) Neurocognitive mechanisms of cognitive control: The role of prefrontal cortex in action selection, response inhibition, performance monitoring, and reward-based learning. Brain and Cognition 56:129-140.

Rushworth MF, Hadland KA, Paus T, Sipila PK (2002) Role of the human medial frontal cortex in task switching: a combined fMRI and TMS study. Journal of Neurophysiology 87:2577-2592.

Sakai K, Hikosaka O, Miyauchi S, Takino R, Sasaki Y, Putz B (1998) Transition of Brain Activation from Frontal to Parietal Areas in Visuomotor Sequence Learning. The Journal of Neuroscience 18:1827-1840.

Schendan HE, Searl MM, Melrose RJ, Stern CE (2003) An fMRI Study of the Role of the Medial Temporal Lobe in Implicit and Explicit Sequence Learning. Neuron 37:1013-1025.

Shrout PE, Fleiss JL (1979) Intraclass correlations: uses in assessing rater reliability. Psychological Bulletin 86:420-428.

van der Graaf FHCE, de Jong BM, Maguire RP, Meiners LC, Leenders KL (2004) Cerebral activatio related to skills practice in a double serial reaction time task: striatal involvement in random-order sequence learning. Cognitive Brain Research 20:120-131.

von Fersen L, Wynne CDL, Delius JD, Staddon JER (1991) Transitive inference formation in pigeons. Journal of Experimental Psychology: Animal Behavior Processes 17:334-341.

Chapter 6: Conclusion, implications for schizophrenia, and future directions

Conclusion

The main goal for the research effort described in this thesis was to elucidate the neural correlares of transitive inference (TI) in humans. We conducted three fMRI experiments in the healthy population to understand normal function of the hippocampus and other brain areas that have been previously implicated in TI.

TI refers to the process whereby we make inferences about relations between separate items, based on other relations among those items. In the classical experiment for TI, where participants learn the ordered sequence A>B>C>D>E by exposure to the overlapping pairs (A>B, B>C, C>D, D>E) from the sequence, TI is tested using the novel pair BD. Using this experiment, capacity for TI has been demonstrated in birds (Strasser et al., 2004), rodents (Davis, 1992; Dusek and Eichenbaum, 1997; Van Elzakker et al., 2003), monkeys (McGonigle and Chalmers, 1986; Buckmaster et al., 2004) and in behavioral experiments in humans (Greene et al., 2001; Martin and Alsop, 2004). This classical experiment was also used to show the indispensable role of the hippocampus in TI as animals with lesions in this structure cannot correctly pick B over D, whereas their capacity to learn the original overlapping pairs and pick A over E (the end-items of the sequence) is spared (Dusek and Eichenbaum, 1997; Buckmaster et al., 2004).

We set out to elucidate the role of the hippocampus in TI in humans using an adaptation of the the ordered sequence paradigm. In our first study, participants were trained on a set of overlapping pairs (A>B, B>C, C>D, and D>E) that constituted the ordered sequence A-E and a set of non-overlapping individual pairs (a>b, c>d, e>f, and g>h). We first

demonstrated that TIs on the ordered sequence (e.g., A>C and B>D) elicited hippocampal activation compared with non-TIs on individual pairs (e.g., a>d and e>h) (Experiment 1). The block-design nature of the experiment, however, did not permit comparison of non end-item TI (NETI, e.g., B>D) and end-item TI (ETI, e.g., A>C). We, therefore, designed an event-related fMRI study (Experiment 2), which used the six-item ordered sequence A>B>C>D>E>F that permitted testing on three NETI pairs (i.e., B>D, B>E and C>E). We showed increased hippocampal activity for NETI compared with ETI. Further, we detected greater hippocampal activity for hard NETI trials with a symbolic distance of one (i.e., one intervening item in the sequence, namely B>D and C>D) than with a symbolic distance of two (i.e., two intervening items in the sequence, namely B>E). In our third experiment (Experiment 3), we showed hippocampal activation during training on the overlapping pairs that comprise the sequence, compared with training on the non-overlapping individual pairs.

