A FUNCTIONAL NEUROIMAGING STUDY OF WORKING MEMORY IN CHILDREN WITH CHRONIC KIDNEY DISEASE

Waverly Rachel Harrell

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Approved by:

Steven Knotek, Ph.D.

Stephen R. Hooper, Ph.D.

Debbie S. Gipson, M.D.

Aysenil Belger, Ph.D.

Jeffrey A. Greene, Ph.D.

ABSTRACT

Waverly R. Harrell: A Functional Neuroimaging Study of Working Memory in Children with Chronic Kidney Disease
(Under the direction of Stephen Hooper, Ph.D.)

Pediatric chronic kidney disease is associated with deficits in general intellectual functioning, and some evidence indicates specific deficits in memory and executive functions. Most research has focused on children with end-stage renal disease. In the current study, children with moderate as well as severe chronic kidney disease were compared with healthy controls in terms of brain activation patterns during a visual-spatial working memory task. Accuracy was similar among all groups. Reaction time was slower in patients with chronic kidney disease than in healthy controls. Patients with chronic kidney disease showed reduced activity in posterior parietal regions compared with controls, and patients with severe chronic kidney disease showed lower activation over the course of the task in numerous regions of the brain compared with controls. This dissertation is dedicated to my parents, Evans and Janice Harrell, whose love and support have been invaluable on the long journey through graduate school.

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LIST OF ABBREVIATIONS

ABC	Adaptive Behavior Composite
ADHD	Attention Deficit/Hyperactivity Disorder
ANCA	Anti-neutrophil cytoplasmic autoantibody
ANOVAs	Analyses of variance
BA	Brodmann Area
BIAC	Brain Imaging and Analysis Center
BIRN	Biomedical informatics research network
BOLD	Blood oxygenation level dependent
CHD	Congenital heart disease
CKD	chronic kidney disease
CKiD	Chronic Kidney Disease in Children Prospective Cohort Study
CVLT-C	California Verbal Learning Test, Children's Version
DLPFC	Dorsolateral prefrontal cortex
DTI	Diffusion tensor imaging
EEG	Electroencephalogram
ESA	Erythropoiesis-stimulating agent
ESRD	End-stage renal disease
eGFR	Estimated GFR
FEAT	fMRI expert analysis tool
fMRI	Functional magnetic resonance imaging
FSGS	Focal segmental glomerulosclerosis

GLM	General linear modeling
HD	Hemodialysis
MCMC	Markov chain Monte Carlo
MDAT	Modified Developmental Assessment Test
MNI	Montreal Neurological Institute
MR	Magnetic resonance
NHANES	National Health and Nutrition Examination Survey
NIH	National Institutes of Health
NKF-K/DOQI	National Kidney Foundation's Kidney Disease Outcomes Quality Initiative
PD	Peritoneal dialysis
PET	Positron emission technology
PET	Positron emission tomography
PFC	Prefrontal cortex
QA	Quality analysis
rCBF	Regional cerebral blood flow
ROI	Region-of-interest
T	Tesla
TMS	Transcranial magnetic stimulation
TR	Repetition time
Vineland	Vineland Adaptive Behavior Scales-II
VLPFC	Ventrolateral prefrontal cortex
VUR	Vesicoureteral reflux
WASI	Wechsler Abbreviated Scales of Intelligence

WISC-III	Wechsler Intelligence Scales for Children, Third Edition
WJ-R	Woodcock-Johnson Tests of Achievement Revised
WRAT	Wide Range Achievement Test
VAS	Visual Analogue Scale

CHAPTER I

INTRODUCTION

Chronic kidney disease

The kidneys filter waste products and excess water out of the blood and send them to the bladder to be excreted as urine. This process helps to maintain blood pressure at a healthy level and regulates levels of ions important to bodily functions, such as sodium and potassium ("Treatment methods for kidney failure in children," 2006). The kidneys also produce hormones that stimulate red blood cell production, promote calcium absorption, and regulate blood pressure. Consequently, chronic kidney disease (CKD) has far reaching effects on multiple body systems (Harmon, 1999; Wassner & Baum, 1999).

Kidney function is typically assessed using glomerular filtration rate (GFR; Dalton & Haycock, 1999). Filtration of blood by the glomeruli is the first step in producing urine and is reduced when renal function is diminished. GFR is determined not through direct measurement but through measurement of the rate at which the kidneys clear certain compounds from the blood (Dalton & Haycock, 1999). The current gold standard for estimating GFR is based on the rate at which the contrast agent iohexol is cleared from plasma (Schwartz et al., 2009). Iohexol is used for this purpose because it is excreted almost entirely by the kidneys (Nilsson-Ehle & Grubb, 1994; Sterner et al., 2000; Frennby et al., 1995), is not reabsorbed or secreted by the kidneys (Bäck, Krutzén & Nilsson-Ehle, 1988), and binds plasma proteins to only a small extent (Krutzén, Bäck,

Nilsson-Ehle & Nilsson-Ehle, 1984). However, because injection of a contrast agent is inconvenient for routine, repeated GFR assessments, efforts have been made to find a way of calculating GFR based on demographic variables and endogenous substances (Schwartz et al., 2009).

GFR is frequently calculated based on blood levels of creatinine, a waste product of metabolism which is not reused and is normally excreted almost entirely by the kidneys. Serum creatinine is inversely related to glomerular filtration rate. Because creatinine is produced primarily by muscle tissue, expected creatinine production depends on a person's muscle mass. One established method used to estimate GFR in children with CKD is the Schwartz formula (Schwartz, Haycock, Edelmann & Spitzer, 1976), which takes into account serum creatinine, age, height, and gender, using different multipliers for adolescent boys versus children or adolescent girls to account for the increase in muscle mass during male adolescence (Fadem & Rosenthal, 2000). Although this formula has been reported to generate a good clinical estimate of GFR (Furth et al., 2006), it has recently been found to overestimate GFR, perhaps because methods of determining serum creatinine have changed since its development (Schwartz et al., 2009). The current study estimates patients' GFRs using a modified version of the Schwartz formula, which has been found to generate estimated GFR (eGFR) values within 30% of estimates based on iohexol clearance (iGFR) in 79.4% of cases (Schwartz et al., 2009).

Etiology and prevalence

Causes of chronic kidney disease include malformation of the kidneys or urinary tract, cystic or inflammatory conditions that damage or block renal tubules, and metabolic or autoimmune disorders, such as diabetes or lupus ("Treatment methods for kidney

failure in children," 2006). In children, the most common causes are hereditary, congenital, or cystic diseases, to which 32.7% of cases are attributed; glomerulonephritis (25.2%); and secondary glomerulonephritis or vasculitis (11.4%; United States Renal Data System, 2008b). Structural causes are more common in younger patients, while patients older than 12 are more likely to have glomerulonephritis (Warady & Chadha, 2007). The most frequent single cause of glomerulonephritis is focal segmental glomerulosclerosis (FSGS), which accounts for 13.2% of pediatric ESRD cases (United States Renal Data System, 2008b).

For reasons not well understood, FSGS is three times more common in African-American patients than in white patients (United States Renal Data System, 2005). ESRD in general is two to three times more common in African-American children than in white children (United States Renal Data System, 2001). CKD incidence and prevalence are consistently higher in males than females due to the higher prevalence of relevant congenital disorders in males, including obstructive uropathy, renal dysplasia, and prune belly syndrome (Warady & Chadha, 2007).

The 2003–2006 National Health and Nutrition Examination Survey (NHANES) Update estimated that 15.2% of U.S. adults had CKD (United States Renal Data System, 2008a). ESRD was the least common stage, identified in 0.2% of the U.S. population, or 506,256 people. CKD prevalence has been increasing in the U.S. and worldwide, largely due to aging of populations in developed countries and increasing prevalence of type II diabetes (Warady & Chadha, 2007). In 2001, the worldwide cost of maintenance ESRD therapy, excluding transplants, was estimated at 70 to 75 billion U.S. dollars (Lysaght, 2002).

Chronic kidney disease is much less common in children (Warady & Chadha, 2007). In 2003, the number of ESRD patients in the United States aged 0 to 19 years was estimated to be approximately 7,000 (United States Renal Data System, 2005). ESRD rates in children have increased in the last twenty years, but they have increased much less than those in adults. It is difficult to estimate the prevalence of pediatric CKD in earlier stages because early stages may be asymptomatic (Warady & Chadha, 2007). More than one-quarter of children and adolescents with CKD do not see a nephrologist until they progress to end-stage renal disease (United States Renal Data System, 2008b; Warady & Chadha, 2007).

Staging and progression

CKD is classified according to the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (NKF-K/DOQI) as stages 1 (mild) through 5 (end-stage renal disease). Stages 1 through 4 are often referred to collectively as chronic kidney disease, chronic renal insufficiency, or chronic renal failure (Warady & Chadha, 2007). Table 1 shows the GFR limits for CKD stages 1–5, which apply to children ages 2 years and older (National Kidney Foundation, 2002; Warady & Chadha, 2007).

Table 1

Classification of chronic kidney disease based on glomerular filtration rates.

Stage	GFR (ml/min/1.73 m^2)
1	$\geq$90 with demonstrated kidney damage
2	60–89
3	30–59
4	15–29

5 (ESRD)	<15 or dialysis dependence

Regardless of its original cause, CKD progresses toward end-stage renal disease through a predictable series of events (Fogo & Kon, 1999). In a process called glomerulosclerosis, the glomeruli develop scar tissue, eventually eliminating their ability to filter blood. Perhaps as a compensatory mechanism, cells of the glomeruli enlarge in a process called glomerular hypertrophy. Hypertrophy appears to accelerate glomerulosclerosis, so accommodations helpful in the short term may be ultimately deleterious (Harmon, 1999; Wassner & Baum, 1999).

Progression is more rapid following greater initial loss of functional renal tissue (e.g., from injury); prolonged or recurrent injury (as may occur in diabetes or lupus) as opposed to a single episode of injury; or renal damage earlier in life, perhaps because young children respond with more pronounced hypertrophy. For an individual patient, the rate of progression of CKD depends on the patient's specific disease, individual genetic factors, and treatment. Treatment that alleviates hypertension or prevents or alleviates secondary obstructions and infections can slow progression (Harmon, 1999). Early disease identification and early initiation of treatment are thought to slow progression, and in recent years these processes have improved (Fogo & Kon, 1999). Nonetheless, kidney disease diagnosed in childhood typically progresses to ESRD by early adulthood (Gerson et al., 2006).

Treatment

In end-stage renal disease, the normal waste filtration and excretion functions of the kidneys must be replaced using dialysis or transplantation (Harmon, 1999). Dialysis methods consist of hemodialysis (HD) and peritoneal dialysis (PD). In PD, a solution is

poured into the abdominal cavity through a catheter and later drained along with waste products and excess fluid that would in a healthy person be excreted in urine ("Treatment methods for kidney failure in children," 2006). PD can be done at home by a trained parent or older patient. In HD, the patient's blood is removed and cycled through a machine that filters out wastes before returning the blood to the body. HD is usually performed at an outpatient facility three times per week. Particularly toward the beginning of treatment, children may experience side effects due to rapid changes in blood pressure and metabolites. HD can filter out small waste particles more effectively and is the more common dialysis method, but because of the risk of side effects with HD and inconvenience of treatment, PD is typically preferred for young children (Harmon, 1999; United States Renal Data System, 2008b).

Transplantation is considered the best treatment option for most children with ESRD, and many of these children are sustained by renal transplants (Harmon, 1999; United States Renal Data System, 2008b). A kidney can be provided by a living or deceased donor. Patient survival rates are higher with living donor transplants, which may be performed preemptively, prior to dialysis. Up to 25% of children with ESRD receive preemptive renal transplants (Harmon, 1999). Five years after beginning ESRD treatment, up to 80% of pediatric patients are being maintained on kidney transplants (United States Renal Data System, 2008b).

In general, children with ESRD who have received transplants fare better than those maintained on dialysis, because dialysis replaces only some of the filtration functions of the kidneys and none of their endocrine functions (Harmon, 1999; Bargman & Skorecki, 2008). Patients undergoing dialysis still show uremia (accumulation of waste

products in the blood, including urea, a breakdown product of protein metabolism) along with endocrine and metabolic disturbances. Growth and development are impaired, and difficulties with school have been noted (Harmon, 1999). Five-year survival odds are 0.77 for pediatric patients on hemodialysis, compared with 0.82 for pediatric patients on peritoneal dialysis. Renal transplantation increases patients' five-year survival odds to 0.93 (United States Renal Data System, 2008b).

Ten-year survival for pediatric ESRD has been estimated at 79%, with 20-year survival at 66% (McDonald & Craig, 2004). McDonald and Craig examined survival of 1634 children and adolescents who had begun receiving renal replacement therapy before the age of 20 years, using data from the Australia and New Zealand Transplant Registry from the years 1963 to 2002. Although a trend toward improved survival was observed over the decades studied, mortality rates for children with CKD over the entire time period were 30 times those in children without end-stage renal disease. In the last decade, 5-year survival of pediatric ESRD patients has not improved, and in certain populations 5-year survival rates have actually declined (United States Renal Data System, 2008b).

Effects of CKD

A decline in GFR representing stage 1 or 2 CKD may be asymptomatic, although the underlying illness may cause noticeable signs or symptoms (Bargman & Skorecki, 2008). In later stages, CKD is associated with alterations to nearly all organ systems. The most prominent signs and symptoms are anemia; fatigue; reduced appetite, which can lead to malnutrition; and changes to levels of ions and hormones that result in homeostatic and metabolic disturbances. Some of these complications are treatable, but their multiplicity makes the treatment of CKD complex. Pediatric CKD presents

particular challenges to health care providers because it affects many aspects of a child's growth and development (Gerson et al., 2006).

CKD disturbs the normal balance of fluids, electrolytes, and acids and bases in the blood stream. Many renal disorders result in reduced excretion of sodium (Bargman & Skorecki, 2008). Higher blood levels of sodium then promote fluid retention. This contributes to hypertension, which is one of the most common complications of CKD and often develops early in the course of the disease. Hypertension accelerates loss of renal function and increases a patient's risk for cardiovascular mortality. CKD is also sometimes associated with hyperkalemia, increased potassium, which can occur due to decreased renal clearance of potassium, as a side effect of antihypertensive medications, or as a consequence of metabolic acidosis. Metabolic acidosis, an acidification of the blood, can result from inadequate excretion of protons and is common in severe CKD.

CKD also disrupts bone turnover and mineral metabolism. Reduced GFR reduces excretion of phosphate, causing hyperphosphatemia, which increases synthesis of parathyroid hormone (PTH; Bargman & Skorecki, 2008). Under normal circumstances, PTH stimulates renal excretion of phosphate, so this is a compensatory mechanism. PTH also stimulates bone turnover. Failing kidneys produce lower levels of calcitriol, a form of vitamin D which acts to increase calcium absorption. The resulting lower levels of ionized calcium additionally increase PTH production, causing further bone turnover, abnormal bone remodeling, and in severe cases bone pain and fragility. Of all the compounds present in high levels in the blood of patients with CKD, PTH has been most clearly established as a toxin (Wassner & Baum, 1999). High levels of PTH are associated with muscle weakness and damage to cardiac muscle (Bargman & Skorecki,

2008). Hyperphosphatemia has been linked with higher rates of cardiovascular mortality in CKD.

Because damaged kidneys often do not produce enough erythropoietin, a hormone that stimulates red blood cell production, patients with CKD may show anemia by stage 3 and nearly always have anemia by stage 4 (Bargman & Skorecki, 2008). In some cases, hyperparathyroidism, insufficient iron intake, chronic inflammation, and reduced survival of red blood cells contribute to anemia. Anemia reduces delivery of oxygen to tissues, causing fatigue, impairing cognition, diminishing the body's response to infections, and perhaps contributing to growth retardation. In response to anemia, the heart pumps harder, resulting in ventricular dilatation and hypertrophy. This can contribute to heart failure.

Cardiovascular complications are the leading cause of death in pediatric ESRD patients, followed by complications from infection (United States Renal Data System, 2008b). Hypertension, anemia, hyperphosphatemia, and hyperparathyroidism all increase patients' risk for cardiovascular disease (Bargman & Skorecki, 2008). Reduced renal function is also associated with generalized inflammation and with high blood levels of cholesterol and triglycerides, which are additional risk factors for cardiovascular disease. Cardiovascular complications may include ischemic vascular disease, myocardial ischemia, left ventricular hypertrophy, dilated cardiomyopathy, pericardial disease, and heart failure.

In pediatric patients, CKD tends to retard growth and delay puberty (United States Renal Data System, 2008b). In a 2002 sample of 602 pediatric patients receiving hemodialysis, two-thirds (67%) fell into the lowest quintile (20%) of height for the

general population. On average, puberty has been reported to be delayed 2 years in children with CKD, even including those with functioning transplants (Wühl & Schaefer, 1999). Height gain during puberty is diminished by an average of 50%.

Uremia is also associated with gastrointestinal and neuromuscular abnormalities (Bargman & Skorecki, 2008). Uremia may damage the mucosa lining the gastrointestinal tract, causing GI bleeding, abdominal pain, nausea, vomiting, and loss of appetite. These gastrointestinal complications can lead to malnutrition. In later stages of CKD, neuromuscular complications may include muscle twitching, cramps, and peripheral neuropathy (Bargman & Skorecki, 2008; Fraser & Arieff, 1988). Untreated, advanced kidney failure can result in seizures and coma. In earlier stages, often by stage 3 CKD, disturbances in sleep, memory, and concentration are observed.

CKD in infants has historically been associated with high rates of profound neurologic abnormalities (Rotundo et al., 1982). Over the past two decades, improvements in treatment—higher rates of transplantation, improved nutrition, and avoidance of aluminum as a phosphate binder during dialysis—have improved developmental outcomes (Warady, Belden & Kohaut, 1999). However, pediatric CKD is still associated with cognitive impairments. As a group, children and adolescents with CKD show a distribution of IQ scores that is shifted downward from that seen in the general population. Their IQs fall predominantly in the low average (80–89) and average (90–109) ranges (Gipson, Wetherington, Duquette, & Hooper, 2004). Memory and attention are areas of particular concern. Memory deficits in children with CKD have been widely reported, and more severe deficits have been found to correlate with more severe kidney disease (Gerson et al., 2006; Rasbury, Fennell & Morris, 1983; Fennell et

al., 1990b; Mendley & Zelko, 1999; Slickers, Duquette, Hooper & Gipson, 2007). Impairments in attention have been observed in children with CKD even after controlling for IQ and age (Gipson et al., 2006). The reasons for these cognitive impairments are not well established but may include uremia and complications such as anemia and hypertension (Gerson et al., 2006).

Although research concerning cognitive function in children and adolescents with CKD has focused primarily on those with ESRD, several studies including patients with CKD stages 1–4 suggest that less severe CKD may result in less pronounced cognitive impairments (Fennell et al., 1990a; Hulstijn-Dirkmaat, Damhuis, Jetten, Koster & Schröder, 1995; Slickers et al., 2007). Children who had kidney failure in infancy but received transplants early in life have been found to have normal or near-normal school performance in elementary school (Qvist et al., 2002), and compared with children on dialysis, children who received kidney transplants have earned higher scores on some tests of academic achievement (Lawry, Brouhard & Cunningham, 1994). Children assessed before and after transplantation have shown post-transplantation improvement in IQ (Davis, Chang & Nevins, 1990), sustained attention, working memory (Mendley & Zelko, 1999), and other aspects of cognitive performance (Rasbury et al., 1983; Mendley & Zelko, 1999).

However, not all studies have found transplantation to be associated with improved cognitive performance (Rasbury, Fennell, Fennell & Morris, 1986; Brouhard et al., 2000), perhaps due to small sample sizes or heterogeneous patient groups. Additional research is necessary to establish the risk for cognitive impairment associated with mild to moderate CKD and the extent to which transplantation remedies cognitive impairment

(Slickers et al., 2007).

Because previous research has suggested that pediatric CKD patients show impairments in memory, and because relatively few studies have enrolled patients with mild to moderate CKD, in the current study I examine the process of working memory in patients with CKD stages 2–5, including patients with mild to moderate CKD, dialysis-dependent patients and patients with renal transplants. This study will use functional magnetic resonance imaging (fMRI) to evaluate whether abnormalities in brain activation patterns are associated with impairments in working memory in children and adolescents with CKD. Functional MRI is a noninvasive technique that assesses changes in blood oxygenation in the brain, which are taken to indicate patterns of neuronal activity (Huettel, Song & McCarthy, 2004). I hypothesize that during repeated trials of a working memory task, participants with CKD will show less change in blood oxygenation level dependent (BOLD) signal in brain regions typically activated by working memory, compared with healthy children of similar ages. This is a pilot study, and to my knowledge it is the first fMRI study conducted in children with CKD. I hope that the results may inform future research into the mechanisms underlying memory impairments in children with moderate to severe CKD.

Research questions and hypotheses

Question 1: During the time period representing the hemodynamic response to encoding of object locations during the working memory task, will patients with chronic kidney disease, defined as those having GFR $\leq$ 90 ml/min/1.73m^2, dialysis dependence, or transplant dependence, differ significantly from healthy participants in terms of percent BOLD signal change in functionally activated clusters in the ventrolateral

prefrontal cortex (VLPFC), dorsolateral prefrontal cortex (DLPFC), or right posterior parietal cortex? *Hypothesis*: Percent BOLD signal change over the encoding period will be significantly lower in VLPFC, DLPFC, and right posterior parietal cortex in patients with CKD as compared to controls.

Question 1b: Will any of the three groups—patients with moderate CKD, patients with severe CKD, or healthy participants—differ significantly from each other in terms of percent signal change in the VLPFC, DLPFC, or right posterior parietal cortex during the encoding period? *Hypothesis*: Percent signal change in these brain regions during the encoding period will be significantly lower in each group of patients with CKD as compared to healthy participants.

Question 2: During the time period representing the hemodynamic response to retrieval of object locations during the working memory task, will patients with chronic kidney disease, defined as those having GFR $\leq$ 90 ml/min/1.73m^2, dialysis dependence, or transplant dependence, differ significantly from healthy participants in terms of percent BOLD signal change in functionally activated clusters in the ventrolateral prefrontal cortex (VLPFC), dorsolateral prefrontal cortex (DLPFC), or right posterior parietal cortex? *Hypothesis*: Percent BOLD signal change over the retrieval period will be significantly lower in VLPFC, DLPFC, and right posterior parietal cortex in patients with CKD as compared to controls.

Question 2b: Will any of the three groups—patients with moderate CKD, patients with severe CKD, or healthy participants—differ significantly from each other in terms of percent signal change in the VLPFC, DLPFC, or right posterior parietal cortex during the retrieval period? *Hypothesis*: Percent signal change in these brain regions during the

retrieval period will be significantly lower in each group of patients with CKD as compared to healthy participants.

Question 3: Over the entire course of the working memory task, will patients with CKD differ significantly from healthy participants in terms of percent BOLD signal change in functionally activated clusters in the ventrolateral prefrontal cortex (VLPFC), dorsolateral prefrontal cortex (DLPFC), or right posterior parietal cortex? *Hypothesis*: Percent BOLD signal change over the course of the task will be significantly lower in these regions in patients with CKD as compared to controls.

Question 3b: Will any of the three groups—patients with moderate CKD, patients with severe CKD, or healthy participants—differ significantly from each other in terms of percent signal change over the course of the task in the VLPFC, DLPFC, or right posterior parietal cortex? *Hypothesis*: Percent signal change in these brain regions during the retrieval period will be significantly lower in each group of patients with CKD as compared to healthy participants.

Question 4: Will healthy controls differ significantly from the entire group of patients with CKD in terms of accuracy (number of correct answers) on the working memory task? *Hypothesis*: Accuracy will be higher in controls as compared to patients with CKD.

Question 5: Will healthy controls differ significantly from the entire group of patients with CKD in terms of reaction time on the working memory task? *Hypothesis*: Reaction time will be lower—that is, performance will be faster—in controls as compared to patients with CKD.

CHAPTER II

REVIEW OF THE LITERATURE

General cognitive function in children and adolescents with CKD

Children and adolescents with chronic kidney disease have generally been shown to score slightly lower than the average for the general population on measures of IQ or global cognitive functioning (Brouhard et al., 2000; Fennell et al., 1990b; Gipson et al., 2006). In addition to evaluations comparing children with CKD with healthy children of comparable age, our understanding of the cognitive effects of CKD is informed by research comparing children with more impaired renal function to those with less impaired renal function. This research includes studies tracing the same participants over time, often pre- and post-dialysis or pre- and post-transplantation. In a number of studies, standardized test scores serve as a basis for comparison to healthy age-matched controls.

One recent study by Gipson et al. assessed memory, executive function, and IQ in 20 children and adolescents with chronic kidney disease (CKD) compared with 18 healthy controls (Gipson et al., 2006). Participants ranged in age from 7.5 to 19 years at the time of testing. Of the 20 participants with CKD, 12 were on maintenance dialysis and 8 were being managed with conservative therapy. IQ was assessed using the Wechsler Abbreviated Scales of Intelligence (WASI). The CKD group demonstrated lower IQs (mean = 89, vs. 112 in controls; $p < 0.0001$).

Brouhard and colleagues compared 62 children with ESRD with their 62 siblings,

to control for ethnicity and socioeconomic status (Brouhard et al., 2000). The groups were similar in terms of whether they attended school full-time, but fewer of the patients were in regular education classes compared with the controls (61% vs. 84%). Both nonverbal intelligence and academic achievement were assessed. The sibling group had a significantly higher average IQ as measured by the Test of Nonverbal Intelligence-2 (TONI-2; $p < 0.001$). Average sibling IQ was in the 44^{th} percentile (50^{th} being average), while the average IQ for patients was in the 31^{st} percentile. The ESRD patients tended to score lower on subtests of the Wide Range Achievement Test (WRAT) assessing spelling, reading, and arithmetic. Predictors of lower achievement scores were identified as younger age at diagnosis, time on dialysis, and lower educational levels achieved by mothers or caregivers. This may have been because children receiving dialysis spent more time with their caregivers and less time in school.

Sibling controls were also used by Bawden et al. in a neuropsychological evaluation of 22 children with ESRD (Bawden et al., 2004). IQ was assessed using the Wechsler Intelligence Scales for Children, Third Edition (WISC-III), and academic achievement was assessed using the WRAT-3. Compared with their siblings, the ESRD patients had lower verbal ($p = 0.01$), performance ($p < 0.01$), and full scale IQs ($p < 0.01$) by 8.6, 11.7, and 10.9 points, respectively. Patient and sibling groups did not differ in terms of academic achievement.

Another comparison of children with CKD and healthy controls was reported by Fennell and colleagues (1990b). A total of 56 children with creatinine clearance equal to or less than 50 ml/min/1.73m2 were compared in a longitudinal study with a group of healthy controls matched by age, race, and gender (Fennell et al., 1990b). Participants

were 10 renal transplant recipients, 9 patients receiving continuous ambulatory peritoneal dialysis, 7 receiving hemodialysis, 5 patients with moderate CKD, and 22 with advanced renal failure. Average participant age was 13.6 years (range 6 to 18 years). Average age at onset of renal failure was 6.05 years, and average duration of renal failure was 8.32 years. IQ was not assessed, but verbal reasoning was assessed using the Similarities subtest of the WISC-R or WAIS-R, as appropriate for the patient's age, and visual perceptual reasoning was assessed using Raven's Color Progressive Matrices or Raven's Standard Progressive Matrices. These measures were chosen partly because performance was expected to be relatively independent of educational achievement, but an IQ test such as the WASI assesses the same constructs. Compared with matched controls, the group of renal failure patients showed lower performance in verbal abstract reasoning and visual perceptual reasoning ($p < 0.05$).

In an effort to identify clinical predictors of neurocognitive deficits, a recent study used IQ tests along with other neurocognitive assessments to assess children with mild CKD, moderate CKD, and ESRD (Slickers, Duquette, Hooper & Gipson, 2007). Participants included 29 children, ages 7 to 19 years, with estimated creatinine clearance of <90 ml/min/1.73m2. Approximately half had mild to moderate CKD, defined as creatinine clearance of 31 to 89 ml/min/1.73m2. IQ was assessed using the WASI. Lower IQ was found to correlate with more severe CKD ($r = 0.57$, $p = 0.001$). For the subset of 10 patients for whom age at disease onset was known, lower IQ also correlated with longer duration of CKD ($r = -0.66$, $p = 0.04$) and younger age at disease onset ($r = 0.69$, $p = 0.03$).

Fennell and colleagues (1990a) also reported an investigation of patients

representing a continuum of renal function, although their cutoff point for enrollment was a lower level of function (creatinine clearance equal to or less than 50 ml/min/1.73m2). The group was similar to the group of patients in the previously discussed study by the same research group, but the sample was somewhat smaller, including 45 instead of 56 patients, and included fewer patients whose CKD was being medically managed. Children who were mentally retarded or had seizure disorders were excluded. The same test battery was reported. Participants were tested upon study entry and then every 6 months for 2 years. In younger patients, ages 6 to 11, visual perceptual scores deteriorated over the testing period, but this deterioration did not indicate a significant association between performance and duration of renal failure. However, in younger patients, longer duration of renal failure was associated with reduced verbal scores on the WISC-R ($p < 0.05$).

Lawry, Brouhard, and Cunningham (1994) compared 11 dialysis patients (mean age 14.9 years) with 13 transplant patients (mean age 13.7 years, mean duration of transplant 2.4 years) in terms of IQ as measured by the WISC-R or WAIS-R and academic achievement as measured by the Woodcock-Johnson Tests of Achievement Revised (WJ-R), school grade point average, and mathematics and English grades. Days absent from school were similar in both groups. The two groups did not differ significantly on IQ or subtest scores. English grades were lower in the transplant group, but mathematics grades and total GPA were similar in both groups. On the WJ-R achievement tests, however, transplant patients achieved significantly higher scores in written language and mathematics ($p < 0.05$), suggesting a possible improvement in academic skills post-transplant. However, because this difference was not reflected in

school grades, the results should be interpreted with caution.

Rasbury and colleagues (Rasbury, Fennell & Morris, 1983) chose to control for IQ but assessed problem-solving using the WISC-R and Halstead-Reitan Category test and academic achievement using the Peabody Individual Achievement Test. This study compared 14 children with ESRD with 14 healthy controls. The patients' ESRD diagnoses were chronic glomerulonephritis (n = 6), obstructive uropathy (3), dysplasia (3), congenital cystinosis (3), and Alport syndrome (3). The groups were similar in terms of IQ (approximately 91 in the children with ESRD and 92 for the controls), age (approximately 11 years), and parental education level. Patients were tested shortly after being evaluated for renal transplant, which was typically shortly before they began dialysis, and then one month after transplantation. Controls were tested at a comparable interval. The most significant post-transplant improvement was on the Performance subtests of the WISC-R and the Halstead-Reitan Category test, indicating improvements in problem-solving. Children in the control group improved over time on this measure, indicating a practice effect, but children in the ESRD group improved more than twice as much (correlated t for patients = 5.85, df = 13, $p < 0.0001$; correlated t for controls: t = 2.60, df = 13, $p < 0.05$). Surprisingly, ESRD patients improved slightly on subtests assessing mathematics and reading achievement, but controls did not improve. This may indicate that the initial performance of ESRD patients on these measures was impaired by memory, attention, or problem-solving deficits; their performance is unlikely to have improved through learning at school because they were in school less than the controls were over the relevant time period.

Cognitive effects of ESRD beginning in early childhood

Several studies have reported long-term cognitive deficits in children who experienced end-stage renal disease in infancy or early childhood. Hulstijn-Dirkmaat assessed cognitive development over 3 years in 31 patients who were at enrollment under the age of 5 with serum creatinine clearance less than 20% of normal (Hulstijn-Dirkmaat, Damhuis, Jetten, Koster & Schröder, 1995). At the beginning of the study, 16 patients were on dialysis and 15 were receiving conservative treatment. Over the course of the study, 12 patients were transferred to dialysis. Instruments used were the Bayley Developmental Scales (mental scale only) and McCarthy Developmental Scales (verbal, perceptual-performance and quantitative scales), as appropriate for each patient's age. Patients' average cognitive development was significantly delayed when the study began (mean developmental index = 78.5, 100 being average for the general population). Patients undergoing conservative treatment had significantly higher scores than those on dialysis; the dialysis group average fell in the range of mild mental retardation. Transferring to dialysis resulted in a significant short-term improvement ($p = 0.001$), but follow-up over 2 years with 16 patients (7 being conservatively managed, 9 on dialysis) showed no significant change in cognitive development over time, indicating that development did not normalize.

In very young children, improvements in IQ have been reported following transplantation. Davis, Chang, and Nevins (1990) used the Stanford-Binet IQ test or the Bayley Mental Development Index to evaluate pre- and post-transplant functioning of 33 children who received renal transplants at or before 30 months of age. Testing was performed an average of 4 months before transplantation and an average of 14 months

after transplantation. IQ or mental development scores increased significantly, by an average of 12.6 points after transplantation ($p < 0.001$). Of 18 patients initially classified as having mild delay (mental development or IQ score of 50 to 69), two-thirds were classified post-transplant as being in the average range (scores greater than or equal to 70). In 24 of the 33 participants, cephalic growth as assessed by occipital-frontal head circumference also improved significantly ($p < 0.001$).

Warady, Belden, and Kohaut (1999) used age-appropriate cognitive measures to examine the efficacy of transplantation in normalizing neurodevelopmental outcomes for children who presented with ESRD in infancy. Participants were 28 children who had begun long-term peritoneal dialysis at or prior to 3 months of age, as determined by retrospective review. The most common diagnoses were posterior urethral valves, hypoplastic/dysplastic kidney, and autosomal recessive polycystic kidney disease. All the children were assessed at age 1 year, and 23 of them were subsequently reassessed, with ages at reassessment falling between 2 and 9 years (mean age 5.9 years). Two patients, including the most cognitively impaired patient, were still being maintained on peritoneal dialysis when they were reassessed. All the others had received renal transplants, at an average age of 2.1 years. One-year assessments were performed using either the Modified Developmental Assessment Test (MDAT) or Bayley Scales of Infant Development. Later testing consisted of the WISC-III or Stanford-Binet. At age 1 year, 22 patients (79%) patients performed in the average range and 1 child (5%) showed significant developmental delay. Of the 19 children reassessed at 5 years of age or older, 15 (79%) had IQs in the average range, and only the child who had initially tested as impaired had an IQ of <70. However, 3 children who had at 1 year tested in the average range were by

age 6 or 7 years testing in the low average or borderline range (IQ 70 to 90). Also, fewer children achieved an average nonverbal IQ than an average verbal IQ (56% vs. 72%; Warady et al., 1999).

Although transplants may improve developmental outcomes, transplant-dependent patients are still likely to fall below the mean for their ages in terms of cognitive performance. Qvist and colleagues assessed neurodevelopmental outcomes in 33 school-age children who had had renal transplants an average of 6 years previously at an age younger than 5 years. Most of these children had a particular type of congenital nephrotic syndrome. In this group, the mean IQ as assessed by the WISC-R was 87, in the low average range. Nine percent of patients were classified as mentally retarded (IQ < 70), and 42% had IQs in the average range (90 to 110; Qvist et al., 2002).

Another study evaluating long-term developmental outcomes in children who as infants had received peritoneal dialysis found that of 20 infants, 16 survived and 14 were achieving normal developmental milestones or attending mainstream school (Ledermann et al., 2000). Assessments used were the Griffiths Mental Development test and WISC. These patients were followed up in a 2003 study by the same research group (Madden, Ledermann, Guerrero-Blanco, Bruce & Trompeter, 2003). By this evaluation the mean patient age was 5.84 years, and 12 of the 16 patients had functioning transplants. Ten of the patients (67%) had IQs in the average range, and another three scored within 2 standard deviations of the mean, leaving two patients in the IQ range indicating mental retardation.

These results of these studies suggest that although transplantation helps to normalize neurodevelopmental outcomes, experiencing ESRD as an infant puts a child at

greater than average risk of long-term cognitive deficits. Approximately 20–25% of patients presenting with ESRD are estimated to show long-term developmental delay (Gipson et al., 2007).

A child's age at the onset of chronic kidney disease might consistently correlate with cognitive difficulties due to either repeated insults to the central nervous system over time or insults at particularly vulnerable periods in infancy or early childhood (Gerson et al., 2006). In the Lawry et al. study, age at onset correlated positively and significantly with IQ. Of the six participants whose IQ fell at least one standard deviation below average, five were diagnosed with renal failure before they were 5 years old. Mean duration of chronic renal failure in this study was 2.8 years in the transplant group and 4.0 years in the dialysis group (Lawry et al., 1994). However, neither Davis et al. (1990) nor Fennell et al. (1990b) found a correlation between age at onset and IQ. In the Davis study, the range of ages at which participants were diagnosed was relatively narrow: birth to 13 months, with a mean of 3 months. The Fennell study was skewed toward long-standing chronic kidney disease, with an average age at onset of 6.05 years and an average disease duration of 8.32 years. It is possible either that cognitive differences based on age of onset are more apparent when comparing patients with quite different ages at onset or that kidney disease is more damaging at certain critical periods.

Attention and other executive functions

Attention has been considered in several studies of children with CKD, but other executive functions have not been frequently studied. Attention problems in children are a concern because they can interfere not only with behavioral performance but also with acquiring new skills (Gerson et al., 2006). Evidence on attention problems in CKD has

been mixed.

Slickers et al. (2007) measured attention using the Gordon Diagnostic System, a computerized continuous performance test. The participants' mean attention score fell in the average range, although there was substantial variability. Attention was not linearly associated with creatinine clearance or age at onset, but patients with ESRD had poorer attention than patients with mild to moderate CKD ($p = 0.012$). Similarly, Qvist et al. (2002) used the NEPSY to assess simple attention in school-age children with ESRD and found that the group mean was in the average range, but 24% of participants had scores at least one standard deviation below the mean for the general population.

Rasbury et al. (1983) compared attention in healthy controls and ESRD patients pre- and post-transplant using an auditory continuous performance task. The groups did not differ in terms of attention as assessed by the continuous performance test, nor did either group improve over time. In contrast, Mendley et al. (1999) found within-subject improvements following renal transplant in sustained attention as measured by the Connors Continuous Performance Test (CPT; $p = 0.039$ for discrimination sensitivity and for reaction time). Fennell et al. (1990a), assessing children diagnosed in infancy with ESRD, found impairments in attention as assessed by a visual continuous performance test.

Gipson et al. (2006) assessed sustained attention and other executive functions using the Gordon Diagnostic System, Tower of London Test, Controlled Oral Word Association Test, Ruff Figural Fluency Test, and the Woodcock-Johnson-III Numbers Reversed Subtest. The executive function domains were based on the model proposed by Denckla (1996) and consisted of initiation (assessed by Tower First Move Initiation

Time, Controlled Oral Word Association FAS Total, Ruff Figural Fluency Total), sustaining (Gordon Diagnostic System Correct Total, Woodcock-Johnson Numbers Reversed), inhibition (Gordon Diagnostic System Commission Total, Tower Rule Violations), and set-shifting (Tower Total, Ruff Perseverations). Performance of CKD patients and controls was similar on subtests assessing set-shifting and inhibition and on learning tasks involving multiple repetitions over which feedback on a participant's performance was provided. Controlling for chronological age, the group with CKD showed impairments in initiation and sustained attention. Although the CKD patients had lower IQs than the healthy controls, differences in executive function remained even after controlling for age and IQ ($p < .04$, ES = .57).

Memory

Memory deficits have been more consistently reported in children with CKD (Gerson et al., 2006). The type of memory most often assessed has been immediate recall, but some studies have assessed memory using additional measures. In the longitudinal study by Fennell et al. (1990b), children and adolescents with CKD showed memory impairments compared with age-matched controls. The CKD group had lower scores on immediate recall as measured by the Digit Span subtest on the WISC-R or WAIS-R, memory prompted by minimal feedback as measured by the Buschke Restricted Reminding Memory Procedure, and memory challenged by interference as measured by Auditory Consonant Trigrams with the Peterson-Brown Distraction Paradigm ($p < 0.05$). On some measures of memory, renal patients' performance improved less over time than the performance of controls, while on some measures their performance actually declined over time, suggesting progressive deterioration of function.

Memory deficits have repeatedly been correlated with more severe kidney disease. In the study enrolling 45 children and adolescents with CKD, Fennell et al. found differences in memory between CKD patients with differing levels of renal function (Fennell et al., 1990a). Higher creatinine clearance (i.e., better renal function) was associated with better immediate recall, and lower blood urea nitrogen (another indication of better renal function) was associated with better memory prompted by minimal feedback ($p < 0.05$ for both measures). Mendley et al. (1999) found within-subject improvements after renal transplantation in working memory as measured by the Paced Auditory Serial Addition Test or Children's Paced Auditory Serial Addition Test ($p = 0.016$). In the study by Slickers et al. (2007), greater severity of CKD predicted poorer memory as measured by the Wide Range Assessment of Memory and Learning (WRAML; $p = 0.009$), and longer duration of CKD predicted lower memory scores ($p = 0.03$). Rasbury et al. (1983), using the Digit Span subtest of the WISC-R, found post-transplant improvement in ESRD patients in terms of immediate recall ($p < 0.05$).

In contrast, a later study by Rasbury, Fennell, Fennell and Morris (1986) comparing 18 ESRD patients with controls before and after transplantation found no difference between groups in terms of immediate recall and no difference between pre- and post-dialysis performance in the group with ESRD. The earlier study found this pattern on a free recall memory task in which 16 common nouns were presented repeatedly and the child was repeatedly asked to recall as many as possible in 60 seconds: the ESRD patients neither differed from controls nor improved after transplantation. In the study of sibling pairs by Bawden et al. (2004), memory was assessed using the Nonverbal Selective Reminding Test, which requires subjects to recall locations of

objects, and the Verbal Learning, Sentence Memory, and Finger Windows subtests of the WRAML. The Verbal Learning subtest gives the subject multiple trials over which to learn and recall words, the Sentence Memory subtest assesses immediate verbal recall, and the Finger Windows subtest assesses immediate visual recall. On none of these measures did the patients with ESRD differ from their siblings. In the group of transplant-dependent school-age children assessed by Qvist et al. (2002), 20% had memory scores on the NEPSY that fell at least one standard deviation below the mean, although the mean memory score for the group was in the average range.

Electrophysiology findings

In adults with CKD, electroencephalogram (EEG) studies and have shown lower baseline total power and abnormal distribution of power, including P300 latency, compared with healthy controls (Evers et al., 1998; Marsh et al., 1991; Sagalés, Gimeno, Planella, Raguer & Bartolome, 1993; Teschan et al., 1979). As serum creatinine levels rise, EEG waves slow (Teschan et al., 1979). Hemodialysis (Evers et al., 1998) and treatment of anemia with erythropoietin (Marsh et al., 1991; Sagalés et al., 1993) have been shown to help normalize EEG results. Defects in peripheral nerve conduction are also common in adults with ESRD, occurring in up to 75% of patients (Suppiej et al., 1991).

Chronic kidney disease in children has also been associated with abnormalities in cerebral and peripheral nerve conduction. Several EEG studies have been conducted, and a few studies have evaluated brain stem evoked responses or peripheral nerve conduction (Gipson et al., 2007).

In the 1994 study by Elzouki et al., abnormal EEG findings were observed in the

three children with microcephaly and three additional children, for a total of 43% of the 14 receiving EEGs. However, all of the 12 children tested for auditory brain stem evoked response had a normal brain stem response. In the Qvist et al. study (Qvist et al., 2002) enrolling primarily patients with congenital nephrotic syndrome, abnormal EEGs were found in 11 children (33%). All of these patients showed ischemic lesions on MRI, and five were receiving anticonvulsant medications. A larger study enrolled 22 children with chronic renal failure who were younger than 5 years of age and assessed them every 6 months for 3 years using auditory brain stem evoked potentials and somatosensory evoked potentials (Hurkx et al., 1995). Brain stem conduction was normal, although a delay in peak I suggested possible peripheral conduction disturbance. No differences were found between patients who were dialysis dependent and those who were not. In the group of 10 patients younger than 2.5 years, increased N13 to N20 interpeak latency was observed, suggesting delayed thalamocortical conduction and perhaps delayed myelination or synaptogenesis of the somatosensory pathway.

One study has assessed children with ESRD using median nerve somatosensory evoked potentials (Suppiej et al., 1991). A group of 10 children with ESRD ages 9 to 19 years were assessed. Patients were maintained on hemodialysis and had no clinical neurological signs or symptoms. Compared with age-matched controls, three children (30%) demonstrated abnormal peripheral nerve conduction.

Factors potentially contributing to cognitive impairment in chronic kidney disease

Factors that may contribute to cognitive deficits in children with CKD include uremia (CKD severity); modality of renal therapy; structural abnormalities; hypoxia; and complications of CKD (Gerson et al., 2006). Each of these factors will be reviewed

individually.

Disease severity, dialysis, and transplantation

There is evidence that the severity of kidney disease correlates with the severity of cognitive dysfunction (Gerson et al., 2006). In the study by Slickers et al. in which approximately half the 29 participants had mild to moderate CKD (creatinine clearance of 31 to 89 ml/min/1.73m^2), more severe renal impairment, younger age at disease onset, and longer duration of disease predicted more pronounced neurocognitive deficits (Slickers et al., 2007). Fennell et al. (1990b) also evaluated patients with a range of kidney function (all with creatinine clearance equal to or less than 50 ml/min/1.73m^2) and found an association between renal function and memory. Hulstijn-Dirkmaat et al. (1995) found that ESRD patients undergoing conservative treatment, suggesting lesser disease severity, had higher mental development scores than those on dialysis. In contrast, Rasbury and colleagues found no differences in neurocognitive performance before and after dialysis, although they had expected the reduced uremia following dialysis to improve performance (Rasbury et al., 1986).

Children with mild to moderate CKD, who have not yet reached the stage at which they are dialysis- or transplant-dependent, have been studied far less than those with ESRD (Slickers et al., 2007). Although current severity of kidney disease or treatment modality may be an important predictor of cognitive impairment, it is also possible that age at onset is more relevant, either as a predictor of time spent with CKD or as it relates to neurological insult during a critical period. More study is needed to determine to what extent children with mild to moderate CKD are at risk for cognitive impairment and what factors predict that risk (Slickers et al., 2007). One study in

progress aimed at addressing this issue is the Chronic Kidney Disease in Children Prospective Cohort Study (CKiD), a multicenter, prospective, longitudinal study funded by the National Institutes of Health and examining developmental outcomes of mild to moderate pediatric CKD. It is hoped that these findings will ultimately aid in detection and treatment of developmental delays (Gerson et al., 2006).

In general, research suggests improvements in some aspects of neurocognitive performance following renal transplant. Qvist et al. (2002) reported that for 33 pediatric ESRD patients who had received renal transplants before the age of 5, group mean scores on tests of attention, language, and visual-spatial abilities were in the average range. Other studies of school-age children have not found improvements in IQ post-transplant but have found improvements in attention, memory (Mendley & Zelko, 1999; Rasbury et al., 1983), problem-solving (Rasbury et al., 1983), or achievement test scores (Lawry et al., 1994). However, one study enrolling 26 dialysis-dependent and 36 transplant-dependent ESRD patients (mean age 13.7 years) found similar nonverbal intelligence and academic achievement in both groups, suggesting no cognitive benefit to transplant (Brouhard et al., 2000).

Individual responses to transplantation are likely to vary substantially. Many factors may play into a particular child's level functioning after transplant, including age at disease onset, chronicity and severity of disease, previous responses to treatment, rate of deterioration, and cognitive function preceding the illness (Gerson et al., 2006).

Structural abnormalities and hypoxia

Hypoxia or structural malformations of the central nervous system are associated with certain disorders that also result in renal disease. Hypoxia, ischemia, and asphyxia

are established causes of acute kidney injury in neonates, children, and adolescents, and patients who have recovered from acute kidney injury are at increased risk for later chronic kidney disease (Andreoli, 2009). These early insults to the brain may result in later cognitive difficulties (Gipson, Wetherington, Duquette & Hooper, 2004). The pattern of vascularization in the frontal lobe makes the watershed areas between the anterior and middle cerebral arteries particularly susceptible to injury when blood flow to the brain is reduced (Caine & Watson, 2000). A review of 58 studies of cerebral anoxia found that the watershed cerebral cortex and basal ganglia were most often damaged, and the neurocognitive deficits most often resulting were in memory (54%), personality and behavior (46.2%) and visuospatial performance (31.3%). In one study assessing cognitive development in 16 children who had presented with ESRD during infancy (Ledermann et al., 2000), developmental delay of two children was attributed to perinatal asphyxia or hypoxia associated with transposition of arteries. Prune belly syndrome, which impairs kidney function beginning in infancy and in some cases leads to ESRD, also leads to clinically significant pulmonary difficulties in approximately half of survivors (Geary, MacLusky, Churchill & McLorie, 1986), which could cause hypoxia.

Other congenital syndromes that cause CKD are also associated with structural irregularities of the central nervous system. For instance, Joubert syndrome, which causes cystic kidney disease, is associated with specific brain malformations and with developmental delay or mental retardation (Parisi, Doherty, Chance & Glass, 2007). Higher than average rates of structural abnormalities of the central nervous system have been reported in children with CKD. One 1994 study assessed neurological outcomes in 15 children who had been diagnosed with chronic renal failure in infancy and had

received nutritional therapy and no aluminum salts (Elzouki, Carroll, Butinar & Moosa, 1994). They were assessed at an average of approximately 4 years (range approximately 1 to 12 years). Three of the 13 children who received CT scans (23%) presented with both microcephaly and developmental delay.

A number of other studies have focused on groups with particular types of CKD and have therefore perhaps overestimated the rates of CNS abnormalities in the general population of children with CKD. However, their results are useful in assessing risk in these particular patient groups. For instance, in one study of 33 patients published by a group of Finnish researchers, 29 of the patients had mutations in the nephrin gene leading the severe Finnish type of congenital nephrotic syndrome (Qvist et al., 2002). These patients had received transplants by 5 years of age and these patients and were tested at school age, an average of 6 years after transplant. MRI determined that five patients (15%) showed brain atrophy, and 19 (58%) had ischemic lesions in watershed areas or brain infarcts.

Several investigations have correlated Lowe syndrome, a syndrome involving renal tubular dysfunction among other features, with defects in myelination. In one study, all of the three patients assessed displayed some degree of mental retardation along with white matter lesions as evidenced by diffuse high T2 signals periventricularly (Pueschel, Brem & Nittoli, 1992).

Cystinosis, an autosomal recessive disorder causing cystine to accumulate in lysosome, is associated with renal failure as well as other abnormalities. Cystinosis has also been associated with high rates of cerebral atrophy and other structural abnormalities of the brain, including demyelination and hydrocephalus. In one study of 11 children and

adolescents diagnosed with infantile nephrotic cystinosis, 10 (91%) displayed cerebral atrophy (Nichols, Press, Schneider & Trauner, 1990).

Medical complications that may affect cognition

Chronic kidney disease frequently leads to medical complications that may impair cognition. These include anemia, hypertension, cardiovascular disease, and malnutrition (Gerson et al., 2006).

Anemia

Another probable contributor to cognitive deficits in early studies of children with CKD was anemia (Marsh et al., 1991). Anemia management improved in the early 1990s, after treatment with erythropoietin entered standard practice (Gipson et al., 2004). Studies in adults found that after 3 months' treatment with erythropoietin, hematocrit approached normal levels, and results of both EEG (Sagalés et al., 1993) and neurocognitive tests improved, although not to normal levels (Marsh et al., 1991). However, anemia continues to be a concern in patients with chronic kidney disease (Gerson et al., 2006). A study published in 1999 (Pickett, Theberge, Brown, Schweitzer & Nissenson, 1999) reported that current practices did not typically achieve normal hematocrit. Normalizing hematocrit levels was associated with reduced EEG slowing and reduced P300 latency on an auditory oddball task assessing sustained attention and working memory.

Iron deficiency anemia can cause developmental delay in infants (Booth & Aukett, 1997) and long term cognitive deficits may be observed even following iron therapy (Lozoff & Georgieff, 2006). Halterman and colleagues, using the large sample of children and adolescents ages 6 to 16 years old from the NHANES III Survey, found an association between iron deficiency anemia and lower scores on standardized math tests

(Halterman, Kaczorowski, Aligne, Auinger & Szilagyi, 2001). In a sample of 24 adult patients with chronic renal failure, raising hematocrit to near normal significantly improved performance on the Symbol Digit Modalities Test and Trail Making Test Part B (Marsh et al., 1991). Anemia is also an independent risk factor for mortality in patients on dialysis (Foley et al., 1996). However, in a recent study evaluating factors affecting neurocognition in children with mild to severe CKD, anemia was not a significant predictor of neurocognitive performance (Slickers et al., 2007).