Together, these experiments convincingly supported the relational memory account of hippocampal function. In this account, the hippocampus acts to rapidly bind common features into a unified representation that supports flexible inferential memory expression (Eichenbaum, 2004). We confirmed its role in explicit learning and organizing sequence information (Experiment 3), and its role in flexible expression of the sequence for TI (Experiment 1). Further, we demonstrated the specificity of its function in TI for situations where the previously learned sequence of overlapping pairs had to be manipulated flexibly to solve NETI (Experiment 2). Hippocampal recruitment is also enhanced under circumstances that call for a greater degree of flexible manipulation of

the sequence as when the two items in the novel pair are closer together on the sequence continuum (smaller symbolic distance) (Experiment 2). In addition, the behavioral results presented in this thesis provide further support for the relational flexibility account compared with the associative strength/value transfer account. Under the value transfer account, individual items acquire different associative weights during training and novel pairs are then solved by implicit comparison of these weights. This view is in contrast to the relational flexibility account where a mental representation of the sequence exists, and novels pairs are solved by referring to this representation.

Besides the hippocampus, our experiments detected a network of brain areas that also supports learning ordered sequences and making TIs on those sequences. Included in this network are PFC, preSMA, SMA, insula, anterior and posterior cingulate cortices, lateral temporal cortex, precuneus, posterior parietal cortex, cerebellum, thalamus, ventral striatum, and midbrain (the TI network). The role of the network may differ between learning and structuring of the ordered sequence, and TI judgments. During learning, PFC, the cingulate cortex, insula, preSMA, SMA, and the thalamus support manipulation and integration of information from the overlapping pairs into a sequence. Posterior parietal cortex contributes to learning by representing the ordered sequence in a visuospatial dimension. In TI judgments, PFC supports reasoning, whereas activation of the thalamus and the insula reflects decisions under uncertainty. Parietal activation supports comparisons that are made between items in the novel pairs in the visuospatial representation of the ordered sequence. The midbrain codes the salience and/or predictability information about the winning item from the novel pair. This information

together with information from the hippocampus may be integrated in the ventral striatum to guide the appropriate choice when faced with the novel pair in TI.

Given that two of the three fMRI experiments described in this thesis were conducted under high magnetic field strength (3.0T) where susceptibility artifacts such as geometric distortions and signal loss could become pronounced, we set out to evaluate their effect on our data, and to investigate methods that could correct for this effect. These artifacts lead to a mismatch between participants' structural and functional images, which are coregistered together. Because geometric distortions and signal loss are particularly prominent in MTL, we examined the use of field maps for distortion correction. This correction did not affect data from the hippocampus significantly. We concluded that field maps are not sensitive to the moderate-sized susceptibility gradients throughout the hippocampus. Susceptibility artifacts and inter-subject anatomical variability together contribute to uncertainty in localization of activations in group analysis, which uses data normalized to a common template. Given the high degree of anatomical variability of the hippocampus across the population (Pruessner et al., 2000; Miller et al., 2005), the use of normalization is of particular concern in this structure. We, therefore, supplemented the whole-brain analysis in Experiments 2 and 3 with a region-of-interest (ROI) analysis in individual participant's native rather than normalized space, thereby ensuring that hippocampal activation would be reliably localized. The hippocampal activations related to the ordered sequence learning detected in Experiment 3 and to TI on the ordered sequence reported in Experiment 2 can, therefore, be localized to the hippocampus within

the same degree of uncertainty associated with registration mismatch, which was found to be approximately 3 mm throughout the hippocampus.

Implications for schizophrenia

An implementation of the ordered sequence experiment demonstrated a TI impairment in SZ (Titone et al., 2004). SZ patients were able to learn the original overlapping pairs and correctly pick A over E in the subsequent test, but they failed to pick B over D. This impairment was seen as evidence of a specific deficit of memory, which in itself is an area of a specific and differential impairment of cognitive function in SZ (Harrison, 1999; Kuperberg and Heckers, 2000; Antonova et al., 2004; Emilien et al., 2004). Intriguingly, abnormalities of hippocampal structure and function are amongst the most frequently cited findings in SZ research (Harrison, 1999; Heckers, 2001; Heckers and Konradi, 2002; Harrison, 2004). Can we make a link between the TI impairment and abnormalities in hippocampal structure and function in SZ?