The 2008 Annual Report of the United States Renal Data System reported mean hemoglobin in pediatric ESRD patients to be approximately 10 g/dL. Since hemoglobin <11.8/dL marks iron deficiency in a 6-year-old female (Halterman et al., 2001), and 11 g/dL hemoglobin has been identified as the target level for adults with ESRD (Fadrowski, Furth & Fivush, 2004), many children with ESRD can be concluded to be anemic. In a sample of 99 dialysis-dependent children (mean age 12.6 years), Fadrowski and colleagues found 36.6% of hemodialysis patients and 39.5% of peritoneal dialysis patients to be anemic based on a standard of hemoglobin 11 g/dL (Fadrowski et al., 2004). Patients who had been on dialysis less than 6 months were more likely to be anemic than longer term dialysis-dependent patients, and teenagers in this population were more than 4 times as likely as younger children to be classified as anemic (Fadrowski et al., 2004). Before reaching the renal failure stage, 50% of children with CKD receive an erythropoiesis-stimulating agent (ESA) to treat anemia (United States Renal Data System, 2008b). However, the optimal hematocrit in children with ESRD whose anemia is treated with recombinant human erythropoietin has not been clearly established (Fadrowski et al., 2004; Yorgin, Belson, Al-Uzri & Alexander, 2001).

Anemia also occurs frequently pre- and post-transplant. A study of 162 pediatric transplant recipients found that nearly 67% were anemic, and after transplantation the rate rose to 84.3% (Yorgin et al., 2002). Anemia rates began to go down 3 months after transplant but remained high for at least 5 years after transplant, varying from 64.2% (at 6 months post-transplant) to 84.3% (at 5 years). Only 4 of the assessed patients (2.7%) never tested as anemic. Certain immunosuppressive drugs administered to guard against rejection post-transplant suppress generalized bone marrow production and can therefore result in anemia (Yorgin et al., 2002).

Hypertension and cardiovascular disease

Hypertension may be another factor contributing to cognitive deficits in children with CKD. Either episodes of hyper- or hypotension or side effects of antihypertensive therapy may result in brain injury (Gerson et al., 2006). A study of the general pediatric population, based on NHANES-III data, examined IQ on the WISC-R and academic achievement as assessed by the WRAT-R in normotensive children compared with hypertensive children (Lande, Kaczorowski, Auinger, Schwartz & Weitzman, 2003). A large sample (n = 4860) of children with blood pressure in the normal range was compared with a smaller sample (n = 217) of children whose systolic blood pressure was in the 90th percentile for their age and gender. Hypertension was associated with lower IQ scores and lower scores on the memory, attention, and arithmetic subtests of the WISC-R (Lande et al., 2003). In adolescents with ESRD, ages 16 to 18 years, higher systolic blood pressure has been associated with poorer immediate recall (Fennell et al., 1990a). However, other studies have failed to find a relationship between neurocognition and hypertension or blood pressure in patients with CKD (Slickers et al., 2007).

Hypertension is a frequent complication of CKD and in pediatric CKD is often undertreated or untreated (Flynn et al., 2008). A recent report from the Chronic Kidney Disease in Children cohort, which assessed blood pressure in 432 children (mean age 11 years, mean GFR 44 ml/min/1.73m2), defined hypertension as blood pressure equal to or greater than the 95th percentile for age, gender, and height, or by self-report in patients prescribed antihypertensive medications (Flynn et al., 2008). In this population, 54% of patients had either systolic or diastolic hypertension or were taking antihypertensive medications based on a history of hypertension. Another 20% were prehypertensive, defined as blood pressure in the 90th to 95th percentile. More than one-third of the CKD patients with elevated blood pressure were not being treated for it (Flynn et al., 2008). A smaller study enrolling 42 children ages 2 to 19 years, with CKD stages 2 to 5 of heterogeneous etiology, found somewhat lower rates despite using ambulatory blood pressure monitoring (Dionne, Turik & Hurley, 2008). In this sample, 8% of patients had daytime systolic hypertension, 5% daytime diastolic hypertension, 14% nighttime systolic hypertension, and 24% nighttime diastolic hypertension. In 4.7% of pediatric ESRD patients, hypertension or large vessel disease is the primary diagnosis (United States Renal Data System, 2008b) and is a particularly common diagnosis among African-American patients (United States Renal Data System, 2006).

Hypertension contributes to cardiovascular disease, which in both adult and pediatric CKD populations is a major cause of death (Saran & DuBose, 2008; United States Renal Data System, 2008a). Most adults with CKD will die from cardiovascular disease before requiring dialysis (Saran & DuBose, 2008). An examination of 1380 deaths recorded by the U.S. Renal Data Systems found that in children and adolescents

who started ESRD therapy in childhood and died before age 30 years, cardiovascular events accounted for 23% of the deaths (Parekh, Carroll, Wolfe & Port, 2002). This represents a 1,000-fold higher risk of cardiac death for children with ESRD compared with children in the general population. In this analysis, African-American patients were 1.6 times as likely as white patients to die of cardiac cause, while dialysis patients were 78% less likely than transplant patients to die of a cardiac cause. Congenital heart disease (CHD) is associated with developmental delay and cognitive deficits (Griffin, Elkin & Smith, 2003; Mahle & Wernovsky, 2001), and cardiovascular disease can affect neurocognitive function (Gerson et al., 2006).

Malnutrition

Inadequate nutrition has also been reported in children with CKD, including those in relatively early disease stages. Norman et al. (2000) compared nutritional intake and growth in 35 children with normal GFR (defined as GFR >75 ml/min/1.73m2) with 60 children representing a range of CKD severity—35 with 23 with mild CKD (defined as GFR 51 to 75), 19 with moderate CKD (GFR 25 to 50), and 18 with severe CKD (GFR <25). They found that as renal function worsened, weight, height, body mass index, and mean total energy intake diverged from normal values. Mean total energy intake decreased from an estimated average requirement of 103% in the normal GFR group to 85% estimated average requirement in children with severe CKD ($p < 0.01$). Mean serum parathyroid hormone levels were also higher in moderate and severe CKD. Malnutrition early in life affects cognitive function into early adulthood (Lucas, 2005), and malnutrition may be another contributor to cognitive dysfunction in pediatric CKD (Gerson et al., 2006).

Any number of these risk factors—anemia, hypertension, cardiovascular disease, or malnutrition—may act singly or synergistically with other risk factors to cause neurological insults at one or more points along the developmental course of a child with CKD (Gerson et al., 2006).

Models of working memory

The idea that certain human functions require participation of certain regions of the brain came initially from observations of patients with focal brain injuries (Shallice, 1988). One early report by Paul Broca, in 1861, concerned the autopsy of a patient who had only been able to articulate the word "tan." The man was found to have a lesion in the left frontal lobe, leading Broca to conclude that this part of the brain (now known as Broca's area) was essential for speech production. The publication of this theory stimulated a multitude of case studies and theoretical models explaining observed clinical disorders as interruptions to specific cognitive processes. However, in many cases the evidence for anatomical specificity of function was weak (for instance because patients' lesions were large or diffuse), or published descriptions of either the relevant cognitive processes or the postulated disorder's clinical signs were vague or misleading.

By the early 1900s, academic discourse was dominated by the voices of critics who claimed these ideas had no practical import (Shallice, 1988). Case studies fell out of favor, and researchers instead began to record data from groups of patients with similar brain lesions or functional deficits. Clinical observations were deemed insufficient and tests producing quantitative scores were developed (such as the Halstead-Reitan Neuropsychological Test Battery, which was designed to determine whether a patient had sustained a brain injury and if so, in which part of the brain). Some results—e.g., findings

that left- vs. right-brain lesions corresponded with different deficits—supported earlier theories. However, since finding large numbers of patients with comparable brain injuries is not easy, and since many patients are needed to achieve significant group differences, progress in the field of neuropsychology slowed considerably.

In the 1960s and 70s, change came from another quarter. Cognitive psychology was moving away from Gestalt theory, a holistic theory of brain function, and toward information processing models, which in some ways resembled the neuropsychology diagrams of the late 1800s (Shallice, 1988). Single-case studies regained respect as relevant information, if not as reliable and generalizable as group studies. Cognitive psychologists expressed interest in evidence from brain lesions as it informed theories of normal brain function. This change in attitudes in cognitive psychology rejuvenated neuropsychology.

It is now widely accepted that the brain is to some extent modular—that rather than working as a single unit, the brain employs selected physical units to perform certain functions (Shallice, 1988). This theory was developed from several lines of evidence. As outlined by Simon (1969) and Marr (1976) in a computational argument, separating processes is practical so that a breakdown at one point does not shut down the entire machine, or the entire brain—and lesion studies amply demonstrate that deficits due to brain injuries can be limited, even subtle. Linguistic theory, as propounded by Chomsky (1980), among others, held that since grammar and syntax are defined by a limited set of rules which do not apply to other human functions and which can function in the absence of other competencies, it would be logical for grammatical competence to reside in a separate part of the brain than, say, vision.

In addition to these theoretical arguments, Sternberg (1969), elaborating on a method first proposed by Donders in 1868 (during the early flush of information processing diagrams), developed an additive factor method for postulating separability of mental processes based on reaction time. He found that reaction times in response to tasks requiring two different identifiable processes (e.g., the ability to recognize a number, as signified by stating its name, vs. the ability to select a less automatic response) could in some cases be summed to generate the reaction time for a task requiring both of those processes. Investigating the previous example, Sanders, Wijnen & van Arkel (1982) found that loss of sleep affected response time for stimulus identification but not for response selection. Since the additive reaction times suggest the processes are completed separately, and the sleep loss findings suggest that the processes are separately modifiable, these results were taken as evidence of modularity. For instance, McLeod, McLaughlin & Nimmo-Smith (1985) found that the physical attempt to catch a ball triggered by an expanding image on the retina (such as an approaching ball would produce) is accurate within an average of <5 ms. The authors concluded that a response so precise must be controlled by a relatively small and independent portion of the brain.

Further evidence for modularity came from primate studies showing that different parts of the primate cortex displayed different anatomy, physiology, and specialized function; for example, a portion of the visual cortex responds specifically to color (Zeki, 1980). More pertinently to this study, bilateral lesions of the prefrontal cortex have repeatedly been found, in several primate species and other animals, to severely impair performance on a task requiring the subject to briefly remember the location of food

(Fuster, 1997).

Based on these various lines of reasoning and experimentation—computational theory, linguistic theory, behavioral evidence from humans, and anatomical and behavioral evidence from animals—the idea developed that parts of the brain can be modular, that is, that they can function with some level of independence, requiring neither effort nor information from other parts of the brain (Shallice, 1988). Which functions of the brain comprise separate modules, and to what extent those modules are independent or interconnected, are some of the major questions of cognitive neuropsychology. Anatomical, physiological, and behavioral evidence from animals and humans has been brought to bear on these questions. The current study was designed based on behavioral evidence, that is, evidence from cognitive and neuropsychological test scores, that chronic kidney disease in children is associated with impairments in short-term memory, and the particular module of interest for purposes of this study is the system responsible for spatial working memory.

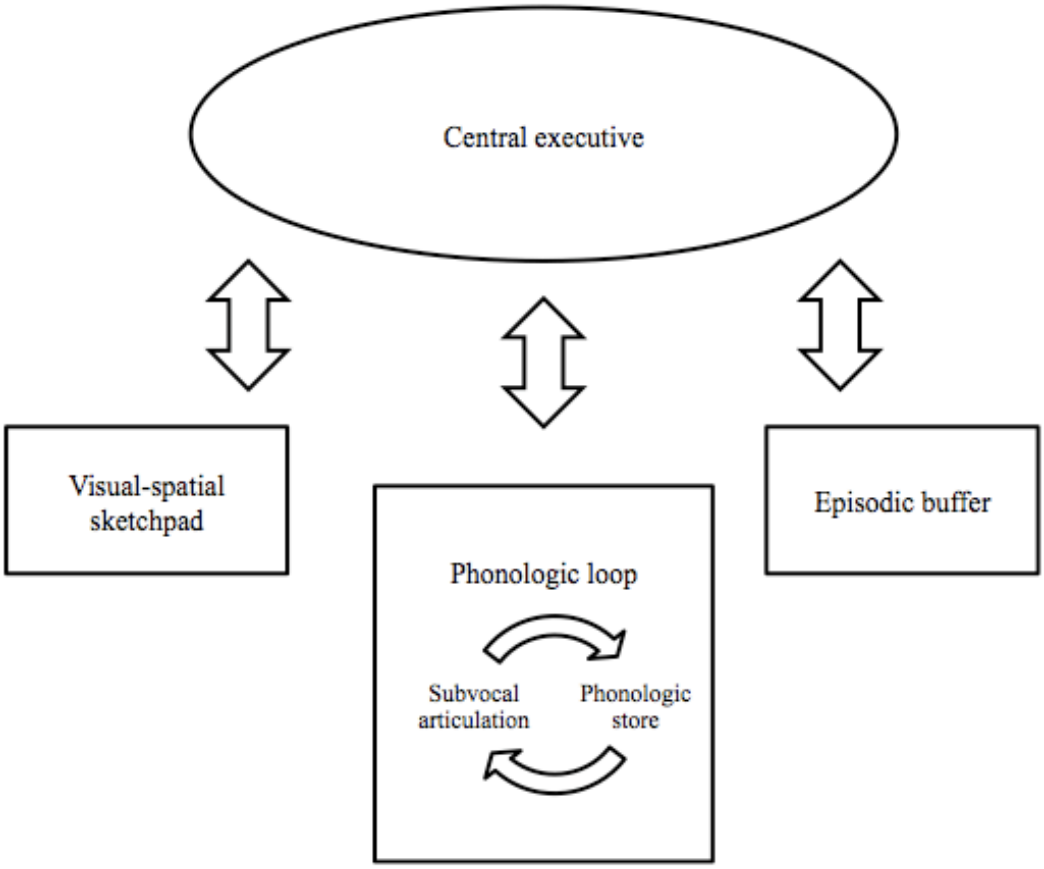
Long-term memory was distinguished from short-term memory as early as 1900, when Müller & Pilzecker introduced the term "consolidation" to describe the process by which an experience comes to be stored as a relatively permanent memory (cited in Moscovitch et al., 2005). In 1957, evidence concerning the neural substrates involved in memory was provided by the case of H.M., a man who had undergone bilateral excision of the anterior and medial temporal lobes, including the hippocampus and parahippocampal region, in order to treat severe epilepsy (Scoville & Milner, 1957). Although the surgery controlled his epilepsy and did not clearly affect his intelligence, sensory or motor functions, or short-term memory, it left him with a dramatic inability to

commit information to long-term memory. Similar syndromes were later observed in other patients with damage to the medial temporal lope or midline thalamic nuclei (Moscovitch et al., 2005). The medial temporal lobes and diencephalic structures were concluded to be necessary for consolidation.

The term "working memory" was used at least by 1960 to refer to a short-term storage and retrieval process distinct from the process of long-term memory (Miller, Galanter & Pribram, 1960). As with the idea of modularity, the concept of working memory was based partly on studies of patients with brain damage. Although H.M. and patients with similar lesions showed severely impaired long-term memory but normal short-term memory, in others the reverse was observed (Shallice, 1988). An early model suggested that a short-term memory process funneled information into and retrieved information from long-term memory (Waugh & Norman, 1965; Atkinson & Shiffrin, 1968). However, this did not account for the long-term learning abilities of patients with extremely impaired short-term memory (Baddeley, 1992).

An influential model was then proposed by Baddeley and Hitch (1974), who defined working memory as a mental system separate from long-term memory in which a limited amount of information can be held and manipulated for a limited time. The short-term storage system holds information in the mind only for seconds and requires active maintenance, by such processes as phonological rehearsal. Baddeley and Hitch proposed a tripartite model for working memory comprising two rehearsal systems, one phonological and the other visual, that hold onto information, and a system called the "central executive" that maintains attention and processes or manipulates information (Baddeley, 1986; Baddeley, 1981). Baddeley later added a third, "episodic" rehearsal

system that is posited to maintain complex information in a story-like form (Baddeley & Lieberman, 1980). This system is illustrated below.



Processes taking place in the "central executive" have since been elaborated upon. The relevant executive processes have been defined as attention (focusing on relevant information), inhibition (ignoring irrelevant information), task management (scheduling subtasks), planning (defining subtasks), monitoring (checking working memory contents to determine the appropriate next step), and coding (adding values of time and place to remembered information; Smith & Jonides, 1999).

The Baddeley and Hitch model has been challenged on the grounds that the two or three proposed rehearsal systems, or "buffers," would not encompass all possible types

of information; for instance, information gathered from the senses of smell and taste remains unaccounted for. Also, having different rehearsal systems dedicated to different sensory modalities seems inefficient (D'Esposito, 2007). Other theories have considered the rehearsal systems to be subsumed under one short-term storage system, so that working memory comprises executive processes and short-term storage (Smith & Jonides, 1999). Regardless of how the storage systems are delineated, these models suggest that information is held in working memory as it would be in a computer's RAM, which holds a subset of information from the computer's hard drive (D'Esposito, 2007). The models suggesting that some set of domain-specific buffers provide temporary storage have been termed the memory system models (Feredoes & Postle, 2007).

In contrast, Cowan (Cowan, 1988) proposed a model in which information is focused on, not held by, working memory. In this model, the information that is the focus of attention is a subset of information that is activated, and that activated information is a subset of the information stored in long-term memory. Similarly, Andersen (Andersen, 1983) described a model in which certain representations are activated at higher levels than other representations. These ideas of working memory, which have been termed the emergent processes models, do not suggest that the buffer containing the representations is limited to any particular size (Feredoes & Postle, 2007). Conceptualizing attention rather than storage as the crucial process helps to explain the broad predictive utility of working memory performance, in that a single process seems more likely than a series of processes to contribute to performance in numerous different tasks (Kane et al., 2004).

Current ideas about working memory have developed based on the integration of psychological theory with neuroscientific research (Postle, 2006). Although theoretical

models of working memory did not predict which areas of the brain might be active during or critical to working memory, neuroscience provided avenues for investigating the neural substrates of executive and short-term storage processes. This approach was pioneered by Goldman-Rakic (Goldman-Rakic, 1987), who first linked neuronal activity sustained over a delay period in the brains of non-human primates to the idea of rehearsal systems proposed by Baddeley and Hitch.

The buffer model, with its multiple rehearsal systems, suggested that specialized neural systems might work to maintain active mental representations of information gathered by different sensory modalities (Postle, 2006). However, evidence from functional neuroimaging studies in humans and electrophysiologic and lesion studies in both primates and humans, taken together, suggest that a network of brain regions is critical for actively maintaining mental representations of information, and that these brain regions are active in other processes as well. In recent years it has therefore been proposed that working memory is most accurately conceived of neither as a dedicated system, as implied by the buffer models, nor as a unitary system, as implied by attentional models. Instead, working memory may most accurately be viewed as an emergent property of functional interactions between several regions of the brain (D'Esposito, 2007; Postle, 2006). These include attentional systems, which act in recruitment and coordination of additional neural regions, and regions evolved for sensory and action-related as well as representational functions (Postle, 2006).

Neural systems involved in working memory

The prefrontal cortex (PFC) is the brain region most clearly and consistently associated with working memory. It is thought to be necessary for the executive

processes, such as attention, that are needed to perform well on working memory tasks (Miller & Cohen, 2001). Parietal regions have also been established to be active during both verbal and spatial working memory tasks and are thought to maintain representations of the items to be remembered (Crone, Wendelken, Donohue, van Leijenhorst & Bunge, 2006; Marshuetz, Smith, Jonides, DeGutis & Chenevert, 2000). Temporal and occipital regions are believed to function in image processing and perception, respectively (Awh, Jonides & Reuter-Lorenz, 1998; Awh, Smith & Jonides, 1995; Belger et al., 1998).

Efforts have been made to more narrowly define the prefrontal regions active during different types of working memory tasks. The idea that more dorsal regions process spatial information and more ventral regions process nonspatial information is termed the domain-specific model (Goldman-Rakic, 1995). The process-specific model, in contrast, holds that the dorsolateral prefrontal cortex is preferentially activated not by spatial tasks but by more complex tasks, such as those requiring manipulation or monitoring in addition to recall (Petrides, Alivisatos, Evans & Meyer, 1993). Evidence for each of these models will be reviewed. Another theory of brain organization holds that spatial tasks predominately activate the right hemisphere and verbal tasks the left (Belger et al., 1998; Smith & Jonides, 1999). Studies considering hemispheric specialization by domain will also be reviewed.

The neural systems involved in working memory have been examined using primate unit recording studies, primate lesion studies, human lesion studies, and neuroimaging in humans. Each approach has its benefits. Informative experiments have been carried out on primates that would be considered unethical in humans. Lesion

studies in humans are hampered by the lesions' variability in size and location, but they have the potential to reveal areas critical to a task, whereas neuroimaging studies may reveal areas that are involved but not critical. In healthy humans, neuroimaging can reveal multiple brain regions active during a task, supplying information about functional connectivity; can define those regions with some specificity, although not on the small scale possible in unit recording studies of primates; and can examine the regions active during different task demands, for instance during encoding vs. retrieval.

Primate studies

In the early 1970s, neuronal activity in primates was studied by surgically implanting electrodes in the animals' brains which could record the action potentials associated with neuronal impulses (Fuster & Alexander, 1971). This method showed that individual neurons in the primate prefrontal cortex displayed increased activity during a delay period in which the primates remembered the location of a desirable food item (Funahashi, Bruce & Goldman-Rakic, 1989; Fuster, 1973; Fuster & Alexander, 1971; Kubota & Niki, 1971). For instance, Fuster and Alexander (1971) studied five rhesus monkeys trained to near-perfect performance on a delayed response task that required remembering the location of a desirable food item for at least 15 seconds. They found that neurons in the prefrontal cortex and nucleus medialis dorsalis of the thalamus displayed increased activity while the task stimuli were presented, and some of these cells showed increased activity over the course of the delay. Sustained neuronal firing in the prefrontal cortex during the maintenance period of a delayed-response task was replicated in numerous primate studies conducted over the next 30 years (D'Esposito, 2007). This sustained neuronal activity was theorized to signify the rehearsal system posited by the

Baddeley model of working memory (Goldman-Rakic, 1987).

Additionally, lesioning the principal sulcus of the dorsolateral prefrontal cortex was found to result in impaired performance on delayed response tasks, and the longer the delay, the more impairment was found (Bauer & Fuster, 1976; Funahashi, Bruce & Goldman-Rakic, 1993). Bauer and Fuster (1976) tested monkeys' performance on delayed response tasks under conditions of normal temperature, frontal cooling, and parietal cooling. At normal temperature, as delay time increased, accuracy declined and reaction time increased. These differences were increased by frontal but not parietal cooling, suggesting that the frontal cortex was important to maintenance of the information. Funahashi, Bruce, and Goldman-Rakic (1993) had four rhesus monkeys taught a task requiring memory of spatial locations of peripheral visual cues. Following lesions to areas in or around the principal sulcus, longer delay periods were associated with reduced accuracy on the task. Eye movements were misdirected after lesioning, and lesions on one side of the brain affected tasks in the contralateral hemifield. These and similar results supported the idea that prefrontal cortex was critical to active maintenance of task-relevant information.

Studies in non-human primates predominantly support a domain-specific model of working memory (Levy & Goldman-Rakic, 2000; Goldman-Rakic, 1987). "Spatial memory" cells have been identified in a more dorsal and "object memory" cells in a more ventral region of the prefrontal cortex (Wilson, Scalaidhe & Goldman-Rakic, 1993). More specifically, the dorsolateral prefrontal cortex surrounding the principal sulcus, Walker's area 46, is thought to be specialized for visual-spatial working memory in primates, whereas Walker's areas 12 and 45, which are located below area 46 on the

inferior portion of a convex area, are specialized for working memory involving nonspatial stimuli such as objects and faces.

In monkeys, individual neurons in the dorsolateral prefrontal cortex appear to take in spatial information from different locations, so that a single neuron increases in activity when a target disappears from one location (Goldman-Rakic, Bates & Chafee, 1992). If the activity of that neuron is interrupted, the monkey more often makes an error. Single-unit recording studies have found that many neurons in Walker's area 46 in the primate DLPFC are active in spatial delayed-response tasks, whereas few neurons in this area are active in tasks involving nonspatial visual memory (Funahashi et al., 1993; Carlson, Rämä, Tanila, Linnankoski & Mansikka, 1997; Wilson, Scalaidhe & Goldman-Rakic, 1993; Rao, Rainer & Miller, 1997).

Lesions of the principal sulcus or the middle third of the principal sulcus result in performance deficits on spatial delayed response tasks, including not only visual-spatial tasks but tasks involving spatial auditory and somatic stimuli, and spatial alternation tasks (Butters, Pandya, Sanders & Dye, 1971; Butters et al., 1971; Butters, Pandya, Stein & Rosen, 1972; Goldman-Rakic, 1987; Fuster, 1997). The deficits produced by lesioning this limited area are as large as those produced by lesioning larger areas. However, monkeys with principal sulcus DLPFC lesions perform as well as intact monkeys in tasks requiring short-term memory of patterns, shapes, or colors (Passingham, 1975; Mishkin, 1978; Bachevalier & Mishkin, 1986). Performance on these nonspatial visual tasks is impaired by lesions to Walker's areas 12 and 45 (Iversen, 1970; Passingham, 1975; Mishkin, 1978; Kowalska, Bachevalier & Mishkin, 1991).

At least one study has presented evidence for a process-specific rather than

domain-specific PFC organization in primates. Petrides found that increasing the delay period impaired performance in monkeys with lesions to the anterior inferotemporal cortex, but not in monkey with mid-dorsolateral prefrontal lesions (Petrides, 2000). Increasing the number of stimuli to be monitored, in contrast, impaired performance following mid-dorsolateral PFC lesions but not anterior inferotemporal lesions. More severe lesions in the mid-dorsolateral PFC also resulted in more severe impairments on tasks requiring monitoring.