The neural underpinnings of the TI impairment in SZ were recently investigated in a study conducted by the research group of which I was a member (Ongur et al., 2006). The study adopted the block-design experiment described in Chapter 1, where TIs (inferences on pairs from the ordered sequence A-E) were compared with non-transitive inferences (inferences on individual unrelated pairs). An event-related analysis was also performed to compare brain activations in NETI (on the BD pair devoid of either sequence end-item) and ETI (on pairs including an end-item, e.g., A>C). The study replicated the previously reported (Titone et al., 2004) selective deficit of SZ patients in

hard TI. When NETI and ETI trials were combined, accuracy did not differ between the healthy control group and the SZ group. Further, the control group showed similar accuracy for NETI and ETI, but the SZ group exhibited significantly diminished accuracy on the hard inference pair BD.

Imaging data provided neural correlates of this behavioral deficit. In the contrast between NETI and ETI, a whole-brain analysis revealed that the SZ group showed significantly diminished activation in the inferior parietal cortex (BA 7 and 40) compared with the control group. The whole-brain analysis was supplemented by an anatomically guided ROI analysis, which demonstrated significant activation in the left anterior hippocampus in the healthy group, which was significantly stronger than that in the SZ group.

The experiment thus provided evidence that the TI deficit observed in SZ is related to deficient hippocampal function. Specifically, the deficit in NETI trials, which were shown to elicit left hippocampal activation in the event-related experiment described in Chapter 2, is associated with impaired recruitment of the left hippocampus. NETI requires greater degree of flexible manipulation of the sequence representation and thus elicits greater hippocampal recruitment, as demonstrated by the TI experiment described in Chapter 2. This report of the relationship between a relatively selective behavioral deficit and impaired left hippocampal recruitment during hard inference joins a multitude of other neuroimaging studies that link memory deficits in SZ with abnormal recruitment of the hippocampus (Heckers et al., 1998; Heckers, 2001; Weiss et al., 2003; Weiss et al., 2004). Together, these studies suggest that impaired hippocampal function contributes to

the well documented memory deficits in SZ (Kuperberg and Heckers, 2000; Egeland et al., 2003; Antonova et al., 2004; Emilien et al., 2004; Muller et al., 2004). Along with other domains of cognitive dysfunction, these memory deficits, in turn, may be a key factor in preventing SZ patients from holding jobs and integrating into society (Goff et al., 2001).

What is the nature of the hippocampal abnormality in SZ that underlies the TI deficit? In a multitude of postmortem and imaging studies, hippocampal volume has been shown to be decreased compared to normal samples (Harrison, 2004; Weiss et al., 2005). Abnormalities of hippocampal shape have also been reported (Harrison and Weinberger, 2005). On the level of hippocampal subfields, the CA1 field is relatively spared in SZ compared to the CA2/3 fields that are affected more severely. In the latter, marked cellular and molecular abnormalities have been identified such as increase of GABAergic receptors on interneurons and decreases in mRNA expression of a glutamic acid decarboxylase, a key enzyme in GABAergic neurons (Heckers and Konradi, 2002). Interestingly, the CA2/3 subfield is hypothesized to possess the necessary characteristics that could mediate properties of relational networks (Eichenbaum, 2004). It is possible that the relational memory deficit in SZ, such as the TI impairment investigated here, could be linked to structural and functional abnormalities of the CA2/3 subfield of the hippocampus.