Sensory processing of visual stimuli in the primate posterior cortex has two pathways, a dorsal pathway through posterior parietal cortex that processes location and spatial information and a ventral pathway through the inferior temporal cortex that processes object features, such as shape and color (Kessels, Postma, Wijnalda & de Haan, 2000). The pattern of interconnections between sensory processing areas and prefrontal cortex supports a domain-specific model. The inferior parietal association cortex, which is active in visual-spatial processing, projects to the primate DLPFC (Mesulam, Van Hoesen, Pandya & Geschwind, 1977; Andersen, Essick & Siegel, 1985; Barbas & Mesulam, 1985; Cavada & Goldman-Rakic, 1989; Friedman & Goldman-Rakic, 1994; Chafee & Goldman-Rakic, 1998), whereas the inferior Walker's areas 12 and 45 receive projections from the inferior temporal association cortex, which is thought to be involved in nonspatial visual processing (Kuypers, Szwarcbart, Mishkin & Rosvold, 1965; Chavis & Pandya, 1976; Jacobson & Trojanowski, 1977; Kawamura & Naito, 1984; Shiwa, 1987; Barbas, 1988; Seltzer & Pandya, 1989; Ungerleider, Gaffan & Pelak, 1989; Distler, Boussaoud, Desimone & Ungerleider, 1993; Webster, Bachevalier & Ungerleider, 1994; Baylis, Rolls & Leonard, 1985; Fuster, 1990). In conjunction with the data from single-

unit recording studies and lesion studies, this pattern of connectivity suggests parallel processing of spatial and nonspatial visual stimuli in different parts of the prefrontal cortex (Goldman-Rakic, 1998; Levy & Goldman-Rakic, 2000).

Human studies

Numerous studies have associated prefrontal lesions with impaired memory according to various measures. Lesions of the lateral prefrontal cortex have been associated with difficulty remembering the context in which information was acquired (Janowsky, Shimamura, Kritchevsky & Squire, 1989) and the order in which events occurred or information was presented (Shimamura, Janowsky & Squire, 1990), although in both these studies the patients showed normal recall for the items independent of context. Prefrontal lesions have been associated with impairments in event-based prospective memory (i.e., remembering to do something when an event reminds you of it) though not time-based prospective memory (remembering to do something at a particular time; Cheng, Wang, Xi, Niu & Fu, 2008). Patients with focal lesions in the dorsolateral prefrontal cortex have been found to be more impaired by distractors during a delayed response task than controls or patients with other brain lesions (Chao & Knight, 1995; Chao & Knight, 1998). Patients with prefrontal lesions have been found to produce more false positive responses than patients with hippocampal lesions (Swick & Knight, 1999) or healthy older participants (Swick, Senkfor & Van Petten, 2006), yet have normal recognition rates (i.e., true positive rates). In patients with lesions in the ventromedial prefrontal cortex, higher false positive rates compared with controls have been observed when item format changed but not when item order changed (Ciaramelli & Spaniol, 2009). In people with frontal lobe lesions, factors that would normally improve memory,

such as trying to remember something or learning something related, have been shown to offer no benefit (Mangels, 1997).

Although these results certainly indicate memory impairments, the impairments do not apply strictly to recognition or recall. Indeed, a meta-analysis of 11 studies of memory in patients with prefrontal lesions, encompassing 166 individual patients, found no significant deficits in verbal or spatial span (D'Esposito, Postle, Ballard & Lease, 1999). Rather, frontal patients generally seem to show deficits in executive processes (Müller & Knight, 2006) such as coding (remembering contextual information), inhibition, and attention. In terms of the Baddeley and Hitch model, the observed deficits apply to the central executive rather than to any short-term storage buffer. Notably, patients with prefrontal lesions show deficits not only in memory tasks but in go/no-go (Drewe, 1975) and Stroop tasks (Perret, 1974), which require inhibition; in the Wisconsin card sorting test (Janowsky, Shimamura & Squire, 1989), which requires inhibition and set-shifting; in the category switching demands of word fluency tasks (Troyer, Moscovitch, Winocur, Alexander & Stuss, 1998); and in a spatial working memory task requiring strategy (Owen, Milner, Petrides & Evans, 1996). That is, prefrontal lesions disrupt executive processes. It has therefore been posited that the prefrontal cortex is active in working memory insofar as it is critical to attention, inhibition, and planning (Miller & Cohen, 2001).

A multitude of neuroimaging studies, utilizing both PET scans and fMRI, have made progress toward defining the neural systems involved in working memory. In adults, maintenance of information in working memory has been associated with increased blood flow in the ventrolateral prefrontal cortex (Brodmann’s areas 44, 45, 47),

dorsolateral prefrontal cortex (BA 9, 46; Courtney, Ungerleider, Keil & Haxby, 1997; D'Esposito, Postle, Ballard & Lease, 1999; Owen, Milner, Petrides & Evans, 1996), and inferior parietal regions (Belger et al., 1998).

Some studies have found that spatial working memory tasks activated more dorsal PFC regions than verbal or object working memory tasks, providing evidence for a domain-specific rather than process-specific model of PFC organization (Courtney, Ungerleider, Keil & Haxby, 1996; Köhler, Moscovitch, Winocur, Houle & McIntosh, 1998; Courtney, Petit, Maisog, Ungerleider & Haxby, 1998). Using positron emission technology (PET) scans, Courtney, Ungerleider, Keil, and Haxby (1996) found that working memory for faces increased regional cerebral blood flow (rCBF) in more ventral areas (inferior frontal cortex, anterior cingulate, fusiform and parahippocampal areas, right thalamus, and midline cerebellum) while spatial working memory increased rCBF in more dorsal areas (a caudal portion of the superior frontal sulcus and superior and inferior parietal cortex; Courtney et al., 1996). Kohler, Moscovitch, Winocur, Houle, and McIntosh (1998) also used PET to differentiate areas involved in spatial vs. object memory (Köhler et al., 1998). They found that spatial memory was associated with increased rCBF in the superior temporal sulcus, right middle occipital gyrus, and supramarginal gyrus, whereas rCBF increased during object memory tasks in lingual and fusiform gyri. However, they also found areas in bilateral superior temporal cortex and bilateral middle and inferior frontal gyri that were activated during encoding and retrieval, respectively, in both object and spatial memory tasks. They concluded that some brain areas involved in memory were domain-specific while others were domain-general and process-specific.

A study published in 1998 by Courtney et al. presented evidence in favor of the domain-specific model in humans (Courtney et al., 1998). This fMRI study of 11 healthy volunteers defined an area in the superior frontal sulcus that showed sustained activity during delays in a spatial working memory task. However, many neuroimaging studies in humans have not found a prefrontal dorsal-ventral difference for spatial vs. object working memory tasks (Kessels et al., 2000). Evidence for a dorsal-ventral, spatial-object distinction in posterior cortex, that is, in sensory processing areas, is more robust. One meta-analysis of 60 neuroimaging studies of working memory found a dorsal-ventral difference for spatial vs. nonspatial tasks in the posterior cortex but not in the frontal cortex (Wager & Smith, 2003). For instance, an fMRI study by Belger et al. (1998) found dorsal-ventral differences for spatial vs. object tasks, with spatial tasks resulting in activation of inferior parietal cortex and object memory tasks resulting in activation of inferior occipitotemporal cortex. Activation of these areas was similar for working memory tasks and tasks requiring only perceptual processing.

Neuroimaging in humans has predominantly supported the process specific model, which holds that dorsal vs. ventral prefrontal cortex activity depends not on whether a task taps spatial vs. object memory but on the complexity of the task. Dorsolateral prefrontal cortex activity has been found to increase more than ventrolateral PFC activity in tasks requiring that information be manipulated as well as maintained (D'Esposito et al., 1999; Owen et al., 1996). For instance, Owen, Evans, and Petrides (1996) examined blood flow during five spatial memory tasks using PET and found that tasks requiring memory and performance of spatial movements resulted in significant changes in bilateral ventrolateral frontal cortex (area 47), while task requiring monitoring

and manipulation of spatial information in working memory resulted in additional activation in dorsolateral frontal cortex (areas 46 and 9; Owen et al., 1996). Using fMRI, D'Esposito, Postle, Ballard, and Lease (1999) found that both dorsolateral and ventrolateral PFC were activated during verbal memory tasks, but that dorsolateral activation was increased in a task that required letter sequencing (D'Esposito et al., 1999).

The process-specific theory was supported by a review of 20 neuroimaging studies investigating working memory performance in healthy adults (D'Esposito et al., 1998) and including Talairach coordinates, a system of three-dimensional coordinates used to define locations in the brain with reference to the location of the anterior commissure (Talairach & Tournoux, 1988). D'Esposito and colleagues found that spatial and nonspatial tasks both activated areas throughout the prefrontal cortex, although spatial tasks tended to activate the right hemisphere and nonspatial tasks the left. A dorsal-ventral organization was observed based on task requirements, with tasks requiring information to be manipulated or intervening information to be screened activating more dorsal areas than tasks requiring simple maintenance (D'Esposito et al., 1998). Another review including lesion studies as well as PET and MRI studies concurred with the process-specific model (Kessels, Postma, Wijnalda & de Haan, 2000). Smith and Jonides, reviewing PET and MRI studies of verbal, spatial, and object working memory that reported Talairach coordinates, suggested that both hypotheses could be valid. The prefrontal cortex may be organized both by modality, with more dorsal regions representing spatial memory and more ventral regions representing object memory, and by process, with ventrolateral areas mediating short-term storage and dorsolateral regions mediating manipulation (Smith & Jonides, 1999).

While organization and functions of the prefrontal cortex have been disputed, a broader organization of the brain is somewhat better understood. It is generally agreed that different areas of the brain are recruited by spatial vs. verbal memory tasks, with spatial working memory tasks showing greater activation in the right hemisphere and verbal working memory tasks showing left lateralization (Courtney et al., 1997; Goldman-Rakic, 1987; Smith & Jonides, 1999; Smith & Jonides, 1997). Spatial working memory tasks have been found to activate the right prefrontal cortex, right middle frontal gyrus, right posterior parietal cortex, right occipital cortex, and right premotor cortex (Belger et al., 1998; Smith & Jonides, 1999; Thomas et al., 1999) (Smith, Jonides & Koeppe, 1996). Verbal working memory tasks instead result in left lateralization, with activation of Broca's area, left inferior frontal cortex, left posterior parietal cortex, and left premotor and supplementary motor areas (Kelley et al., 1998; Belger et al., 1998; Smith et al., 1996; Wager & Smith, 2003). Memory for objects has been reported in association with left lateralization (D'Esposito et al., 1998), right lateralization (Wager & Smith, 2003; Courtney et al., 1998), and bilateral activation, perhaps depending on the nature of the objects and whether they can be encoded verbally (McCarthy et al., 1996).

For instance, a PET study enrolling a total of 29 participants for three experiments found that verbal memory tasks primarily activated left-hemisphere regions (BA 40 in the parietal cortex; BA 44, Broca's area, in the frontal cortex; and BA 6, the premotor area and supplementary area), whereas spatial memory tasks activated primarily right-hemisphere regions (ventrolateral frontal cortex, centered in BA 47; BA 19 in occipital cortex; BA 40 in parietal cortex; and BA 6 in premotor cortex), although a task requiring participants to remember the positions of letters activated areas in both hemispheres

(Smith et al., 1996). Another PET study enrolling 19 healthy volunteers found greater left lateralization of cerebral blood flow for word recognition than for face recognition in the mid-temporal region (Gur et al., 1997). Also, left lateralization in amygdala and hippocampus correlated with better performance on the verbal task, and right lateralization in the parahippocampal gyrus was associated with better performance on the face recognition task.

Numerous fMRI studies also support the idea that the two hemispheres act differently to encode verbal, spatial, and object information. One fMRI study of 10 neurologically normal adults found that a spatial working memory task preferentially activated the middle frontal gyrus in the right hemisphere, whereas an object working memory task was associated with bilateral middle frontal gyrus activation (McCarthy et al., 1996). Middle frontal gyrus activation developed within 3-6 seconds after the task began and declined after the task, while cingulate gyrus activation was noted after task completion. In another fMRI study enrolling ten neurologically normal participants, encoding of both words and faces produced dorsal frontal activation, but encoding of words resulted in left-lateralized activation and face encoding resulted in right-lateralized activation (Kelley et al., 1998). When the objects to be remembered were nameable, dorsal frontal activation was bilateral. An fMRI study by Belger et al. (1998) also found right-left differentiation for spatial vs. object perceptual processing, with spatial memory tasks resulting in activation of inferior parietal cortex; right middle frontal gyrus; and intraparietal sulcus, while object memory tasks resulted in activation of inferior occipitotemporal cortex, predominantly in the left hemisphere; left inferior frontal gyrus; intraparietal sulcus, particularly in the left hemisphere; and the middle frontal gyrus

bilaterally. Cingulate gyrus activation was noted to occur at task offset.

Evidence from human lesion studies supports the idea that verbal working memory preferentially activates the left hemisphere and spatial working memory the right. A 1968 study by Cohen, Noblin, Silverman, and Penick found that electroconvulsive shocks administered to the left hemisphere of neurologically normal participants impaired verbal memorization more than nonverbal memorization, while shocks to the right hemisphere had the reverse effect (Cohen, Noblin, Silverman & Penick, 1968). More recently, Floel et al. (2004) used transcranial magnetic stimulation (TMS) to simulate lesions and found that TMS of the left prefrontal cortex disturbed memory for words, whereas TMS of the right prefrontal cortex impaired memory for shapes (Floel et al., 2004). Brodmann area 40, in the left parietal cortex, is the most frequent location of lesions in patients with impaired verbal memory (McCarthy & Warrington, 1990, and Vallar & Shallice, 1990, cited in Smith, Jonides, & Koeppe, 1996).

Left lateralization for verbal working memory and right lateralization for spatial working memory appears to be more pronounced in younger adults compared with older adults (Reuter-Lorenz, Kinsbourne & Moscovitch, 1990). Task complexity may also affect lateralization. A meta-analysis of 60 PET and fMRI studies found that only less complex tasks resulted in left frontal dominance for verbal working memory tasks, whereas with spatial tasks, right frontal lateralization increased along with executive demand (Wager & Smith, 2003). It is possible that one fMRI study's failure to find left-right, verbal-spatial dissociation was caused by the relatively high cognitive demands of that study's n-back tasks (Nystrom et al., 2000).

Parietal and premotor regions have been observed to be active during storage and retrieval in working memory tasks (Courtney, Ungerleider, Keil, & Haxby, 1996; Belger et al., 1998; Jonides et al., 1998; Kelley et al., 1998; Wager & Smith, 2003). Visual-spatial processing appears to involve an exchange of information between premotor and parietal cortex (Cavanna & Trimble, 2006).

The parietal cortex has been hypothesized to hold representations of space or magnitude that allow items in working memory to be organized or manipulated (Smith & Jonides, 1998; Crone, Wendelken, Donohue, van Leijenhorst & Bunge, 2006; Marshuetz, Smith, Jonides, DeGutis & Chenevert, 2000). One PET study found that although the encoding phase of a verbal working memory task did not reliably activate the parietal cortex, the maintenance phase was associated with activation of the posterior parietal, premotor, dorsolateral prefrontal, and inferior frontal cortices, whereas the retrieval phase was associated with activation of posterior parietal cortex as well as dorsolateral prefrontal cortex and anterior cingulate (Jonides, Schumacher, Smith, Koeppe, Awh, & Reuter-Lorenz, 1998).

The premotor cortex has been hypothesized to act as a rehearsal system for either verbal or spatial memory. For instance, when a person is trying to memorize locations of items, the premotor cortex might be involved in shifting attention from one location to another (Awh, Jonides & Reuter-Lorenz, 1998; Awh, Smith & Jonides, 1995). Subvocal rehearsal used to retain verbal information has been suggested to be subserved by Broca's area and left-hemisphere premotor and supplementary motor areas (Smith & Jonides, 1998). The precuneus connects with parietal areas involved in processing visual-spatial information, including the inferior and superior parietal lobules and intraparietal sulcus,

and it has also been implicated in visual-spatial processing and attention (Selemon & Goldman-Rakic, 1988; Cavada & Goldman-Rakic, 1989; Leichnetz, 2001). In PET and fMRI studies, both actual and imagined movement in response to visual-spatial information have been associated with activation of the superior parietal lobule and precuneus activation (Le, Pardo & Hu, 1998; Hanakawa et al., 2003; Malouin, Richards, Jackson, Dumas & Doyon, 2003; Ghaem et al., 1997).

Development of relevant neural systems

During the extended period over which working memory matures, a number of different types of changes take place in the brain. Density of synapses and neurons declines slowly over the ages of 2 years to 16 years, reducing redundancy and increasing efficiency (Huttenlocher, 1979). Cerebral blood flow and oxygen utilization are reduced in young adults compared with preadolescent children (Kennedy & Sokoloff, 1957). Myelination, which speeds conduction of neural impulses, continues into the teenage years (Klingberg, Vaidya, Gabrieli, Moseley & Hedehus, 1999; Paus et al., 1999). Since processing speed increases are thought to underlie working memory improvements, myelination is likely to be important in the development of working memory capacity (Thomas et al., 1999).

Functional neuroimaging studies in children indicate that during working memory tasks, children and adults activate similar, but not identical, brain areas (Casey et al., 1995; Nelson et al., 2000; Thomas et al., 1999). Even in infants less than one year old, working memory (as measured by performance on an A-not-B stage 4 object permanence task) is associated with recruitment of dorsolateral PFC (DLPFC), and maturation of DLPFC is thought to underlie improvements in working memory (Diamond & Goldman-

Rakic, 1989). Both dorsal-ventral differentiation and right-left lateralization have been observed in both adults and children, suggesting that the differential processing of verbal and spatial information during memory tasks has developed at least by 8 years of age (Thomas et al., 1999).

The difficulty of designing tasks that result in similar performance across an age range is a concern in mapping brain activation associated with working memory (Klingberg, Forssberg & Westerberg, 2002). Fry and Hale developed a series of tasks to test verbal and spatial working memory span (the number of items a person can remember) which succeeded in eliciting spans of greater than zero from participants ages 8, 10, and 19 years (Hale et al., 1997). Performance in the three age groups was differentiated by mean length of span and by response to interference. On the spatial working memory task with only one task demand, mean span was 3.89 in the 8-year-olds, 4.48 in the 10-year-olds, and 7.10 for the 19-year-olds. All groups showed interference effects when asked to perform a secondary task in the same domain as the first task, but only 8-year-olds showed a significant amount of interference when the secondary task tapped a different domain. These results suggest that the domain specificity of working memory is still evolving between ages 8 and 10 years.

One 1999 study compared six children ages 8 to 10 years with six adults ages 19 to 26 years in terms of performance and brain activation patterns during a spatial working memory task (Thomas et al., 1999). During the memory condition, both adults and children showed increased activation of the right dorsolateral prefrontal cortex (BA 10/46) and right parietal lobule (BA 7, 40). Deactivation of the left medial superior frontal cortex (BA 9) and inferior frontal gyrus (left BA 47 for adults; right BA 45 for

children) was also observed in both groups. Adults but not children also showed increased activity in the middle temporal gyrus (BA 21) and right cingulate gyrus (BA 24/32), whereas children but not adults demonstrated activation of the left precuneus cortex (BA 7), left and right inferior parietal lobule (BA 40) and right cerebellum. These regions were posited to relate to the mapping and motor learning components of the task rather than the memory component.

Although children are observed to recruit similar frontal-parietal networks as adults during simple working memory tasks, they may fail to do so when presented with more complex tasks (Ciesielski, Lesnik, Savoy, Grant & Ahlfors, 2006; Schweinsburg, Nagel & Tapert, 2005). A complex object working memory task was found in adults to activate bilateral, particularly left, ventral PFC, bilateral fusiform gyrus, posterior cingulate and precuneus, suggesting that the adults were using a semantic labeling strategy to keep track of the objects (Ciesielski et al., 2006). Children activated different regions: premotor and parietal cortex, anterior insula, caudate/putamen, and cerebellum, suggesting use of visual-spatial strategies. These results suggest a shift in strategies and associated brain activation as children mature.

Recruitment of additional brain regions by older children was also reported by Crone et al. (2006). On a working memory task requiring manipulation as well as maintenance, Crone and colleagues attributed superior performance in participants older than 12 years to increased recruitment of dorsolateral prefrontal cortex and superior parietal cortex (Crone et al., 2006). In contrast, activation in the ventrolateral PFC, which has been associated with maintaining received information, appeared stable up to age 25 years.

Even in a task requiring only maintenance, differences in activation patterns have been found between adults and children. Geier et al., comparing visual spatial working memory in 43 children, adolescents, and adults ranging from ages 8 years to 30 years, found that while all participants recruited frontal, parietal, and temporal regions for the task, during an extended delay, activation was more broadly distributed in younger participants, while adults recruited additional posterior parietal cortex (Geier, Garver, Terwilliger & Luna, 2009).

Brain activation response patterns during a spatial working memory task may differ depending on gender as well as age, even when performance is stable (Schweinsburg et al., 2005). In a study of 25 female and 24 male adolescents, ages 12 to 17 years, activation in left prefrontal and bilateral inferior posterior parietal regions increased with age, while activation in bilateral superior parietal cortex decreased with age. That is, older and younger participants showed parietal activation, but activation was more superior with younger participants (i.e., it was physically higher in the brain, with a lot more activation around axial slices 54 and 58 and less around slices 38 and 42). Also, males showed more activation in the frontopolar cortex and females showed less activation in the anterior cingulate. This suggests that frontal and parietal networks involved in spatial working memory change with age and differ between genders, perhaps representing different mnemonic strategies.

A study of 49 typically developing adolescents, aged 12 to 17 years, examined the relationship between results on standardized neuropsychological tests and fMRI response (Nagel, Barlett, Schweinsburg & Tapert, 2005). Brain activation was observed in bilateral dorsolateral prefrontal and intraparietal regions, bilateral subcortical structures, right

caudate, and cerebellum in response to the spatial working memory task. In many brain regions, test scores and brain activation were negatively correlated; adolescents performing better appeared to be using fewer resources. However, blood oxygenation level dependent (BOLD) responses in the left inferior parietal lobe and supramarginal/angular gyrus region were positively correlated with executive function performance, perhaps indicating strategizing during the spatial working memory task, and left dorsolateral prefrontal activation was positively correlated with learning/memory scores, particularly scores on the California Verbal Learning Test, Children's Version (CVLT-C).

During working memory tasks, higher brain activity (i.e., percent signal change in fMRI) has been found to correlate with better performance. Activity in frontal and parietal regions relevant to working memory has been shown to be higher in correct than incorrect trials of a task (Pessoa, Gutierrez, Bandettini & Ungerleider, 2002), and signal change is greater when a person is holding more items in mind than when the same person is remembering fewer items (Vogel & Machizawa, 2004).

Klingberg, Forssberg, & Westerberg (2002) compared performance on a spatial working memory task with brain activation as measured by fMRI in 13 healthy participants, ages 9.4 to 18.5 years (4 girls, 9 boys, mean age 13.4 years). Accuracy on the scanner task was high and neither accuracy nor reaction time differed significantly across the age range. To assess developmental differences in working memory capacity, a related but more demanding task was administered outside the scanner. In this second task children were asked to identify, after a delay, several previously presented locations. After subtracting activity on a control task, the brain areas that appeared activated as a

main effect of working memory included the superior and middle frontal gyri, inferior and superior frontal sulci, cingulate cortex, and areas of the parietal and occipital cortices. The older children showed higher activation of bilateral superior frontal sulcus and intraparietal sulcus, while the participants with higher working memory capacity (as determined by the more demanding task) showed higher activation of the left superior frontal and left intraparietal cortex. These results suggest that the positive correlation of age with brain activity during a working memory task is due to increases in working memory capacity from childhood to early adulthood.

The positive correlation of age, working memory performance, and brain activity was confirmed by Kwon, Reiss, & Menon (2002). This study assessed 23 participants of ages 7 to 22 years using a 2-back spatial working memory task. Accuracy and response time improved gradually into young adulthood. Brain activation increased with age in focal regions of the left and right dorsolateral prefrontal cortex, left ventrolateral prefrontal cortex, left premotor cortex, and left and right posterior parietal cortex. The researchers concluded that these areas represent at least two neural systems, a visual-spatial attentional system in the right hemisphere and a phonologic storage and rehearsal system in the left hemisphere, and that both neural systems mature throughout childhood and adolescence.

The Swedish research group including Klingberg, Westerberg, Olesen, and Nagy has accumulated evidence tying white matter maturation in certain regions with gray matter maturation in nearby regions and with improvements in working memory over the same age span (Klingberg, 2006; Klingberg et al., 2002; Nagy, Westerberg & Klingberg, 2004; Olesen, Nagy, Westerberg & Klingberg, 2003). Increasing activation of frontal and

intraparietal regions has been correlated with increasing working memory capacity in children and adolescents (Klingberg et al., 2002). Increasing activation in the superior frontal sulcus and inferior parietal lobe has been correlated with increasing myelination of nearby fronto-parietal regions over the ages of 8 to 18 years (Olesen et al., 2003). Over this same age span, development of working memory capacity has been positively associated with increasing myelination in the left frontal lobe, including a region between the superior frontal and parietal cortices (Nagy et al., 2004). These studies indicate that improvements in working memory over childhood and adolescence are subserved by the simultaneous development of myelination and gray matter in a superior frontal-intraparietal network (Klingberg, 2006).

Development of working memory skills

Working memory skills are apparent in infancy (Diamond & Goldman-Rakic, 1989) and improve into adolescence (Bayliss, Jarrold, Baddeley & Leigh, 2005; Brocki & Bohlin, 2004; Demetriou, Christou, Spanoudis & Platsidou, 2002; Gathercole, Pickering, Ambridge & Wearing, 2004; De Luca et al., 2003; Luciana, Conklin, Hooper & Yarger, 2005; Luna, Garver, Urban, Lazar & Sweeney, 2004; Siegel & Ryan, 1989; Swanson, 1999). The number of items that can be maintained in working memory (working memory span) increases over childhood and adolescence into adulthood. In fact, Swanson reported that in a study enrolling 778 participants ages 6 to 57 years, working memory improved up until age 45 years. This was true of cued, non-cued, verbal, and visual-spatial working memory tasks. More often studies have reported skills stabilizing in young adulthood. A study of 245 participants ages 8 to 30 years assessing working memory as measured by oculomotor tasks found that performance reached adult levels

and stabilized at approximately 19 years of age (Luna et al., 2004). Similarly, a study of over 700 participants ages 4 to 15 years found that verbal memory span improved until at least age 14, and visual spatial memory span improved until at least age 15 (Gathercole et al., 2004). Another visual spatial working memory study found improvements through at least at 16 (Westerberg, Hirvikoski, Forssberg & Klingberg, 2004).

In early childhood, differences in working memory capacity appear to stem from differences in strategy (Cowan et al., 1994). After approximately the age of 6 years, increases in working memory capacity occur at a linear rate and seem to reflect a quantitative change in capacity instead of a change in strategy (Fry & Hale, 2000; Gathercole et al., 2004). Performance on verbal vs. visual spatial working memory tasks has been found to be highly correlated, suggesting that working memory is a domain-general skill (Kane et al., 2004).