In addition to deficient hippocampal and inferior parietal cortex recruitment in NETI trials, abnormalities in brain activation were also detected in the contrast between TI and

non-TI. In this comparison, healthy participants showed significant brain activation in inferior parietal cortex (BA 7 and 40), inferior frontal gyrus (BA 47), preSMA and SMA (BA 6), pulvinar, anterior cingulate cortex (BA 24 and 32), posterior temporal cortex (BA 21 and 37) and hippocampal formation. The results thus replicated the findings of activation of the hippocampus and the TI network in TI. SZ participants demonstrated significant brain activation in the inferior frontal gyrus (BA 47), the pre-supplementary motor area (BA 6), the posterior temporal cortex (BA 21 and 37). A comparison of the two groups revealed significantly reduced activation in the anterior cingulate cortex and the posterior parietal cortex in SZ.

PreSMA seems to play an integral role in TI tasks based on previous studies (Acuna et al., 2002b; Heckers et al., 2004a) and the experiments described in this thesis. Prior studies had shown robust preSMA activation during cognitive task performance in SZ (Yucel et al., 2002; Heckers et al., 2004b). Results of the TI study indicated that the function of the preSMA was not impaired in SZ during relational memory tasks that required the storage and retrieval of a sequence. This is in contrast with a recent study that linked reduced preSMA volume in SZ with impaired implicit motor sequence learning (Exner et al., 2006). Volume measurements for the preSMA were not obtained in the TI study, but the results suggested that preSMA function during the retrieval of the ordered sequence for TI was not impaired in SZ.

Posterior parietal cortex (Brodmann areas 7 and 40) also plays an important role within the TI network. Decreased recruitment of the parietal cortex in SZ during TI judgments

was found overall, and during the hard inference BD trials in particular. As discussed in the Introduction, parietal lobe is one of the brain areas whose volume abnormalities have been reported in SZ in structural MRI studies (Shenton et al., 2001). Gray matter reductions are thought to be particularly prominent in the inferior parietal lobule, which includes the Broadmann area 40 activated in TI by normal participants (Pearlson et al., 1996; Ross and Pearlson, 1996). Previous studies provided evidence of parietal cortex dysfunction in SZ in two-choice decision-making task that involved uncertainty (Paulus et al., 2002; Paulus et al., 2003). TI can be thought of as such a two-choice decision making task, where the choices are made along a visuospatial continuum of the ordered sequence, which may also be represented in the parietal lobe. The reduced activation of the parietal lobe identified may reflect abnormalities in both the decision-making and the visuospatial representation aspects of its function in TI.

The anterior cingulate cortex is another area that has been implicated in TI. I have interpreted its role in TI in the context of its function in monitoring response errors, decision uncertainty, and overall performance (Ridderinkhof et al., 2004); as well as in the planning, response conflict resolution, selection and execution of correct responses (Quintana et al., 2004). TIs compared to non-TIs s involve more uncertainty in the selection of correct responses, which lead to greater conflicts, thus eliciting greater activity of the anterior cingulate cortex. The deficit of anterior cingulate function in SZ described here is consistent with other reports of its abnormal function in tasks involving conflict resolution in response selection (Yucel et al., 2002; Heckers et al., 2004b; Kerns et al., 2005). These functional deficits, in turn, may stem from the well-documented

structural abnormalities of the anterior cingulate cortex in SZ including overall (Lewis and Lieberman, 2000) and gray matter (Mitelman et al., 2005) volume reductions and cytoarchitectural (Bogerts, 1999) and cellular (Benes, 1995; Clark et al., 2006) abnormalities.

In summary, the three experiments investigating TI in healthy participants pointed to an important role for the hippocampus and a network of brain areas including prefrontal cortex, cingulate cortex, pre-supplementary (preSMA) and supplementary motor areas (SMA), precuneus, posterior parietal cortex, thalamus, and ventral striatum. The results guided my interpretation of the impaired recruitment of the hippocampus, and the anterior cingulate and posterior parietal cortices as underlying the deficit in TI in SZ.