Executive processes important in working memory also develop through adolescence and into young adulthood (Gathercole et al., 2004). Attention improves, and children become less susceptible to interference (Hale, Bronik & Fry, 1997) and better able to inhibit inappropriate responses (Davidson, Amso, Anderson & Diamond, 2006). Working memory development is also probably subserved by increases in processing speed (Demetriou et al., 2002; Thomas et al., 1999). A number of different methods have been used to increase the attentional demands of working memory tasks, including requiring subjects to manipulate as well as recall information, requiring performance of more than one type of task, and introducing irrelevant information to interfere with the task (Klingberg, 2006). For instance, in a study of 325 participants ranging in age from 4 to 45, even the youngest children were able to correctly answer questions tapping

working memory and requiring inhibition of an automatic response (Davidson et al., 2006). However, the younger the participants, the more trouble they had ignoring irrelevant stimuli and inhibiting automatic responses. The oldest children in this study were 13, and those children were not consistently performing at adult levels. Luna et al. found that response inhibition reached adult levels at approximately 14 years (Luna et al., 2004). Luciana et al, assessing 133 participants 9 to 20 years of age, found that while recognition memory stayed stable over this age span, ability to manipulate multiple units of information developed until ages 13 to 15 years, and performance on a task requiring monitoring and strategy improved until ages 16 to 17 years (Luciana et al., 2005). The same research group found a similar pattern of development in verbal working memory (Conklin, Luciana, Hooper & Yarger, 2007). Performance on most tasks improved over the course of adolescence. However, tasks supported primarily by more posterior neural substrates did not show improvement after the age of 12, suggesting that while those regions were fully developed by approximately age 12, development in more frontal regions continued into later adolescence.

Working memory is necessary for reading comprehension (Ransby & Swanson, 2003; Swanson & Trahan, 1996) and predicts mathematical problem-solving skills (Swanson, 2004), logical reasoning skills, and general fluid intelligence (Kane et al., 2004). Children who have difficulties with working memory may struggle in school. In children with chronic kidney disease (CKD), there is the additional concern that difficulties with working memory may impede compliance with complex treatment regimens (Gipson et al., 2006). Establishing whether working memory is particularly affected by CKD is a first step toward developing empirically based interventions that

might improve medical compliance as well as academic success in children and adolescents with CKD.

CHAPTER III

METHODOLOGY

Participants

The study has been approved and renewed by the UNC Institutional Review Board. Patients being enrolled in the study are ages 9 to 19 years old and have either moderate CKD, defined as 90 $\geq$ estimated GFR $\geq$30 ml/min/1.73m^2 , or severe CKD, defined as estimated GFR < 30 ml/min/1.73m^2, dialysis dependence, or transplant dependence. Estimated GFR (eGFR) is calculated using a modified version of the Schwartz formula (Schwartz 2009). In 79% of cases, this formula has been found to generate eGFR values within 30% of those based on iohexol clearance, the current gold standard for estimating GFR.

My goal was to enroll 10 patients with moderate CKD, 10 with ESRD, and 10 healthy participants of comparable age and gender. I obtained usable data from 10 patients with moderate CKD, 11 patients with severe CKD, and 11 control patients. Eight scans were concluded to be unsuitable for analysis as a result of excessive motion, claustrophobia, orthodontics, lack of scanner time due to a family arriving later than scheduled, or scanner failure due to overheating. Of the patients for whom some runs of the task could not be analyzed due to excessive motion, two came from each group.

This sample size is not uncommon for a functional MRI study comparing a group of children with a medical disorder to a group of health controls in terms of brain

activation during a spatial working memory task. For instance, Hart, Davenport, Hooper, and Belger (2006), assessing patients with Turner syndrome and Kwon, Menon, Eliez, Warsofsky, White, Dyer-Friedman, Taylor, Glover, and Reiss (2001), assessing patients with Fragile X, each enrolled 10 patients and 15 healthy controls. Similarly, Vance, Silk, Casey, Rinehart, Bradshaw, Bellgrove, and Cunnington (2007), studying patients with Attention Deficit/Hyperactivity Disorder (ADHD), enrolled 12 patients and 12 controls, while Silk, Rinehart, Bradshaw, Tonge, Egan, O'Boyle, and Cunnington (2006), studying autism spectrum disorders, enrolled 7 patients and 9 controls. Each of these research groups found significant differences in brain activation in certain regions of interest in the patients compared with the healthy control participants during a spatial working memory task.

Patients were recruited from the UNC pediatric nephrology clinics and controls from the UNC catchment area. Controls were found by word of mouth. Exclusion criteria were hospitalization within 1 week, acute illness, claustrophobia, permanent metal devices (e.g., braces, shunts), current pregnancy or breastfeeding, and history of traumatic brain injury. Claustrophobic participants cannot tolerate being scanned, and permanent metal devices interfere with the signal to the scanner if they do not create discomfort or danger to the participant. Pregnant and breastfeeding participants were excluded based on concerns about the health of the fetus or baby. Patients who currently were or recently had been acutely ill or had a history of traumatic brain injury were excluded because these events might impair their performance on the working memory task.

Methods

Functional magnetic resonance imaging (fMRI) provides a noninvasive way of measuring changes in blood oxygenation in the brain (Huettel, Song & McCarthy, 2004). Because active neurons take in oxygen at a higher rate than inactive neurons, the oxygenation of blood flowing through the brain changes rapidly following neuronal activity. The changes in oxygenation detected by fMRI are therefore interpreted as indicating localized neuronal activity over time. The fact that fMRI can be performed without injecting a visualization agent makes it an advance over positron emission tomography (PET), which was previously the most commonly used neuroimaging technique but has the disadvantage of requiring injection of radioactive tracer molecules.

The current study uses blood-oxygenation-level dependent (BOLD) fMRI to examine activity in brain regions relevant to visual-spatial working memory in children with CKD and healthy controls. This technique depends on the difference between the magnetic properties of deoxygenated and oxygenated hemoglobin (Huettel et al., 2004). An MRI scanner produces a strong magnetic field. The current study uses a 3 Tesla (3T) scanner, and the weakest scanner typically used for fMRI generates a 1.5 T magnetic field, while the earth's magnetic field has a strength of approximately 0.00005 T. The magnetic field of an MRI scanner is tuned to the frequency of hydrogen nuclei, which are found in water and therefore found throughout the body. The scanner's oscillating magnetic fields match the spin of the hydrogen nuclei, transferring energy to those nuclei. The response of hydrogen molecules to the magnetic field generates a magnetic resonance (MR) signal. Because deoxygenated hemoglobin, unlike oxygenated

hemoglobin, has unpaired electrons, it reacts differently to a magnetic field, and the MR signal from oxygenated blood is stronger than the MR signal from deoxygenated blood.

Within a few seconds the MR signal decays (Huettel et al., 2004). Among other changes, the nuclei, which the magnetic pulse had caused to spin in sync with each other, fall out of sync. This alters a particular component of the MR signal, the transverse component of net magnetization. The time over which the transverse component of magnetization decays is called T2. T2 is longer for oxygenated hemoglobin, because the magnetic properties of deoxygenated hemoglobin cause the signal to decay more quickly. T2 is also longer for more homogenous tissue, because nuclei fall out of phase more slowly when all the surrounding molecules are alike. For instance, T2 is long in water. In the brain, T2 is longer for gray matter, which is well supplied with blood, than for white matter, which contains less fluid. Certain patterns of magnetic pulses can capitalize on these differences in T2 to produce images that clearly distinguish gray from white matter. T2 weighted images, images that maximize T2 contrast, are created using magnetic field gradients (gradient-echo sequences), long intervals between magnetic pulses (long repetition time [TR]), and moderate intervals between energy transfer and data acquisition (moderate echo time [TE]). Because T2 also varies depending on blood oxygenation, T2 contrast is commonly used in BOLD fMRI.

Imaging for the current study is performed using the UNC research MRI, which is a 3-Tesla MRI (Siemens Magnetom Allegra syngo MR 2004A). Participants' heads are cushioned to minimize movement. Functional images are acquired using gradient echo EPI (24 oblique slices, 3.75 mm, FOV 24 cm; TE: 30 ms; TR: 1500 ms; flip angle 80) over 244 time points. This image acquisition sequence permits imaging of the entire brain

and is sensitive to blood oxygenation level dependent (BOLD) contrast (Hart et al., 2006). Preceding functional image acquisition, structural images are acquired using full brain MP RAGE, T1 weighted (1 mm^3 isotropic voxels, acquisition matrix 192 x 256) and T2 weighted (1 mm^3 isotropic voxels, acquisition matrix 256 x 256). This protocol results in high resolution, 3D anatomical images (Hart et al., 2006). Following functional image acquisition, diffusion tensor images are acquired using a Siemens EPI protocol, TE = 83ms, TR=10s, 2 mm3 isotropic voxels, 128x128 acquisition matrix, with 21 gradient directions and a b-value of 1000.

The task is a delayed-response visual memory task using a CIGAL display. CIGAL is a computer program used to provide stimuli for fMRI tasks and to gather data on participant behavior (e.g., responses to stimuli and reaction time). Ten trials are collected per run, and 4 trials of data are collected for each patient during one MRI session. During each trial, a participant is shown 4 red squares, each for 1 second, each in a different location. After a pause, a green square appears, and the participant is asked to indicate by a button press whether the green square is in the same location as one of the previous 4 red squares or in a different location. The encoding period therefore lasts 4 seconds, followed by a delay of 17 seconds, then retrieval. This task was previously used to assess visuospatial working memory in a study of girls with Turner syndrome (Hart, Davenport, Hooper & Belger, 2006) and is similar to a task used by Klingberg et al. to assess visuospatial working memory (Klingberg et al., 2002). The timing of the presentation and response in this design allows the hemodynamic response correlating with each phase of working memory to be isolated (Hart et al., 2006).

For each participant, a parent or caregiver is asked to complete the Vineland

Adaptive Behavior Scales-II, Parent/Caregiver Rating Form (Vineland). This rating scale assesses communication, daily living skills, socialization, generating composite and domain scores with a mean of 100 and standard deviation of 15. Vineland scores correlate with scores on other adaptive rating scales and on intelligence tests and are commonly used as part of an assessment of mental retardation (Bensberg & Irons, 1986). The Vineland scales were normed on a sample of 3,695 people in the U.S. aged from birth to 90 years, with higher proportions of participants in the younger age brackets in anticipation of greater behavioral changes in those age brackets (Sparrow et al., 2005). The sample was balanced in terms of gender and representative of the U.S. in terms of race/ethnicity, community size, geographic region, and mother's education level. It is hoped that the psychometrics reported in the Vineland manual will be relevant to the study population.

For children and adolescents ages 9 to 19 years in the standardization sample, split-half reliability of the Adaptive Behavior Composite ranged from 0.94 to 0.97, and test-retest reliability ranged from 0.81 to 0.91. For children and adolescents ages 9 to 18 years, interrater reliability for the parent/caregiver form was calculated to be 0.75 (Sparrow et al., 2005).

Participants are also asked to complete a VAS (Bond & Lader, 1974), which entails marking a vertical line between a happy face and a sad face to indicate how they have been feeling for the past few weeks. The distance from the happy face will be measured and taken as a measure of pre-scan affective state. A standard urine test is performed to rule out pregnancy.

Data analysis

Functional imaging data will be analyzed using both the FSL software package 4.1 (FMRIB, Oxford, UK) and eventstats, custom software developed by the Duke-UNC Brain Imaging and Analysis Center (Duke-UNC BIAC, Durham, NC). Random-effects activation and contrast maps for the whole brain will be generated initially, followed by region of interest (ROI) analyses to examine BOLD response in functionally activated clusters in prefrontal cortex and parietal cortex.

ROI analyses will be used to answer research questions 1 through 3, which can be outlined as follows: Will patients with chronic kidney disease differ significantly from healthy participants in terms of percent BOLD signal change in functionally activated clusters in the ventrolateral prefrontal cortex (VLPFC), dorsolateral prefrontal cortex (DLPFC), or right posterior parietal cortex

- during the encoding period? (question 1)
- during the retrieval period? (question 2)
- over the course of the task? (question 3)

A second subset of research questions will explore the difference between patients with severe CKD and those with moderate CKD, who have less often been studied. These questions can be outlined as follows: Will patients with moderate CKD (eGFR $\leq$ 90 ml/min/1.73m^2, $\geq$ 30 ml/min/1.73m^2), patients with severe CKD (eGFR $\leq$ 30 ml/min/1.73m^2 or dialysis dependence), or healthy participants differ significantly from each other in terms of percent signal change in the VLPFC, DLPFC, or right posterior parietal cortex

- during the encoding period? (question 1b)

- during the retrieval period? (question 2b)
- over the course of the task? (question 3b)

Taking advantage of differences between the two programs, FSL will be used to assess average activity in relevant brain regions during the encoding vs. retrieval periods, addressing questions 1 and 2. Eventstats will be used to examine peak activity for individual participants in relevant brain regions during the encoding and retrieval phases and activity over the course of the task, addressing Questions 1 through 3.

Assessing usability of data

Quality analysis (QA) will be conducted using tools developed by the Biomedical Informatics Research Network (BIRN; Keator et al., 2008). QA will assess number of volumes able to be reconstructed (fewer volumes indicating greater motion by the participant during the scan), signal-to-noise ratio, and motion in the x, y, and z planes. Head motion will be assessed based on center of mass measurements in three orthogonal planes. The z-plane is the dimension in which a person in an MRI scanner is most likely to move significantly. Following QA, sets of functional data showing motion in the z-plane exceeding 5 mm will be excluded from both the FSL and eventstats analysis. In eventstats, each behavioral response to the task will be analyzed as a separate event. Any event showing more than 3 mm motion in any plane will be excluded from the eventstats analysis.

Participants who, due to excessive motion or other technical difficulties during some functional runs, produce only one usable set of functional data will be excluded from the FSL analysis because FSL requires that separate sets of data be averaged for each subject. These participants will be included in the eventstats analysis.

Preparing data for analysis

Brain extraction, image registration, motion correction, and t-comparisons will be conducted using the FSL FEAT tool, version 5.92 (Smith et al., 2004). Brain extraction eliminates the imaging data representing participants' skulls, skin, and eyes, so that the data representing the brain remain. In image registration, functional images are registered to a standard image—that is, all the images from different participants' differently sized and shaped brains are aligned to a standard brain using identifiable spatial markers, so that participants can be compared to each other. Although the standard brain template was developed based on adults' brains, it has been found to be usable in fMRI studies for children as young as 7 years old (Burgund, Kang, Kelly, Buckner, Snyder, & Petersen 2002; Kang, Burgund, Lugar, Petersen, Schlaggar 2003). Images will be co-registered first to each participant's high resolution structural scan and then to the MNI 152 person 2-mm template, using a 12-parameter affine transformation. Functional analyses will be overlaid on the participants' average high resolution structural scan in MNI space.

Head motion will be corrected using the MCFLIRT tool, which begins with the middle image in a time series and looks for an identity transformation between that volume and the next, then uses that transformation to estimate the transformation between the next two volumes. These repeated identity transformations are used to average across volumes and create a new template image. The first and last volumes in the z-plane are doubled to capture as much data as possible. Corrections for the interleaved slice acquisition over the 1.5 seconds (TR) will be made using a slice time correction algorithm. Images will be spatially smoothed with a 5 mm full width at half maximum Gaussian kernel to improve signal-to-noise ratio. High pass temporal filtering

will be used to cut off periods longer than 66 s.

Within- and between-subjects analysis in FSL

In the FSL whole-brain analysis, differences between groups will be assessed using a three-level mixed effects general linear modeling (GLM) procedure conducted with the FEAT tool. A first-level GLM was defined for each functional run completed by each participant. Since the encoding and response portions of the task are separated by a relatively long time (17 s), the model used in this analysis will treat them as two separate events, each represented by a basic square waveform shape convolved with a single gamma hemodynamic response function. Temporal derivatives of these convolutions will also be included in order to account for differences in the timing of individual participants' responses. Motion parameters will not be included, determinations of acceptable motion having been made in the QA stage. FEAT uses a nonparametric estimation of time series autocorrelation to "prewhiten" data at each voxel, that is, to adjust analysis of noise in the data to account for some task-related, non-white noise. This prewhitening is intended to improve efficiency of analysis (Smith, Singh & Balsters, 2007). For each participant or group of participants, color images will be generated showing which parts of the brain had significant changes in BOLD signal over time. A second-level fixed effects model will be fit for each participant to account for variability among the four runs of the task.

Third-level analysis will compare the entire group of patients and each separate group of patients—those with moderate and those with severe CKD—to the control group. To compare data across participants, FEAT uses Markov chain Monte Carlo (MCMC) sampling to estimate random-effects variance and degrees of freedom at each

voxel in order to determine mixed-effects variance across participants (Smith et al., 2004). Single-sample t-tests will be used to identify brain areas in which the MR signal changed significantly over the course of the task for each group of participants. Significant activation will be defined by cluster size and corrected for multiple comparisons with cluster correction using a threshold of $z > 2.3$ ($p < 0.05$). Statistical maps will be overlaid onto the MNI 152 person 2-mm standard template brain for activation visualization. Independent t-tests will then be used to identify areas showing significant differences in activation in all patients compared with controls and in each patient group compared with the control group.

The FEATquery tool will be used to examine activation in specific regions of the brain that are expected to be active during a visual-spatial working memory task. FEATquery allows interrogation of FEAT results within a mask, calculating image and time series statistics for MR signal change within the brain area defined by the mask. This tool will be used to generate mean and maximum percent signal change associated with the experimental paradigm in each defined brain region for each individual. Areas of activation will be described in terms corresponding to standard stereotactic (Talairach) coordinates. For the region of interest analyses, masks will be generated using the tools from the Wake Forest University Pick Atlas, which is based on the Talairach Daemon database (Lancaster, Summerlin, Rainey, Freitas, & Fox, 1997; Lancaster, Woldorff, Parsons, Liotti, Freitas, Rainey, Kochunov, Nickerson, Mikiten, & Fox, 2000; Maldjian, Laurienti, Kraft, & Burdette, 2003). Functionally activated regions will be analyzed.

FEATquery results will be analyzed in Microsoft Excel using t-tests to compare activation in each region of interest in the control group with activation in the region in

the group made up of all CKD patients during the encode phase and the retrieve phase. Unpaired t-tests are appropriate when two samples from two different populations are independent and have the same probability distribution. In this case, the control and patient groups are independent of each other, and data for each group are expected to approximately follow a normal distribution.

One-way analyses of variance (ANOVAs) will be conducted using the statistical software SPSS in order to determine whether there are statistically significant differences among all three groups—patients with severe CKD, patients with moderate CKD, and control participants—in either the encode or retrieve phase of the task. These analyses will address Questions 1b, 2b, and 3b. A one-way ANOVA is a statistical test used to determine whether a significant difference exists between two or more means representing two or more populations that are classified according to one characteristic (Moore & McCabe, 2003). In this case, the characteristic is severity of chronic kidney disease, and the controls, patients with CKD, and patients with ESRD represent three populations. ANOVA is appropriate when participants are randomly assigned to groups, when the data are normally distributed, and when there is homogeneity of variance across groups (Moore & McCabe, 2003). In the current study, the participants were of course not randomly assigned to groups; it is an observational rather than experimental study design. However, even in this non-experimental paradigm, ANOVA remains the best statistical option for determining whether more than two groups represented by a discrete variable differ in terms of a given characteristic represented by a continuous variable.

Eventstats analysis

Whole-brain and region-of-interest analyses will also be performed using custom

software called eventstats, which was developed by the Duke-UNC Brain Imaging and Analysis Center (Duke-UNC BIAC, Durham, NC). Eventstats uses a whole-brain voxel-based approach to generate random-effects t-maps for each participant based on the hemodynamic response to each event. In this case, each presentation of the retrieval stimulus, the stimulus for which the participant is to press a button to give a response, is considered a separate event. Epochs beginning 18 image volumes before the retrieval stimulus and continuing for 14 image volumes after the target event will be extracted from the raw functional imaging data and averaged together over all runs to get a mean time course of raw MR signal. The BOLD signal over time for each voxel will be correlated with an empirically determined hemodynamic response waveform (Hart, Davenport, Hooper, & Belger 2006), and t-statistics will be calculated based on correlation coefficients for each voxel. A random-effects analysis will then be performed on the resulting individual t-maps to determine the significance of differences between the patient and control groups, and this analysis will be used to generate contrast maps that can be displayed on a template anatomical image.

The same masks generated from the Wake Forest Pick Atlas will be used for ROI analysis in eventstats as were used in the FSL ROI analysis. ROI analyses will be run using custom scripts in MATLAB. Average percent signal change at each time point in the event epoch will be calculated within each region of interest. Eventstats ROI results will be analyzed in SPSS using one-way ANOVAs to compare average percent signal change in each region of interest across the three groups (moderate patients, severe patients, and controls) over the entire course of the task. Individual participants' peak amplitudes will also be used to assess group differences in the encode and retrieve phases

of the task.

Structural data and descriptive measures

The T1- and T2-weighted images constitute structural rather than functional data. Structural imaging data will be analyzed volumetrically by a collaborating research group.

Descriptive measures (e.g., mean, standard deviation) will be used to determine whether groups differ in terms of age, gender, VAS score, or Adaptive Behavior Composite scores on the Vineland. These values will be used to describe each group for purposes of informal comparison. The Vineland ABC scores, which provide a measure of functioning in ordinary daily life, will also be analyzed using ANOVA in SPSS to determine whether differences among the groups are statistically significant. If the ABC scores differ significantly among groups then that variable will be used as a covariate in the FSL whole-brain analysis of BOLD percent signal change.

Task accuracy (fraction of correct responses) and reaction time in patients with CKD and controls will be compared using t-tests. Results of these comparisons will address research questions 4 and 5, which ask whether patients with CKD differ significantly from healthy controls in terms of either accuracy or reaction time on the working memory task.

Post-hoc analyses

Due to the number of different tests being performed for this study, probability thresholds for the whole-brain analyses will be adjusted post-hoc using the Holm-Bonferroni method. The Bonferroni method tests each hypothesis at a significance value divided by the number of tests conducted (Moore & McCabe, 2003). The Holm-

Bonferroni procedure is less conservative but similarly corrects for the increased probability of finding one significant result among the results of multiple tests. This procedure will be performed by hand and the resulting lower p-values used to assess whether the findings can reasonably be termed significant.

Effect-size calculations will be performed for the behavioral results and for selected imaging results, those that show significant or marginally significant differences between patients with CKD and healthy controls in regions of interest relevant to working memory. Effect size calculations estimate the magnitude of a difference without judging whether or not such a difference would have been likely to occur by chance. Effect size can therefore be used as a complement to measures of statistical significance, which estimate whether a difference might have occurred by chance instead of how large the difference is. Cohen's *d* will be used as a measure of effect size (Cohen, 1988).

Limitations

The samples in this study are not entirely random, in that they consist of patients who can remain still enough to gather fMRI data that can be interpreted. Participants also could not have braces, which may have contributed to sampling bias. However, the participants were not chosen by a systematic method along any dimension other than disease severity. By gathering data regarding their adaptive function and using it as a covariate if necessary, I hope to control for one of the primary variables on which the groups might differ and which might influence the study data. To any extent that the samples are not representative of their respective populations, the results of the study may fail to generalize to other sample populations.

CHAPTER IV

RESULTS

Enrollment and participant characteristics

Patients with CKD and control participants ages 9 to 19 years were enrolled in the study. Exclusionary and inclusionary criteria were as stated in Chapter 3. Informed consent concerning the risks and potential benefits of the research was obtained from the parents of participants or, in the cases where participants were aged 18 or 19 years, from the participants themselves. Assent was obtained from minor participants. When participants became alarmed while in the MRI scanner and wanted to come out before finishing the task, they communicated with the scanner technicians. The scanner technician attempted to reassure the participant and make him or her comfortable, and a parent or caregiver was sent into the scanner room to reassure the participant during the task, but if the participant still felt frightened, he or she was permitted to come out and data collection was terminated.

Pediatric CKD patients who participated in the study were classified as having either moderate or severe CKD. Moderate CKD was defined as eGFR > 30, and severe CKD as eGFR < 30. Estimated GFR was calculated based on a modified version of the Schwartz formula (Schwartz 2009). The causes of CKD in the patients were various and are summarized in Tables 2 and 3. The most common was FSGS, which was the cause of CKD in 4 participants, all of whom had severe CKD. Etiologies among patients whose

data were excluded were represented by patients whose data were included. The causes of CKD for the patients in this study represent the most common causes of CKD in all pediatric patients. FSGS accounts for 13.2% of all pediatric ESRD cases (United States Renal Data System, 2008b).

Table 2

CKD etiologies in participants with moderate disease

Systemic diseases resulting in renal damage	Cystinosis	Genetic disorder in which the amino acid cystine builds up in lysosomes and damages cells, including renal cells
	Anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis	Autoimmune disease in which antibodies attack white blood cells, causing damage to small blood vessels in many tissues, including the kidneys
Conditions causing urinary obstruction	Posterior urethral valves	Malformation of the urethra resulting in urinary obstruction; common cause of urinary obstruction in male infants
	Vesicoureteral reflux (VUR)	Condition in which urine flows from the bladder back into the ureter; reflux nephropathy
	Reflux nephropathy	Association of VUR with scarring of the kidneys
Hereditary or congenital nephropathies	Autosomal dominant polycystic kidney	Inherited condition in which numerous fluid-filled cysts develop in the kidneys
	Right kidney hypodysplasia	Characterized by small kidneys, reduced nephron number, and malformation of renal tissue

Primary glomerulopathies	IgA nephritis	Inflammation of the glomeruli due to presence of IgA antibodies
	Idiopathic cortical necrosis	Death of renal cortex tissues from unknown causes, usually as a result of vascular injury

Table 3

CKD etiologies in participants with severe disease

Systemic diseases resulting in renal damage	Lupus	Inflammation of the kidney caused by the autoimmune disease systemic lupus erythematosus
	Alport syndrome	Inherited disease caused by mutations in type IV collagen, resulting in progressive scarring of glomeruli and tubules; may also impair hearing and vision
Hereditary or congenital nephropathies	Hypoplastic kidney	Characterized by a lower than usual number of filtering elements in the kidney
	Type 1 nephronophthisis	Inherited condition that by childhood or adolescence results in cysts in the renal medulla, tubular atrophy, and scarring
Glomerulopathies	Focal segmental glomerulonephritis (FSGS)	Disease in which parts of the glomeruli are scarred, preventing them from effectively filtering blood
	C1q nephropathy	Characterized by renal deposits of C1q protein

As determined by lab data within a few months of the fMRI scan (labs were taken on the same day in many but not all cases), four of the patients with severe CKD and three with moderate CKD were hypertensive. One patient with moderate CKD and six with severe CKD were anemic. Several other patients were being treated to control hypertension or anemia. Many patients were taking numerous additional medications, including corticosteroids to reduce inflammation, antibiotics to prevent or treat infection, medication to correct overproduction of parathyroid hormone, and medications to prevent rejection of a renal transplant. Only two of the 21 patients with CKD, both patients in the moderate group, were not taking any medication.