Future directions

Given the selectivity of the TI deficit in SZ, and the neural correlates of this deficit in abnormal function of structures that are known to be altered in this disease, one may consider the TI impairment in SZ as a potential endophenotype. Endophenotypes are internal phenotypes within the disease that are discoverable by a neurobiological, neurocognitive, biochemical, or microscopic exam (Gottesman and Gould, 2003). An endophenotype is more formally defined as a phenotype that is (1) associated with illness in the population, (2) heritable, (3) primarily state-independent (is manifested in an individual whether the illness is or is not active), that (4) cosegregates with the illness in multiply affected families, and (5) is found in affected family members at a greater rate than in general population (Owen et al., 2005). The motivation for introducing

endophenotypes lies in the fact that the complex architecture of the disease could thus be reduced to specific abnormalities that separate the disease from normal state and have biological origins that can be readily investigated (Heinrichs, 2005).

Because cognitive deficits are seen as the most stable feature of SZ (Heinrichs, 2005), it is not surprising that cognitive features figure prominently in the list of putative endophenotypes. Measures of attention (Ban, 2004), working memory and executive function (Gottesman and Gould, 2003), and verbal memory (Heinrichs, 2004) have been suggested as endophenotypes. The support for cognitive measures, such attention, verbal declarative memory, perceptual-motor speed, and verbal fluency as potential endophenotypes further comes from studies of well relatives of SZ patients who tend to show impairments in these domains (Hoff and Kremen, 2002; Hoff et al., 2005). At least some of these cognitive measures may, therefore, be heritable and may conform to the definition of endophenotypes (Hallmayer et al., 2005).

TI presents another putative cognitive measure that qualifies as an endophenotype. The next steps that need to be taken to establish it as an endophenotype require demonstrating that it is heritable, that it can be identified in well relatives of SZ patients, and that it co-segregates with the disease in multiply affected families. Ultimately, endophenotypes could also be used as diagnostic measures that would be more reliable than measures based on subjective reporting of symptoms by patients. Whether the TI impairment could serve as such a diagnostic measure needs to be evaluated further.

References

Acuna BD, Eliassen JC, Donoghue JP, Sanes JN (2002) Frontal and parietal lobe activation during transitive inference in humans. Cerebral Cortex 12:1312-1321.

Antonova E, Sharma T, Morris R, Kumari V (2004) The relationship between brain structure and neurocognition in schizophrenia: a selective review. Schizophrenia Res 70:117-145.

Ban TA (2004) Neuropsychopharmacology and the genetics of schizophrenia. A history of the diagnosis of schizophrenia. Progress in Neuro-Psychopharmacology & Biological Psychiatry 28:753-762.

Benes FM (1995) Altered glutamatergic and GABAergic mechanisms in the cingulate cortex of the schizophrenic brain. Arch Gen Psychiatry 52:1015-1018; discussion 1019-1024.

Bogerts B (1999) The neuropathology of schizophrenic diseases: historical aspects and present knowledge. Eur Arch Psychiatry Clin Neurosci:IV/2-IV/13.

Buckmaster CA, Eichenbaum H, Amaral DG, Suzuki WA, Rapp PR (2004) Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. The Journal of Neuroscience 24:9811-9825.

Clark D, Dedova I, Cordwell S, Matsumoto I (2006) A proteome analysis of the anterior cingulate cortex gray matter in schizophrenia. Molecular Psychiatry 11:459-470.

Davis H (1992) Transitive inference in rats (*Rattus norvegicus*). Journal of Comparative Psychology 106:342-349.

Dusek JA, Eichenbaum H (1997) The hippocampus and memory for orderly stimulus relations. Proceedings of the National Academy of Sciences of the United States of America 94:7109-7114.

Egeland J, Sundet K, Rund BR, Asbjornsen A, Hugdahl K, Landro NI, Lund A, Roness A, Stordal KI (2003) Sensitivity and Specificity of Memory Dysfunction in Schizophrenia: A Comparison with Major Depression. Journal of Clinical & Experimental Neuropsychology 25:79-93.

Eichenbaum H (2004) Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 44:109-120.

Emilien G, Durlach C, Antoniadis E, Van Der Linden M, Maloteaux J-M (2004) Memory: neuropsychological, imaging and psychopharmacological perspectives. Hove and New York: Psychology Press.

Exner C, Weniger G, Schmidt-Samoa C, Irle E (2006) Reduced size of the pre-supplementary motor cortex and impaired motor sequence learning in first-episode schizophrenia. Schizophrenia Res.