Thirty-nine participants were scanned, of whom 32 produced usable data (see table below). Data from 3 controls, 3 patients with moderate CKD, and 1 patient with severe CKD were excluded. Of these, data from one control and one patient with moderate CKD were excluded because they had orthodontics that obstructed the MRI signal; after the first case, I realized that a full set of braces resulted in a partial image, and after the second case I found that brackets on the back teeth also obstructed the signal so much as to make the images unusable. One control's data were excluded because pre-processing resulted in misregistration of the brain. Data from one control, two moderate CKD patients, and one severe CKD patient were excluded because excessive head motion made it difficult to interpret the images.

Partial data—3 rather than the standard 4 sets of answers—were obtained from one patient with moderate CKD because the scanner overheated in the middle of the session. From two patients with moderate CKD, only 1 set of data was obtained, in one case because the other sets of data had too much motion to be interpretable, and in the

other case because after the first set the patient became claustrophobic and had to come out of the scanner.

Participant age ranged from 9 years (1 control, 1 patient with moderate CKD) to 19 years (1 control, 1 patient with moderate CKD). A visual analogue scale (VAS, in which a person places a mark on a line between a happy face and a sad face) was used to assess mood at the time of scanning. Participants' adaptive functioning was assessed using the Parent/Caregiver Rating Form of the Vineland Adaptive Behavior Scales-II. The Vineland Adaptive Behavior Composite provides an estimate of a child's social functioning, communication skills, and daily living skills (such as his or her ability to perform household chores). In most cases parents filled out the form while their child was in the MRI scanner. In one case the form was read out to a parent over the phone shortly after the scan because the parent had not been present during the scan. For one patient with moderate CKD, no Vineland scale was obtained because the caregivers did not speak enough English to answer the questions, and translation of the form for this one patient was found to be prohibitively expensive.

Table 4 summarizes the descriptive statistics for participants' age, VAS score, and Adaptive Behavior Composite (ABC). The standard deviation for each measure is shown in parentheses. Based on one-way analyses of variance (ANOVAs), no significant differences were found among groups in terms of age, VAS score, or ABC ($p > 0.2$ for each measure). T-tests showed no significant differences between patients with CKD as a single group and controls ($p > 0.05$ for each measure). As a result, neither the VAS data nor the ABC scores were included as covariates in the imaging analyses.

Table 4

Descriptive statistics for age, VAS score, and Adaptive Behavior Composite.

Group	Number	Mean age	Mean VAS score	Mean ABC
Control	11	14.5 (3.4)	8.0 (1.8)	107 (14)
Patient	21	14.4 (3.0)	8.0 (1.6)	97 (16)
Moderate CKD	10	13.2 (3.1)	7.9 (1.5)	102 (16)
Severe CKD	11	15.5 (2.6)	8.1 (1.7)	93 (16)

A moderate effect size was found for the difference in Vineland ABC scores between patients with CKD and controls ($d = 0.66$; Cohen, 1988). Effect sizes were very small for age ($d = 0.016$) and VAS score ($d = 0.0089$).

Figures 1, 2, and 3 describe the three groups in terms of the gender and race of the participants and the highest education levels attained by participants' mothers. Maternal education level was used to represent socioeconomic status. The three groups were similar in terms of gender balance but differed in racial distribution and maternal education levels. Both patient groups were primarily composed of white and African-American participants. Among patients with severe CKD, most mothers had finished high school, earned a GED, completed some college, or completed vocational school. Most mothers of patients with moderate CKD had attended some college or complete vocational school or college. The control group, having been gathered largely from acquaintances of the researchers and study coordinators, was predominantly white and had a higher average maternal education level.

Figure 1

Racial distribution of the participant groups.

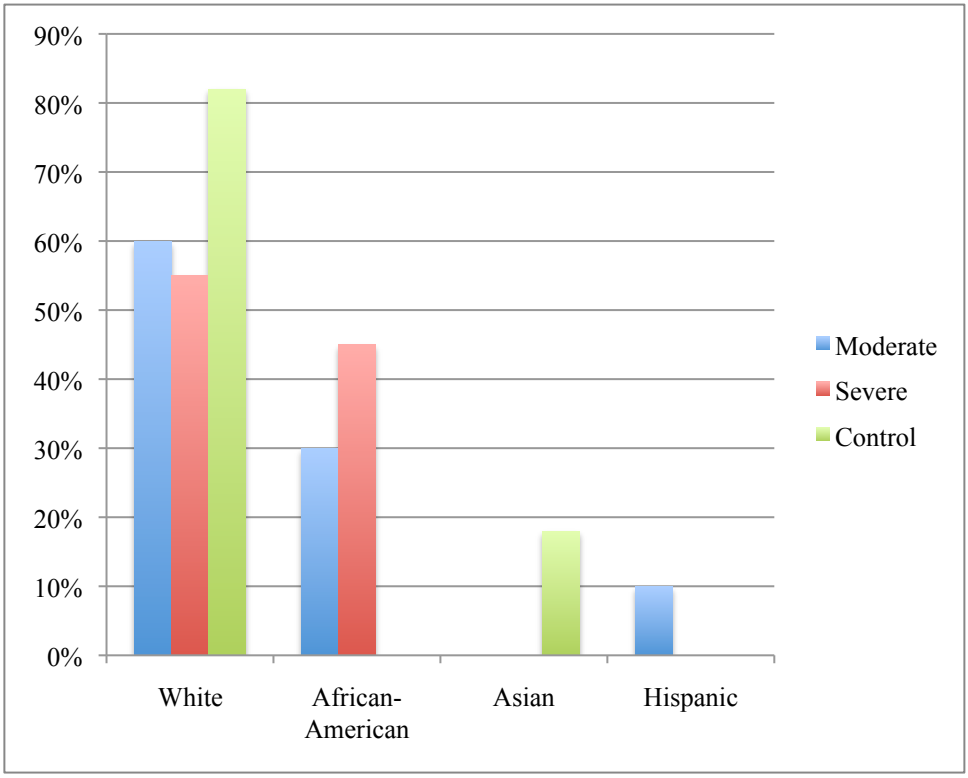


Figure 2

Gender distribution of participants.

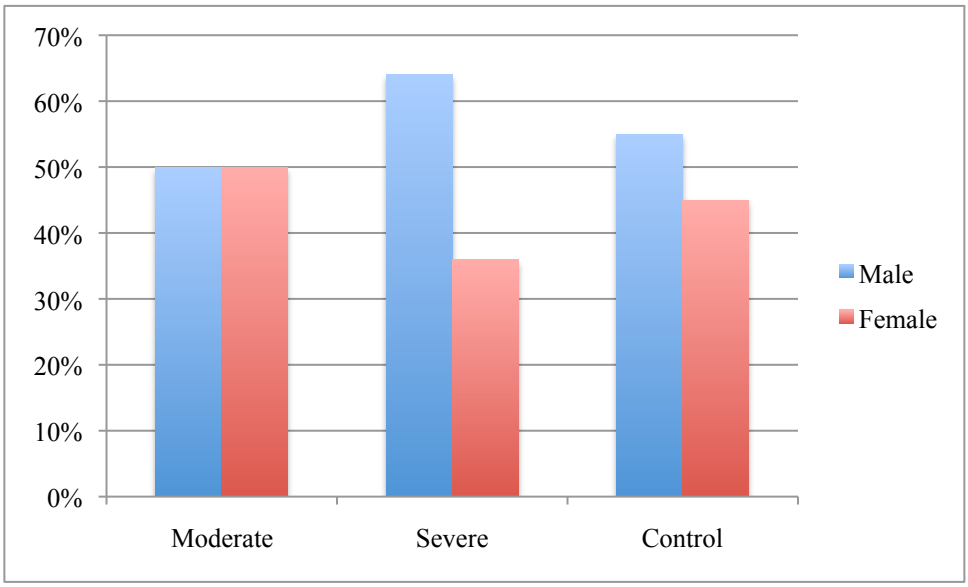
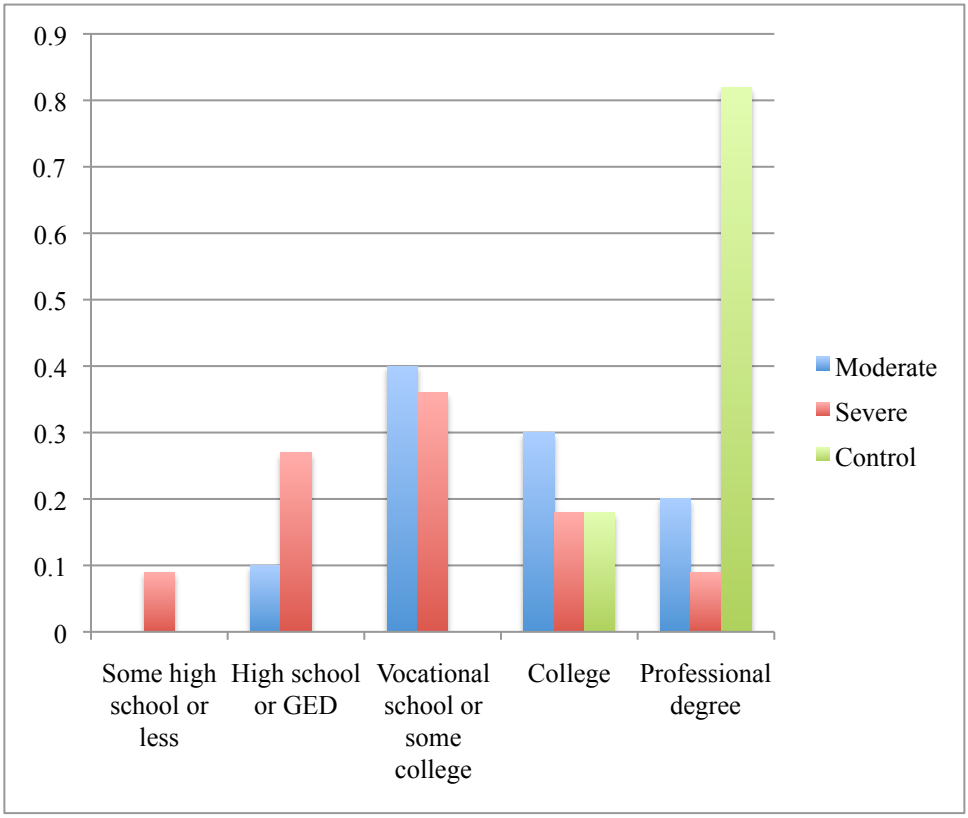


Figure 3

Maternal education levels.



Behavioral data

Most participants completed 4 sets of the visual-spatial memory task, resulting in 40 answers. Data from one patient were excluded due to a recording malfunction. Two hypotheses pertained to the behavioral data.

Hypothesis 4: Accuracy will be higher in controls as compared to patients with CKD.

Contrary to Hypothesis 4, no significant differences in accuracy were found either for all CKD patients compared with controls ($p = 0.17$) or among the three groups ($p = 0.19$). Table 5 shows the mean number of correct answers in each group, out of 10, per run. Standard deviation for each group is in parentheses. Accuracy was highest on the

first run and lowest in the last ($p < 0.01$ for accuracy on run 1 vs. run 4), suggesting that participants might have experienced fatigue. However, the number of questions participants skipped was similar for all four runs ($p = 0.85$). Participants answered just as many questions later in the session, but got more wrong in later data runs.

Table 5

Accuracy on the visual-spatial working memory test.

Group	Mean number of correct answers, out of 10, per run
Control	6.6 (2.5)
Patient	6.0 (2.1)
Moderate CKD	5.7 (2.0)
Severe CKD	6.3 (2.1)

Hypothesis 5: Reaction time will be lower—that is, performance will be faster—in controls as compared to patients with CKD.

Hypothesis 5 was supported. Mean reaction time was significantly faster for the control group compared with all patients (1725 ms vs. 2424 ms, $p = 0.003$). Table 6 shows the mean reaction time for each group. Standard deviations are in parentheses. Control participants also responded more rapidly than either patient group, and the difference between controls and patients with severe CKD was more marked ($p = 0.001$) than the difference between controls and patients with moderate CKD ($p = 0.007$). That is, the controls pressed the answer button more rapidly than the CKD patients, including the patients with moderate CKD. The two patient groups did not differ significantly from each other in terms of reaction time.

Table 6

Reaction times.

Group	Mean reaction time in milliseconds
Control	1725 (558)
Patient	2424 (1034)
Moderate CKD	2378 (560)
Severe CKD	2457 (1270)

Imaging data

Analyses of imaging data were carried out using two different programs, FSL 4.1 (FMRIB, Oxford, UK) and eventstats, eventstats, custom software developed by the Duke-UNC Brain Imaging and Analysis Center (Duke-UNC BIAC, Durham, NC). Imaging data from the two patients with moderate CKD whose scans resulted in only one set of data were analyzed using eventstats but not FSL due to constraints in the FSL program. Consequently, the FSL analysis includes slightly less data than the eventstats analysis. The two methods also lent themselves to different measures of differences among the groups. The eventstats analysis produced values for the percent MR signal change at each time point (every 1.5 seconds) over the course of the working memory task, so eventstats was used to look at average percent signal change over the entire task and at peak signal change for each individual in the encode and retrieve phases of the task. In FSL, the imaging data were analyzed using separate waveforms to represent the encode and retrieve phases for the task. FSL was used to consider the average percent signal change in the encode and retrieve phases, considering the brain regions of interest both bilaterally and in the left and right hemispheres.

Both groups showed activation in brain areas associated with visuospatial working memory: the prefrontal cortex, premotor cortex, parietal regions, and occipital regions. Figures 4 and 5 illustrate significant activation, defined as BOLD signal change of $z \geq 2.3$ ($p = 0.02$), during the time periods representing the hemodynamic response to the encoding and retrieval of object locations during the working memory task.

Figure 4

Horizontal sections showing mean activation above z = 2.3 for control participants (above) and CKD patients (below) during the encode phase.

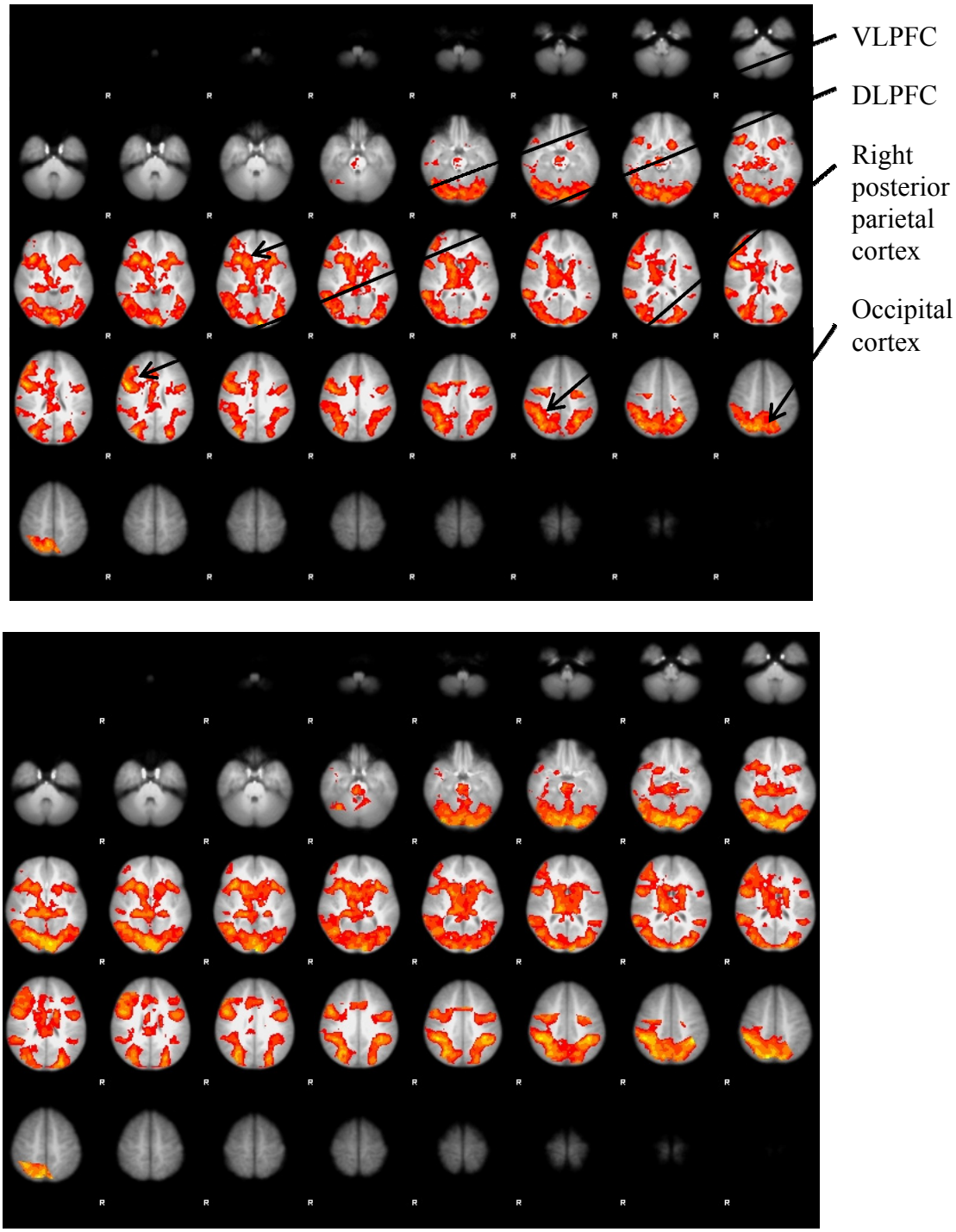
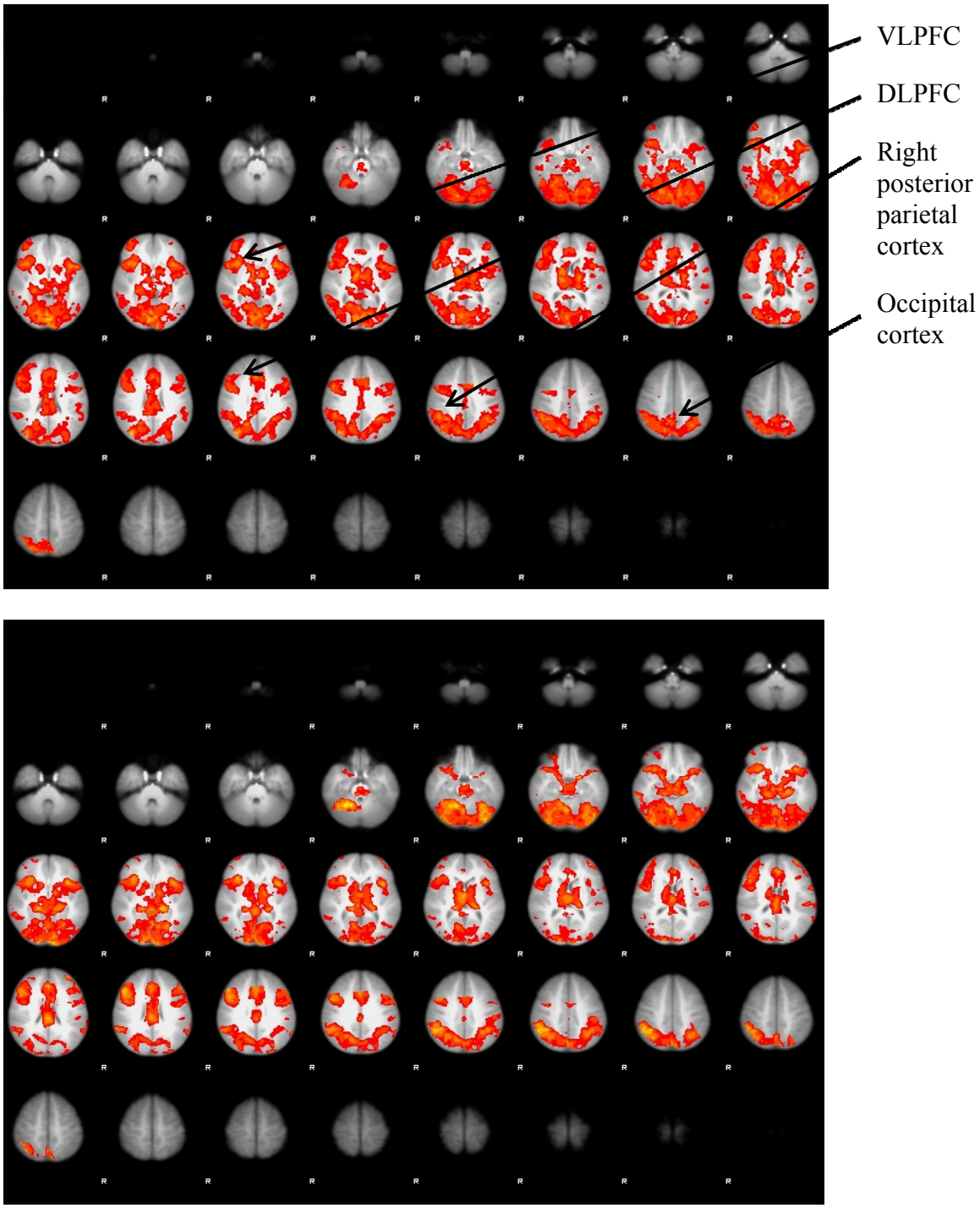


Figure 5

Horizontal sections showing mean activation above z = 2.3 for control participants (above) and CKD patients (below) during the retrieve phase.



Analyses were conducted on functionally activated brain regions. Data provided by patients with CKD as a group were compared with data from control participants using t-tests. Data from patients with severe CKD, patients with moderate CKD, and control participants were compared using ANOVAs. Results of the two-group comparisons are shown in Table 7, while results of the three-group comparisons are shown in Table 10.

Two-group comparisons

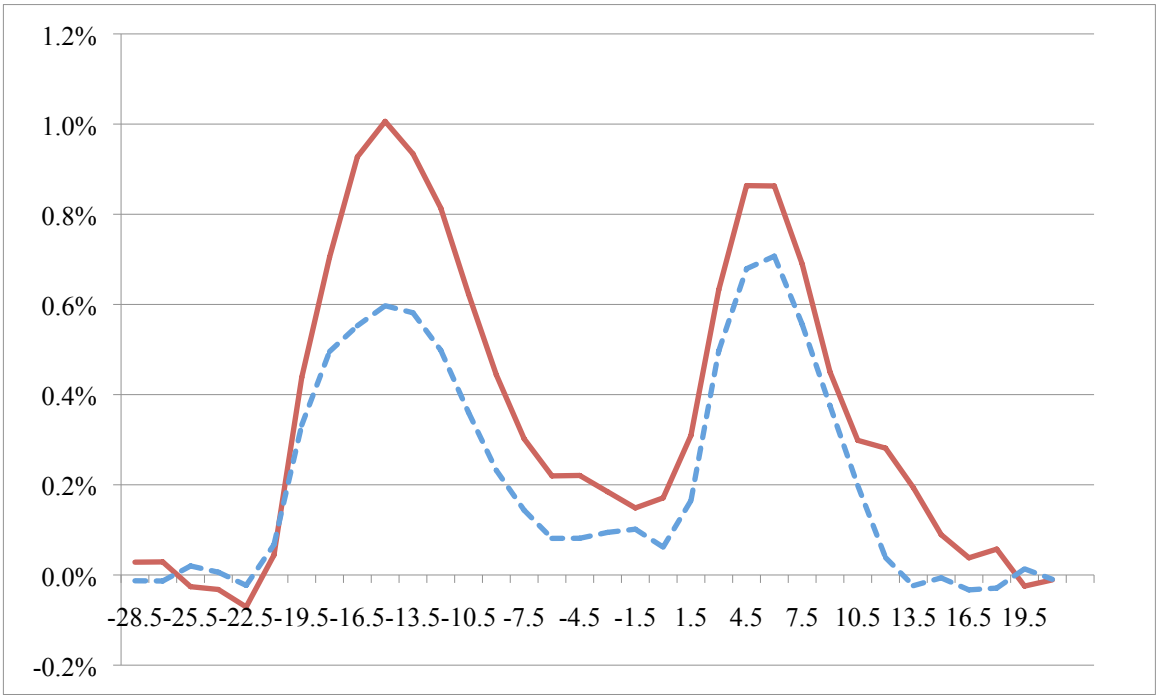
The fMRI results partially supported the hypotheses regarding brain activity. Each hypothesis will be considered individually.

Hypothesis 1: Percent BOLD signal change over the encoding period will be significantly lower in VLPFC, DLPFC, and right posterior parietal cortex in patients with CKD as compared to controls.

When the encode and retrieve phases were considered separately in FSL, a significant difference between control participants and patients with CKD was found in the encode phase in the superior parietal lobule, bilaterally. As illustrated in Figure 6 using the eventstats data, control participants displayed higher average percent signal change in the superior parietal lobule ($p < 0.001$). This finding partially supports Hypothesis 1.

Figure 6

Time course of mean percent signal change in the superior parietal lobule.



When the portions of the superior parietal lobule in the left and right hemispheres were considered separately, trends were evident toward lower mean percent signal change in the patients with CKD, but the difference was not interpreted as significant based on post-hoc tests ($p = 0.004$ for the left hemisphere, $p = 0.007$ for the right). Mean percent signal change in the region was higher in the encoding phase than in the retrieval phase for both groups (0.801 for control participants and 0.494 for patients, vs. 0.442 for controls and 0.254 for patients).

Trends toward differences between the control participants and patients with CKD were found in other regions. Patients with CKD showed lower mean percent signal change during the encode phase in Brodmann Area 7 (BA7; $p = 0.005$), which is part of the posterior parietal cortex adjacent to the superior parietal lobule, suggesting that the

difference in activation between groups extended from the superior parietal lobule to additional parietal regions. Patients with CKD had lower peak percent signal change than control participants during the encode phase in the precentral gyrus ($p = 0.0315$) and superior occipital gyrus ($p = 0.0343$) in addition to the superior parietal lobule and BA7 ($p = 0.0470$ and $p = 0.0314$, respectively).