Goff DC, Heckers S, Freudenreich O (2001) Schizophrenia. In: The Medical Clinics of North America. Advances in the pathophysiology and treatment of psychiatric disorders: Implications for internal medicine (Nemeroff CB, ed), pp 663-689. Philadelphia: W.B. Saunders.

Gottesman II, Gould TD (2003) The Endophenotype Concept in Psychiatry: Etymology and Strategic Intentions. Am J Psychiatry 160:636-645.

Greene AJ, Spellman BA, Dusek JA, Eichenbaum HB, Levy WB (2001) Relational learning with and without awareness: transitive inference using nonverbal stimuli in humans. Memory & Cognition 29:893-902.

Hallmayer JF, Kalaydjieva L, Badcock J, Dragovic M, Howell S, Michie PT, Rock D, Vile D, Williams R, Corder EH, Hollingsworth K, Jablensky A (2005) Genetic Evidence for a Distinct Subtype of Schizophrenia Characterized by Pervasive Cognitive Deficit. American Journal of Human Genetics 77:468-476.

Harrison PJ (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122:593-624.

Harrison PJ (2004) The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications. Psychopharmacology 174:151-162.

Harrison PJ, Weinberger DR (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Molecular Psychiatry 10:40-68.

Heckers S (2001) Neuroimaging studies of the hippocampus in schizophrenia. Hippocampus 11:520-528.

Heckers S, Konradi C (2002) Hippocampal neurons in schizophrenia. Journal of Neural Transmission 109:891-905.

Heckers S, Zalesak M, Weiss AP, Ditman T, Titone D (2004a) Hippocampal activation during transitive inference in humans. Hippocampus 14:153-162.

Heckers S, Weiss AP, Deckersbach T, Goff D, Morecraft RJ, Bush G (2004b) Anterior cingulate cortex activation during cognitive interference in schizophrenia. Am J Psychiatry 161:707-715.

Heckers S, Rauch SL, Goff D, Savage CR, Schacter DL, Fischman AJ, Alpert NM (1998) Impaired recruitment of the hippocampus during conscious recollection in schizophrenia. Nature Neuroscience 1:318-323.

Heinrichs RW (2004) Meta-analysis and the science of schizophrenia: variant evidence or evidence of variants. Neuroscience and Biobehavioral Reviews 28:379-394.

Heinrichs RW (2005) The Primacy of Cognition in Schizophrenia. American Psychologist 60:229-242.

Hoff AL, Kremen WS (2002) Is there a cognitive phenotype for schizophrenia: the nature and course of the disturbance in cognition. Current Opinion in Psychiatry 15:43-48.

Hoff AL, Svetina C, Maurizio AM, Crow TJ, Spokes K, DeLisi LE (2005) Familial Cognitive Deficits in Schizophrenia. American Journal of Medical Genetics Part B 133B:43-49.

Kerns JG, Cohen JD, MacDonald AW, 3rd, Johnson MK, Stenger VA, Aizenstein HJ, Carter CS (2005) Decreased Conflict- and Error-Related Activity in the Anterior Cingulate Cortex in Subjects with Schizophrenia. Am J Psychiatry 162:1833-1839.

Kuperberg G, Heckers S (2000) Schizophrenia and cognitive function. Current Opinion in Neurobiology 10:205-210.

Lewis DA, Lieberman JA (2000) Catching up on schizophrenia: natural history and neurobiology. Neuron 28:325-334.

Martin N, Alsop B (2004) Transitive inference and awareness in humans. Behavioral Processes 67:157-165.

McGonigle B, Chalmers M (1986) Representations and strategies during inference. In: Reasoning and discourse processes (Myers T, Brown K, McGonigle B, eds), pp 141-164. London: Academic Press.
Miller MI, Beg MF, Ceritoglu C, Stark C (2005) Increasing the power of functional maps of the medial temporal lobe by using large deformation diffeomorphic metric mapping. Proc Natl Acad Sci U S A 102:9685-9690.
Mitelman SA, Shihabuddin L, Brickman AM, Hazlett EA, Buchsbaum MS (2005) Volume of the cingulate and outcome in schizophrenia. Schizophrenia Res 72:91-108.
Muller BW, Sartory G, Bender S (2004) Neuropsychological Deficits and Concomitant Clinical Symptoms in Schizophrenia. European Psychologist 9:96-106.
Ongur D, Cullen TJ, Wolf DH, Rohan M, Barreira P, Zalesak M, Heckers S (2006) The Neural Basis of Relational Memory Deficits in Schizophrenia. Arch Gen Psychiatry 63:356-365.
Owen MJ, Craddock N, O'Donovan MC (2005) Schizophrenia: genes at last? Trends in Genetics 21:518-525.
Paulus MP, Frank L, Brown GG, Braff DL (2003) Schizophrenia Subjects Show Intact Success-Related Neural Activation but Impaired Uncertainty Processing during Decision-Making. Neuropsychopharmacology 28:795-806.
Paulus MP, Hozack NE, Zauscher BE, Frank L, Grown GG, McDowell J, Braff DL (2002) Parietal dysfunction is associated with increased outcome-related decision making in schizophrenia patients. Biol Psychiatry 51:995-1004.
Pearlson GD, Petty RG, Ross CA, Tien YA (1996) Schizophrenia: a disease of heteromodal association cortex? Neuropsychopharmacology 14:1-17.
Pruessner JC, Li LM, Serles W, Pruessner M, Collins DL, Kabani N, Lupien S, Evans AC (2000) Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. Cerebral Cortex 10:433-442.
Quintana J, Wong T, Ortiz-Portillo E, Marder S, Mazziotta JC (2004) Anterior cingulate dysfunction during choice anticipation in schizophrenia. Psychiatry Research: Neuroimaging 132:117-130.
Ridderinkhof KR, van den Wildenberg WPM, Segalowitz SJ, Carter CS (2004) Neurocognitive mechanisms of cognitive control: The role of prefrontal cortex in action selection, response inhibition, performance monitoring, and reward-based learning. Brain and Cognition 56:129-140.
Ross CA, Pearlson GD (1996) Schizophrenia, the heteromodal association neocortex and development: potential for a neurogenetic approach. Trends Neurosci 19:171-176.
Shenton ME, Dickey CC, Frumin M, McCarley RW (2001) A review of MRI findings in schizophrenia. Schizophrenia Research 49:1-52.
Strasser R, Ehrlinger JM, Bingman VP (2004) Transitive Behavior in Hippocampal-Lesioned Pigeons. Brain, Behavior & Evolution 63:181-188.
Titone D, Ditman T, Holzman PS, Eichenbaum H, Levy DL (2004) Transitive inference in schizophrenia: impairments in relational memory organization. Schizophrenia Res 68 (2-3):235-247.

Van Elzakker M, O'Reilly RC, Rudy JW (2003) Transitivity, flexibility, conjunctive representations, and the hippocampus. I. An empirical analysis. Hippocampus 13:334-340.

Weiss AP, DeWitt I, Goff D, Ditman T, Heckers S (2005) Anterior and posterior hippocampal volumes in schizophrenia. Schizophrenia Res 73:103-112.

Weiss AP, Zalesak M, DeWitt I, Goff D, Kunkel L, Heckers S (2004) Impaired hippocampal function during the detection of novel words in schizophrenia. Biol Psychiatry 55:668-675.

Weiss AP, Schacter DL, Goff DC, Rauch SL, Alpert NM, Fischman AJ, Heckers S (2003) Impaired hippocampal recruitment during normal modulation of memory performance in schizophrenia. Biol Psychiatry 53:48-55.

Yucel M, Pantelis C, Stuart GW, Wood SJ, Maruff P, Velakoulis D, Pipingas A, Crowe SF, Tochon-Danguy HJ, Egan GF (2002) Anterior cingulate activation during Stroop task performance: a PET to MRI coregistration study of individual patients with schizophrenia. Am J Psychiatry 159:251-254.