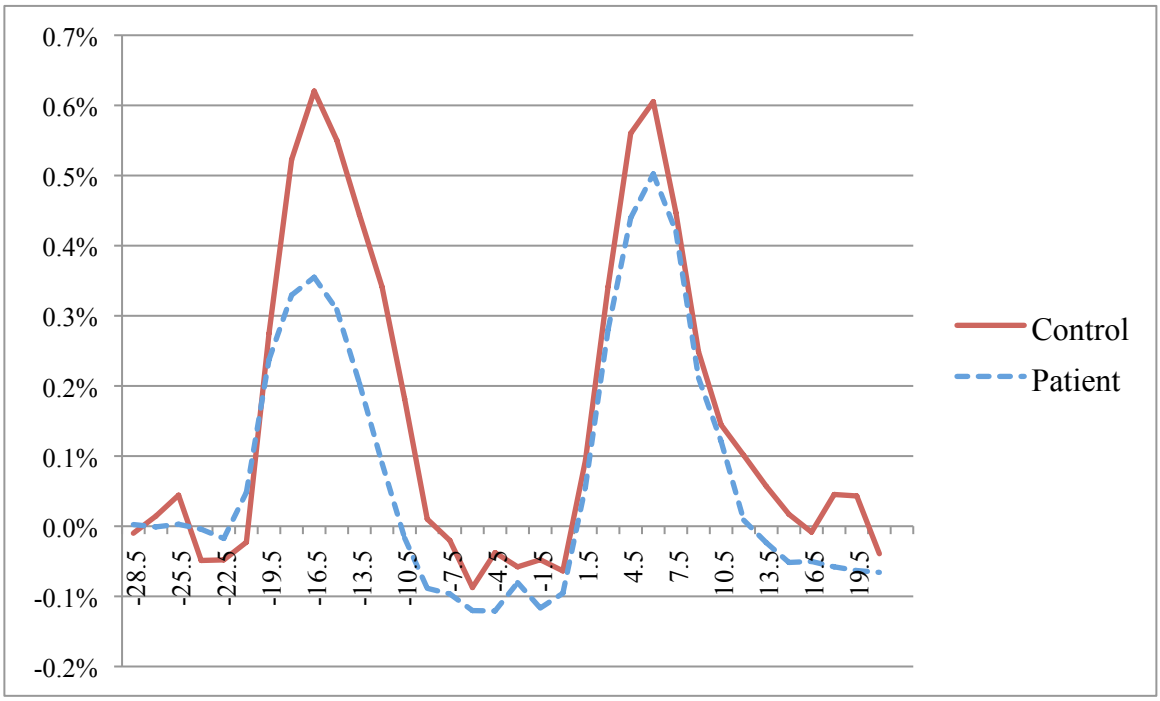
The superior parietal lobule includes part of the posterior parietal cortex and is bounded on one side by the IPS. Activity of parietal regions during visual-spatial working memory tasks is well established. PET and fMRI studies have found activation of parietal regions during storage and retrieval in visual and spatial working memory tasks (Courtney et al., 1996; Belger et al., 1998; Jonides et al., 1998; Kelley et al., 1998; Wager & Smith, 2003). In primates, location and spatial information are processed using a pathway from the visual cortex through the posterior parietal cortex (Kessels, et al., 2000). PET and fMRI studies have found increased regional cerebral blood flow to the superior parietal cortex when attention is shifted from one location to another (Corbetta, Shulman, Miezen, & Petersen, 1995; Corbetta & Shulman, 2002). The posterior parietal cortex is thought to maintain mental representations of information to be remembered (Crone, et al., 2006; Marshuetz, et al., 2000; Smith & Jonides, 1998). Results of this study supported involvement of the superior parietal lobule in a visual-spatial working memory task.

The precentral gyrus is involved in voluntary muscle movements. The precentral sulcus, which forms the anterior boundary of the precentral gyrus, has been found to be activated by eye movements (Courtney et al., 1998) and during visual working memory tasks (Nystrom et al., 2000; Klingberg et al., 2002). The superior and middle occipital

gyri are part of the extrastriate cortex surrounding the primary visual, or striate, cortex. A dorsal pathway moving from the striate to extrastriate cortex and thence to the parietal lobe transmits information about the location and motion of visual stimuli (Gazzaniga et al., 2002). Both these areas are therefore expected to be activated during a visual-spatial working memory task. Figure 7 illustrates the mean percent signal change over time in the patient and control groups in the superior occipital gyrus. Mean percent signal change was higher among controls than patients in the encode phase ($p = 0.03$) and average percent signal change over the course of the task was higher for controls than for patients with CKD ($p < 0.001$).

Figure 7

Time course of mean percent signal change in the superior occipital gyrus.



Hypothesis 2: Percent BOLD signal change over the retrieval period will be significantly lower in VLPFC, DLPFC, and right posterior parietal cortex in patients with CKD as compared to controls.

Hypothesis 2 was not supported. In the retrieval phase, following post-hoc tests, no comparisons between patients and controls were deemed significant. However, as in the encode phase, patients with CKD displayed lower average percent signal change than control participants in the superior parietal lobule ($p = 0.009$) and in BA7 ($p = 0.004$). Patients with CKD also had lower average percent signal change during the retrieval period in the following regions.

- lingual gyrus ($p = 0.03$)
- posterior cingulate ($p = 0.02$)
- precuneus ($p < 0.05$)

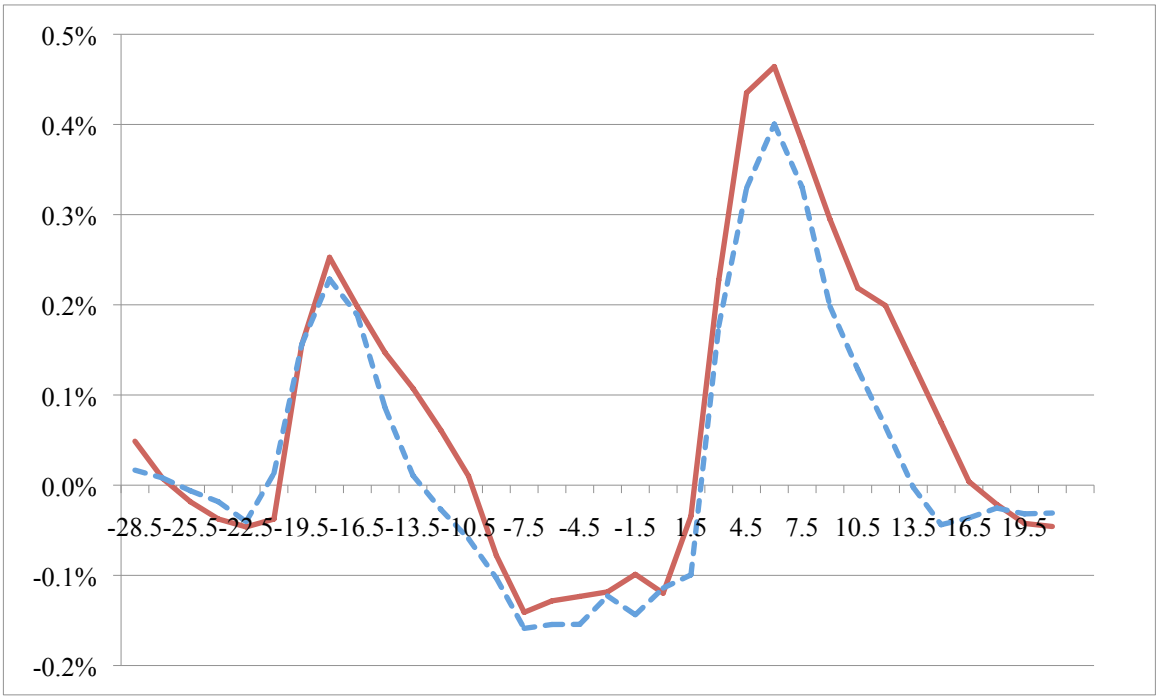
All of these regions are thought to play roles in visual-spatial or working memory tasks. The lingual gyrus is a region active in visual processing, particularly in processing faces (Gazzaniga et al., 2002). It has been noted to be recruited during the delay period of a spatial working memory task (Corbetta et al., 2002). Cingulate cortex has been found to be activated as a main effect of working memory in children and adolescents (Klingberg et al., 2002), and the posterior cingulate is thought to be part of the attentional control network (Gazzaniga et al., 2002). The precuneus connects with parietal areas and is involved in visual-spatial processing and attention (Selemon & Goldman-Rakic, 1988; Cavada & Goldman-Rakic, 1989; Leichnetz, 2001). It is active during spatial (Nystrom et al., 2000; Thomas 1999) and object (Ciesielski et al., 2006) working memory tasks and is thought be part of a system activated more strongly by invalid than valid targets in a spatial task (Corbetta 2002).

Figure 8 illustrates the mean percent signal change over time in the patient and control groups in the posterior cingulate. Mean percent signal change was higher among

controls than patients in the retrieve phase ($p = 0.02$) and average percent signal change over the course of the task was higher for controls than for patients with CKD ($p < 0.001$). As the graph shows, time of peak signal change for the encode and retrieve phases was similar in both groups.

Figure 8

Time course of mean percent signal change in the posterior cingulate.



When right and left sides of the regions were considered separately, patients with CKD showed a trend toward lower average percent signal change than controls in the right lingual gyrus ($p = 0.02$), right medial frontal gyrus ($p = 0.04$), and left BA7 ($p = 0.02$). No differences between patients and controls were found in terms of peak signal change. In no regions did patients demonstrate higher signal change than controls.

Hypothesis 3: Percent BOLD signal change over the course of the task will be significantly lower in the VLPFC, DLPFC, and right posterior parietal cortex in patients

with CKD as compared to controls.

This time period includes not only the encoding and retrieval periods but also the maintenance period, over which information encoded must be kept until a response is requested. The data were analyzed using eventstats rather than FSL because eventstats tracks percent signal change over the entire time course, whereas FSL models the two separate hemodynamic responses created by the encoding and retrieval efforts.

This analysis revealed many significant differences between patients and healthy controls. Patients with CKD demonstrated significantly lower average percent signal change compared with controls over the entire course of the task in numerous regions ($p < 0.001$). These regions are listed below.

Patient < control

- superior occipital gyrus
- middle occipital gyrus
- lingual gyrus
- posterior cingulate
- angular gyrus
- superior parietal lobule
- BA7

A trend ($p = 0.009$) in the same direction was apparent in the precuneus. Based on post-hoc tests to correct for multiple comparisons, this result was not significant. In three regions, the difference was in the other direction: controls had lower average percent signal change over the course of the task than patients with CKD in the insula ($p <$

0.001), medial frontal gyrus ($p = 0.002$) and superior temporal gyrus ($p = 0.006$). This may represent more efficient allocation of resources by healthy controls. The insula, a paralimbic region involved in emotional experiences, has been found to be active during the maintenance period in working memory tasks (Corbetta et al., 2002) but has been reported to be more activated by verbal tasks than visual-spatial tasks (Nystrom et al. 2000). The temporal gyrus is involved in processing time-related and object-recognition information (Smith & Jonides, 2007; Talati & Hirsch, 2005; Gazzaniga et al., 2002; Nystrom et al., 2000) but is not typically preferentially recruited to process locational information, so temporal activation during a spatial memory task may be inefficient. The medial frontal gyrus is active during complex tasks, including decision-making (Talati & Hirsch, 2005). Less activity in this region might indicate that control participants required less extensive recruitment of executive function regions to complete the task.

The angular gyrus, which was activated at a higher level in controls than in patients, is part of the posterior parietal cortex and has been found to be activated in visual-spatial working memory tasks (Kwon et al., 2001). These results support the idea that the patients with CKD showed less activation in the extrastriate cortex and posterior parietal cortex, suggesting that the dorsal stream involved in object and location processing (Gazzaniga et al., 2002) might be disrupted.

Table 7 describes results for the two-group tests. Statistical tables are further detailed in the Appendix.

Table 7

Results of t-tests comparing control participants with patients with CKD.

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over entire task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases, considering left and right sides of the region separately
angular gyrus	Patient significantly lower than control ($p < 0.001$).	NS	NS	NS
inferior occipital gyrus	NS	NS	NS	NS
precentral gyrus	NS	Control higher than patient in the encode phase ($p = 0.0315$).	NS	NS
anterior cingulate	NS	NS	NS	NS
inferior temporal gyrus	NS	NS	NS	NS

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over entire task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases, considering left and right sides of the region separately
precuneus	Patient significantly lower than control ($p = 0.009$).	NS	Control participants showed significantly greater average percent signal change than CKD patients in the retrieve phase ($p < 0.05$).	NS
lingual gyrus	Patient significantly lower than control ($p < 0.001$).	NS	Control participants showed significantly greater average percent signal change than CKD patients in the retrieve phase ($p = 0.03$).	In the right lingual gyrus, control participants showed significantly greater average percent signal change than CKD patients in the retrieve phase ($p = 0.02$).
superior frontal gyrus	NS	NS	NS	NS
superior occipital gyrus	Patient significantly lower than control ($p < 0.001$).	Control higher than patient in the encode phase ($p = 0.0343$).	NS	NS

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over entire task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases, considering left and right sides of the region separately
medial frontal gyrus	Control significantly lower than patient ($p = 0.002$).	NS	NS	In the retrieve phase, in the right hemisphere, control significantly higher than CKD patients in terms of average percent signal change ($p = 0.04$).
fusiform gyrus	NS	NS	NS	NS
middle frontal gyrus	NS	NS	NS	NS
superior temporal gyrus	Control significantly lower than patient ($p = 0.006$).	NS	NS	NS
inferior frontal gyrus	NS	NS	NS	NS
middle occipital gyrus	Patient significantly lower than control ($p < 0.001$).	NS	NS	NS

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over entire task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases, considering left and right sides of the region separately
superior parietal	Patient significantly lower than control ($p < 0.001$).	Control higher than patient in the encode phase ($p = 0.0470$).	Control participants showed significantly greater average percent signal change than CKD patients in the encode phase ($p = 0.0001$) and in the retrieve phase ($p = 0.009$).	In the encode phase, in the left and right hemispheres considered separately, control participants showed significantly greater average percent signal change than CKD patients ($p = 0.004$, $p = 0.007$).
insula	Patient significantly lower than control ($p < 0.001$).	NS	NS	NS
posterior cingulate	Patient significantly lower than control ($p < 0.001$).	NS	In the retrieve phase, control participants showed significantly higher average percent signal change than CKD patients ($p = 0.02$).	NS

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over entire task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases, considering left and right sides of the region separately
BA7	Patient significantly lower than control ($p < 0.001$).	Control higher than patient in the encode phase ($p = 0.0314$).	Control participants showed significantly higher average percent signal change than CKD patients in both encode and retrieve phases ($p = 0.005$, $p = 0.004$).	In the left hemisphere, in both encode and retrieve phases, average percent signal change was significantly higher in controls than in CKD patients ($p = 0.02$ for each). In the right hemisphere, in the encode phase, average percent signal change was significantly higher in controls than in CKD patients ($p = 0.03$).
BA46+9	NS	NS	NS	NS

Cohen's *d* tests were conducted as a measure of effect size of mean percent BOLD signal change in selected regions of interest for which the difference between patients with CKD and healthy controls reached or approached statistical significance. Table 8 shows the results of effect size calculations.

Table 8

Results of effect size calculations comparing control participants with patients with CKD.

Measure comparing patients with CKD vs. controls	Cohen's *d*	Interpretation of effect size
Superior parietal lobule, mean percent signal change in the encoding period	1.099	large
BA7, mean percent signal change in the encoding period	0.808	large
Precuneus mean percent signal change over course of task	0.123	very small
Posterior cingulate mean percent signal change over course of task	0.164	very small
Medial frontal gyrus, mean percent signal change over course of task	-0.099	very small (patients > controls)

Based on Cohen's classification of effect size results (1988), the differences between patients with CKD and controls in terms of mean percent signal change in posterior parietal cortex (that is, in both the superior parietal lobule and BA7) in the encoding period were large differences. Effect size differences in activation over the course of the task were also considered in three regions of the brain thought to be associated with attention or visual-spatial processing: the precuneus, posterior cingulate, and medial frontal gyrus. In none of these regions was the effect size as high as $d = 0.2$, which Cohen defined as "small." In short, the effect size calculations underscored the significance results, showing the clearest difference between groups to be in mean percent signal change in posterior parietal regions during encoding.

Figures 9 and 10 illustrate brain areas where activation was greater in the control group. Figures 11 and 12 show areas where activation was greater in patients with CKD.

Figure 9

Controls > patients with CKD, encode phase, z = 2.0 (p = 0.05).

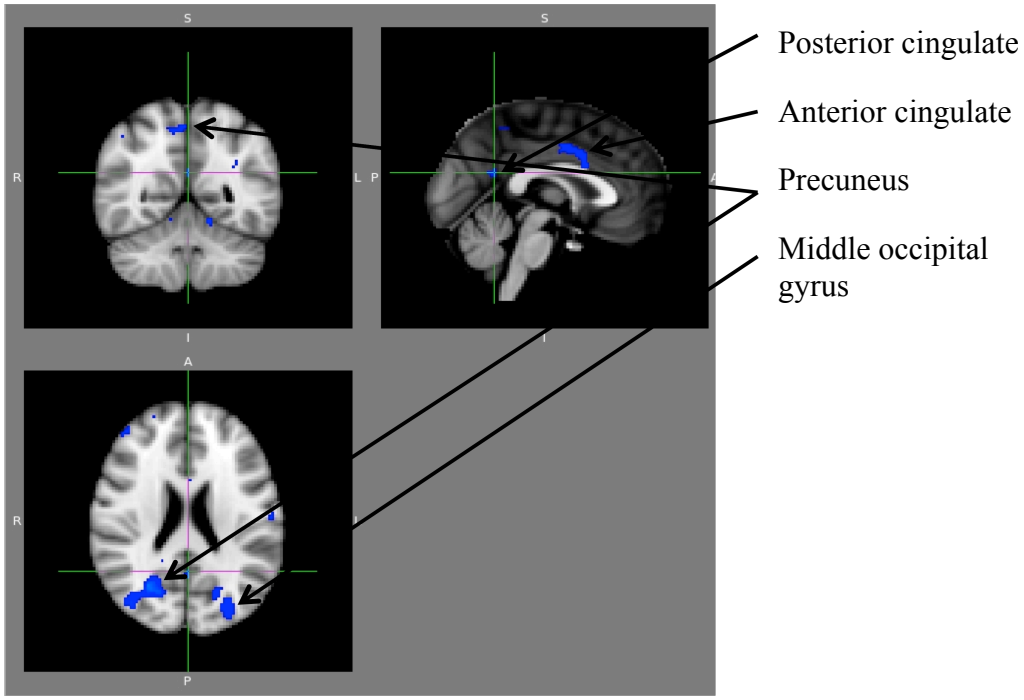


Figure 10

Controls > patients with CKD, retrieve phase, z = 2.0.

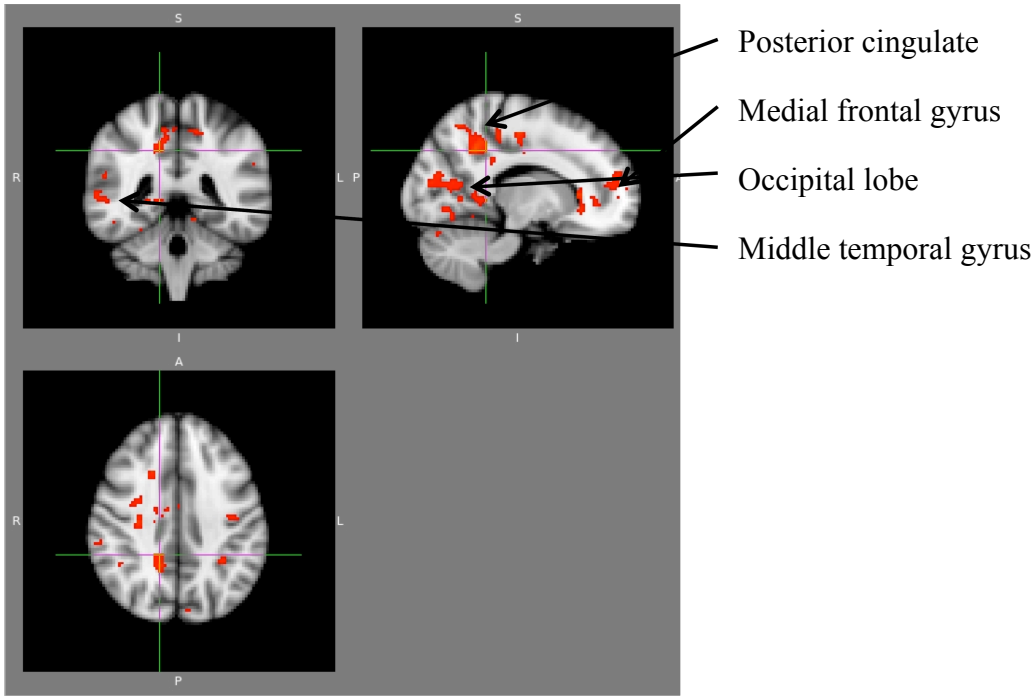


Figure 11

Patients > controls, encode phase, z = 2.0.

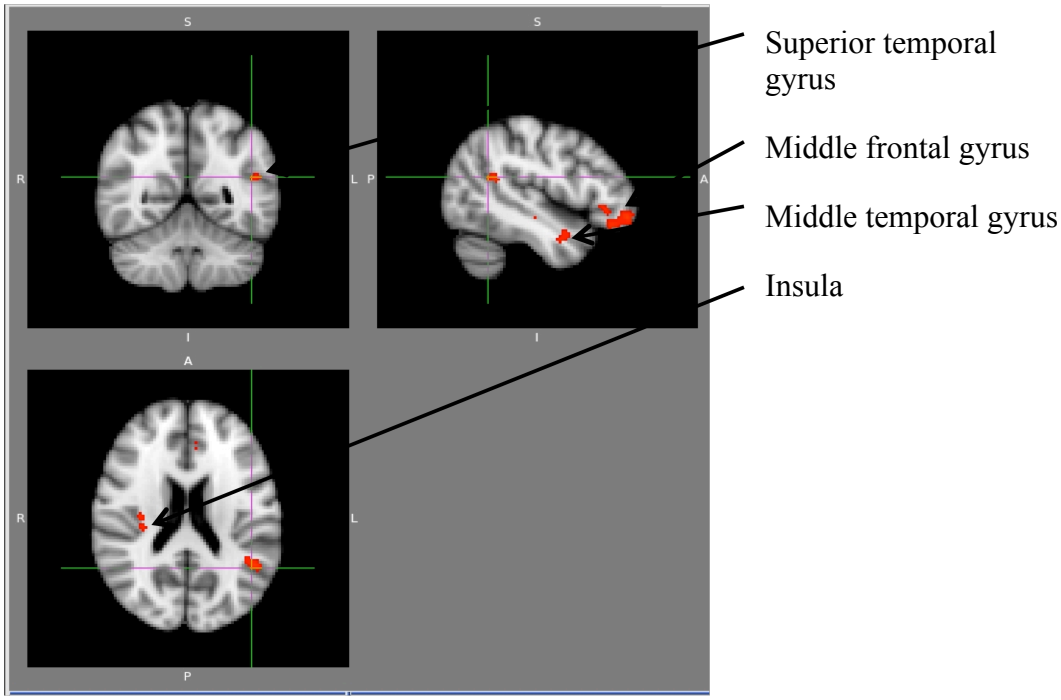
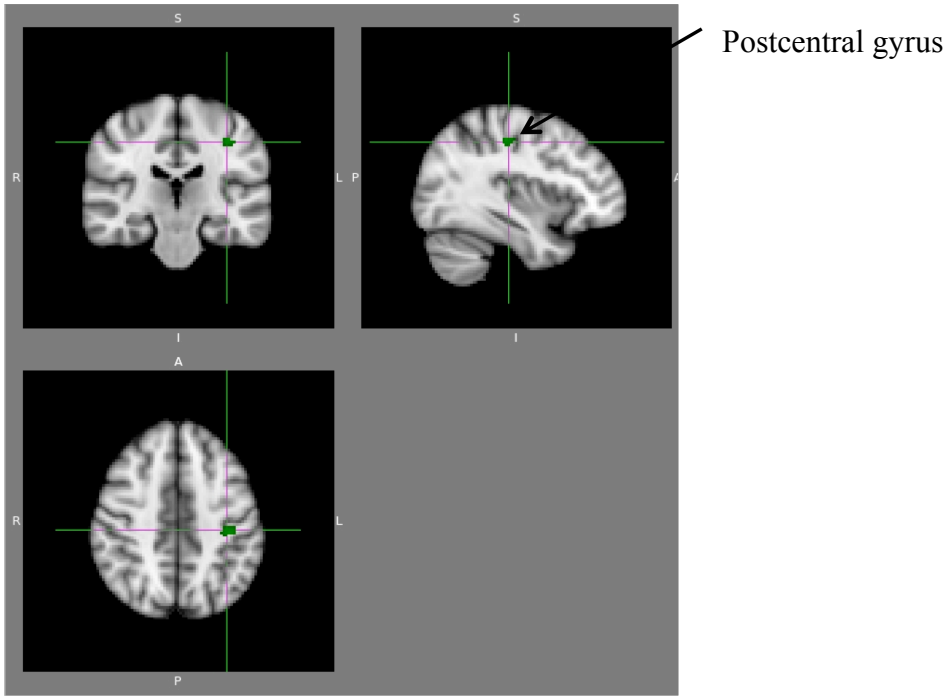


Figure 12

Patients > controls, retrieve phase, z = 2.0.



Three-group comparisons

Hypothesis 1b: Percent signal change in these brain regions during the encoding period will be significantly lower in each group of patients with CKD as compared to healthy participants.

Hypothesis 1b was partially supported. As in the two-group comparisons, patients displayed lower activation of the posterior parietal cortex. In the superior parietal lobule, patients with severe CKD had significantly lower average percent signal change and peak signal change compared with controls ($p = 0.001$ for each). Patients with moderate CKD also showed lower signal change than control participants in this region ($p = 0.007$ for average percent signal change, $p = 0.02$ for peak signal change). In the adjacent region BA7, peak signal change was significantly lower among both patients with severe CKD and patients with moderate CKD compared with control participants ($p < 0.001$ for each).

Patients with severe CKD had lower peak signal change in the encode phase compared with control participants in the following regions. These differences did not reach significance based on the adjusted critical p-values used to correct for multiple comparisons.

- superior frontal gyrus ($p = 0.03$)
- middle frontal gyrus ($p = 0.02$)
- insula ($p = 0.02$)
- superior occipital gyrus ($p = 0.02$)
- angular gyrus ($p < 0.01$)
- precentral gyrus ($p < 0.01$)

In the angular gyrus and superior frontal gyrus, participants with severe CKD had

lower peak signal change not only compared with controls but also compared with patients with moderate CKD ($p = 0.03$ and $p = 0.02$, respectively).

The middle and superior frontal gyri are part of the dorsolateral prefrontal cortex (DLPFC). However, no significant differences were observed in BA9+46, which encompass a larger area and more clearly define the DLPFC. Results of the current study therefore showed a nonsignificant trend toward patients with severe CKD having lower activation than controls in parts of DLPFC as well as in posterior parietal cortex. Maintaining information in working memory has been associated with increased blood flow in DLPFC (Courtney et al., 1997; D'Esposito et al., 1999; Owen et al., 1996). Different theories have associated more dorsal PFC activation with spatial working memory tasks (Courtney et al., 1996; Courtney et al., 1998; Köhler et al., 1998) or with more complex tasks (D'Esposito et al., 1999; Owen et al., 1996).

Few differences were noted between patients with moderate CKD and controls. Moderate patients did show lower peak signal change during the encode phase in the superior occipital gyrus ($p = 0.001$), but in no other regions were differences apparent in peak or mean signal change between controls and patients with moderate CKD.

When the left and right sides of each brain region were considered separately, trends toward differences among groups were observed, but none reached significance following post-hoc tests. On the right side of the following regions, patients with severe CKD showed lower average percent signal change than control participants.

Severe < control

- superior parietal lobule ($p = 0.02$)
- BA7 ($p = 0.02$)

There is therefore some suggestion that the differences observed in posterior parietal cortex, with patients with severe CKD showing significantly less activation than controls, are more concentrated in the right hemisphere. Spatial working memory tasks have been found to preferentially activate right hemispheric regions (Belger et al., 1998; Smith & Jonides, 1999; Thomas et al., 1999; Smith et al., 1996).

In the right hemisphere portion of the following regions, patients with severe CKD showed lower average percent signal change than patients with moderate CKD.

Severe < moderate

- angular gyrus ($p < 0.01$)
- inferior occipital gyrus ($p < 0.01$)
- lingual gyrus ($p = 0.02$)
- superior frontal gyrus ($p = 0.009$)
- medial frontal gyrus ($p = 0.009$)

These regions encompass parts of the DLPFC (superior and medial frontal gyri) and posterior parietal cortex (angular gyrus), and areas involved in visual processing (inferior occipital gyrus, lingual gyrus).

On the left side of the following regions, patients with severe CKD showed lower average percent signal change than control participants.

Severe < control

- middle occipital gyrus ($p < 0.05$)
- superior parietal lobule ($p = 0.04$)
- BA7 ($p = 0.003$)

In the left hemisphere portion of the following regions, patients with severe CKD

showed lower average percent signal change than patients with moderate CKD.

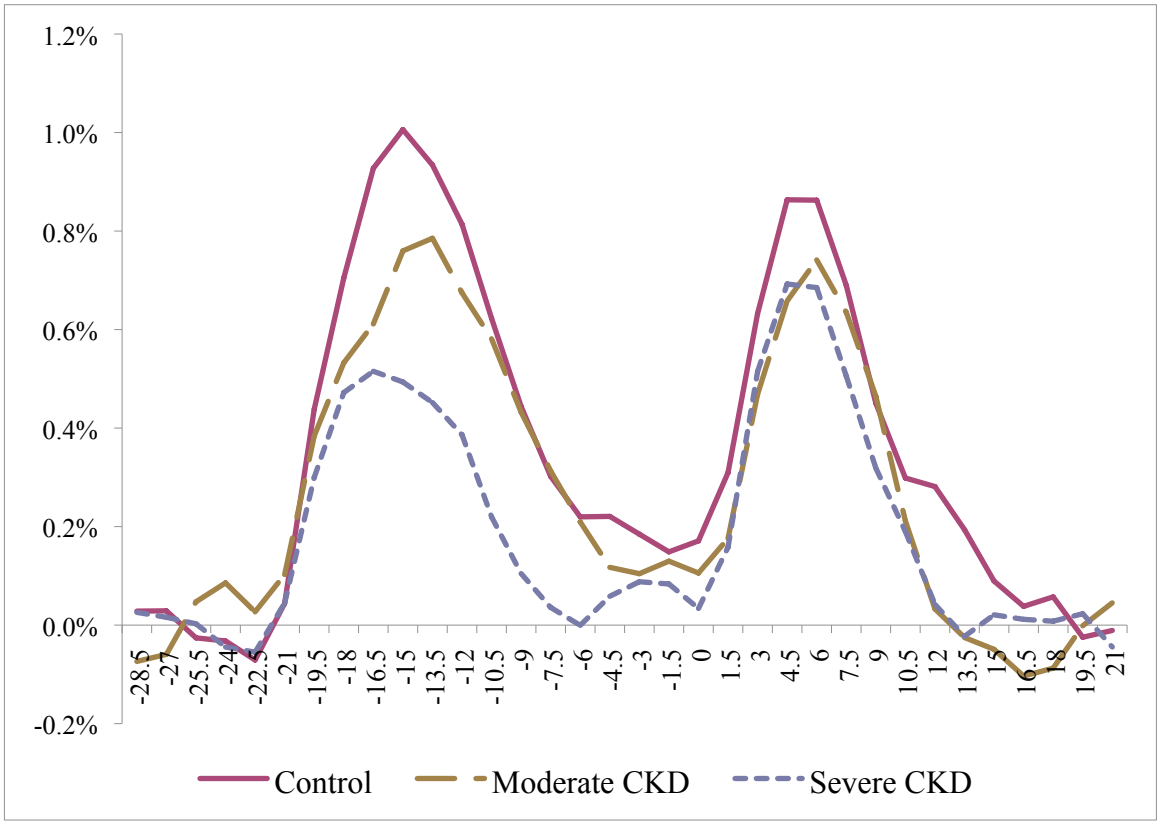
Severe < moderate

- lingual gyrus ($p < 0.01$)
- superior frontal gyrus ($p < 0.01$)
- medial frontal gyrus ($p = 0.02$)
- BA7 ($p = 0.048$)

Although the left hemisphere is expected to be less active than the right during visual-spatial tasks, and the differences in this hemisphere may therefore be less relevant to the task at hand, these results fit into a general pattern of lower activation in the severe CKD group and higher activation in the moderate CKD group, with trends toward higher activation in the moderate CKD group than in healthy controls. Patients with moderate CKD showed lower average percent signal change than controls during the encode phase in the left hemisphere portion of only the superior parietal lobule ($p = 0.04$). The time course is illustrated in Figure 13. In the encode phase, significant differences were found among all three groups.

Figure 13

Time course of mean percent signal change in the superior parietal lobule.



Hypothesis 2b: Percent signal change in VLPFC, DLPFC, and right posterior parietal cortex during the retrieval period will be significantly lower in each group of patients with CKD as compared to healthy participants.

No differences found in the retrieval period among the three participant groups reached significance based on post-hoc tests. However, numerous differences were observed at a level of $p < 0.05$.

Notably, patients with moderate CKD showed lower activation of left hemispheric frontal regions during the retrieval period. Patients with moderate CKD had lower average percent signal change compared with healthy controls in the left middle and medial frontal gyri ($p = 0.04$ for each) and the left posterior cingulate ($p = 0.02$), and

they had lower average percent signal change than patients with severe CKD in the left superior ($p < 0.01$), middle ($p < 0.01$), and inferior ($p = 0.03$) frontal gyri. This reduced blood flow to left frontal regions could suggest preferential allocation of available resources (i.e., healthy oxygenated blood) to right hemispheric regions during retrieval of visual-spatial information, possibly representing efficient distribution of limited resources in patients with moderate CKD. Patients with moderate CKD also had lower average percent signal change than patients with severe CKD in the right posterior cingulate ($p = 0.03$), signifying a nonsignificant trend toward lower activation in part of the attentional control network.

When bilateral regions were considered, patients with moderate CKD, compared with healthy controls, had lower peak signal change in the superior occipital gyrus ($p = 0.01$) and lower average percent signal change in BA7 ($p = 0.02$) and superior parietal lobule ($p = 0.02$). Along with results in the encoding phase, this suggests reduced activation of posterior parietal cortex throughout the task. They also had lower average percent signal change than patients with severe CKD in BA46+9 ($p = 0.03$), which comprise the DLPFC. These results contrast with the high activation shown by patients with moderate CKD in the encoding period.

Controls had lower peak signal change compared with patients with severe CKD in the medial frontal gyrus ($p = 0.02$), an unusual result. Patients with severe CKD had lower average percent signal change compared with controls in the lingual gyrus ($p = 0.03$), lower peak signal change compared with controls in the superior parietal lobule ($p = 0.04$), and lower peak signal change compared with patients with moderate CKD in the precentral gyrus ($p = 0.04$). These results faintly echo some results of the encoding

period, suggesting that patients with severe CKD often had lower activation than either controls or patients with moderate CKD.

Hypothesis 3b: Percent signal change in the VLPFC, DLPFC, and right posterior parietal cortex during the retrieval period will be significantly lower in each group of patients with CKD as compared to healthy participants.

Two main patterns emerged from this analysis. Over the entire course of the task, average percent signal change was significantly lower ($p < 0.001$) in patients with severe CKD than in control participants in the following regions of interest.

Severe < control

- superior frontal gyrus
- inferior temporal gyrus
- precuneus
- lingual gyrus
- superior occipital gyrus
- middle occipital gyrus
- angular gyrus
- superior parietal lobule
- BA7
- posterior cingulate

These include areas associated with maintenance of information in working memory (DLPFC), maintenance of spatial information in particular (posterior parietal

cortex), and attention (posterior cingulate, precuneus), and areas associated with visual processing and object recognition (occipital regions and inferior temporal gyrus).

Nonsignificant trends in the same direction were observed in the following regions.

Severe < control

- inferior occipital gyrus ($p = 0.02$)
- precentral gyrus ($p < 0.01$)
- fusiform gyrus ($p < 0.01$)
- middle frontal gyrus ($p = 0.03$)

Significant differences were apparent between control participants and patients with moderate CKD, with control participants showing higher average percent signal change over the course of the task, in the following regions ($p < 0.001$). No regions showed nonsignificant trends in this direction.

Moderate < control

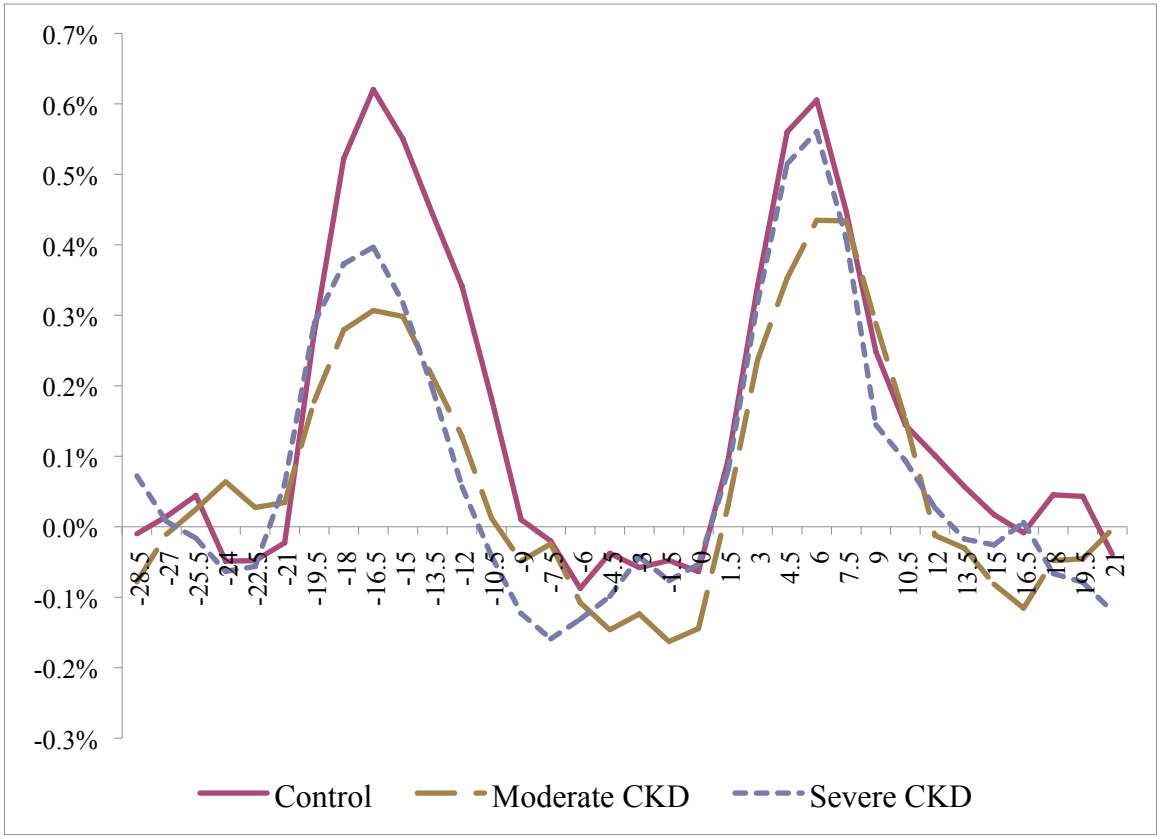
- superior parietal lobule
- superior occipital gyrus

That is, when broken out of the general patient population, patients with severe CKD (modified eGFR $\leq$ 30 ml/min/1.73m^2 or dialysis dependence) showed lower activation in a number of regions of interest compared with control participants. Comparing patients with moderate CKD to controls resulted in far fewer significant differences. These results partially support Hypothesis 3. Figure 14 illustrates average percent signal change in each group in the superior occipital gyrus over the course of the

task. Controls showed higher average percent change over the entire task than either patients with severe CKD or patients with moderate CKD ($p < 0.001$ for each comparison). In the encode phase, control participants had higher peak signal change than patients with moderate CKD ($p = 0.001$).

Figure 14

Time course of mean percent signal change in the superior occipital gyrus.



The second pattern was unexpected. In the following regions, average percent signal change was significantly higher ($p < 0.001$) over the entire course of the task in patients with moderate CKD not only compared with patients with severe CKD but also compared with control participants.

Moderate > severe, control

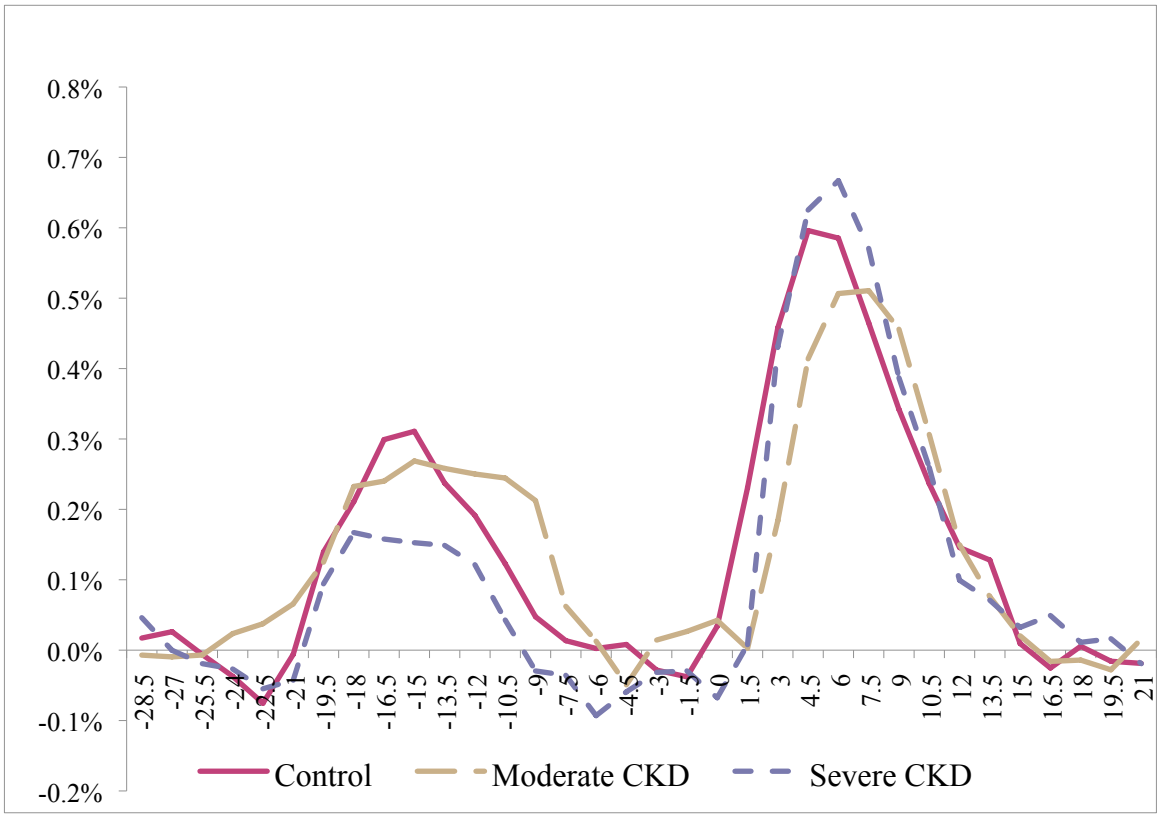
- anterior cingulate
- medial frontal gyrus
- superior temporal gyrus

The anterior cingulate is involved in executive functions including making decisions between conflicting responses (Gazzaniga et al., 2002). The medial frontal gyrus is active during decision-making and is thought be part of the attentional control network (Talati & Hirsch, 2005). The superior temporal gyrus has been found to be active during target detection and so might be expected to be activated during the retrieval period (Corbetta et al., 2002). This pattern of activation may indicate increased recruitment by patients with moderate CKD of brain regions needed for attention and decision-making while responding to the task.

Figure 15 shows the mean percent signal change over time for control participants, patients with moderate CKD, and patients with severe CKD in the medial frontal gyrus. Patients with moderate CKD had greater average percent signal change over the entire task ($p < 0.001$). This time course graph shows that they exhibited a more prolonged peak during the encode phase.

Figure 15

Time course of mean percent signal change in the medial frontal gyrus.



In addition, in the following regions, average percent signal change was significantly higher ($p < 0.001$) over the course of the task in patients with moderate CKD compared with patients with severe CKD (but not compared with control participants).

Moderate > severe

- inferior temporal gyrus
- fusiform gyrus
- inferior occipital gyrus

- middle occipital gyrus
- superior frontal gyrus
- superior parietal lobule
- angular gyrus

The fusiform gyrus is part of the medial/inferior temporal lobe and like the inferior temporal gyrus is involved in object recognition (Gazzaniga et al., 2002). Moderate patients therefore showed significantly greater activation over the course of the task in regions associated with object recognition, visual processing, attention, and maintaining information in working memory. A nonsignificant trend in the same direction was seen in the posterior cingulate, which is also thought to be involved in attentional control ($p = 0.02$).

In short, clear differences were observed between the two patient groups, and what was noticeable about the patients with moderate CKD was that in several regions, they showed higher average percent signal change than the control participants. This may suggest that patients with moderate CKD worked harder to generate accurate answers, recruiting additional resources to maintain attention and aid decision-making. When control participants were compared with all patients, some differences between patients with severe CKD and patients with moderate CKD canceled each other out. Table 9 shows the average percent signal change in each participant group for each analyzed brain region.

Table 9

Average percent signal change over entire task

Region	Control	Moderate CKD	Severe CKD
angular gyrus	0.001445	0.001234	0.000352**
inferior occipital gyrus	0.000557	0.000729	0.000197†
precentral gyrus	0.001165	0.001182	0.000859
anterior cingulate	0.000709	0.001091††	0.000528†
inferior temporal gyrus	0.000949	0.001195	0.000619**
precuneus	0.001661	0.0016	0.001132*
lingual gyrus	0.001244	0.00092	0.000731*
superior frontal gyrus	0.000988	0.001415	0.000458†
superior occipital gyrus	0.001535	0.00068	0.000809
medial frontal gyrus	0.000632	0.001144††	0.000684†
fusiform gyrus	0.000645	0.000853	0.000422†
middle frontal gyrus	0.001356	0.001361	0.001073
superior temporal gyrus	0.000704	0.001302††	0.000706†
inferior frontal gyrus	0.000963	0.001247	0.000852
middle occipital gyrus	0.000866	0.000819	0.000331†
superior parietal	0.003483	0.002658	0.001864**
insula	0.000984	0.001065	0.000966
posterior cingulate	0.000684	0.000494	0.00013*
BA7	0.002337	0.002056	0.001526**
BA46+9	0.001169	0.001233	0.001502

* $p < 0.001$ vs. control participants

† $p < 0.001$ vs. patients with moderate CKD

** $p < 0.001$ vs. both controls and patients with moderate CKD

†† $p < 0.001$ vs. both controls and patients with severe CKD

Figure 16 shows regions in which activation was higher for control participants than for patients with severe CKD, which reached significance prior to post-hoc tests. Figure 17 illustrates regions in which patients with moderate CKD showed a trend toward greater activation than patients with severe CKD. Table 10 details the results of the three-group comparisons conducted for each functionally activated region.

Figure 16

Controls > patients with severe CKD, retrieve phase, z = 2.3 (p = 0.02).

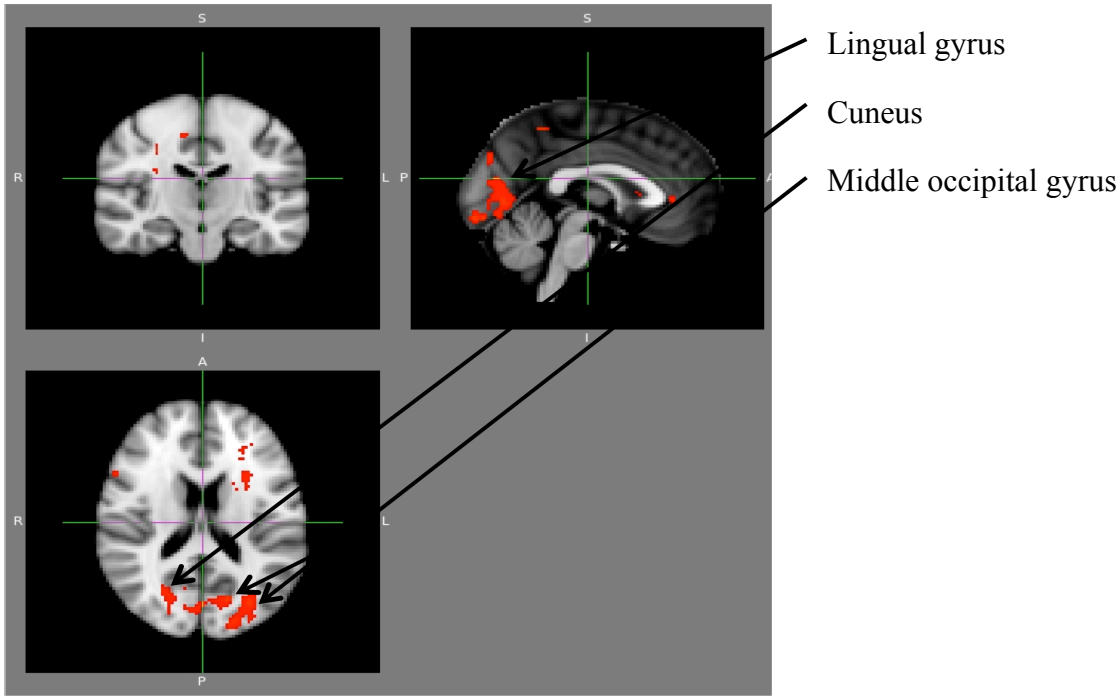


Figure 17

Moderate > severe, encode phase, z = 1.9.

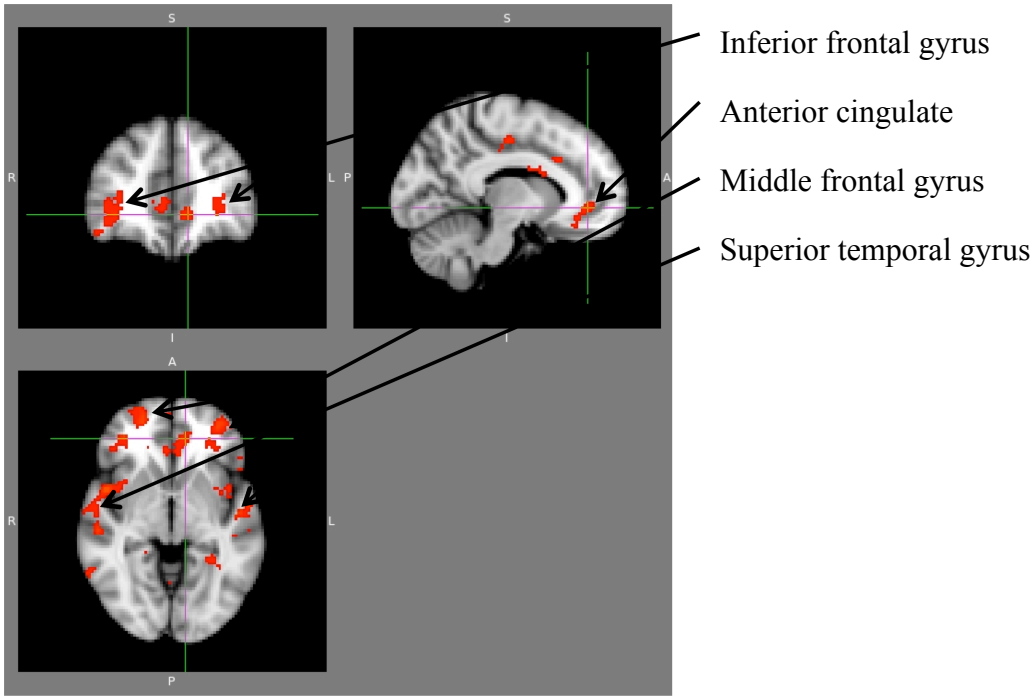


Table 10

Results of ANOVAs comparing control participants, patients with moderate CKD, and patients with severe CKD.

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
Angular gyrus	Severe significantly lower than control or moderate ($p < 0.001$ for each).	Severe significantly lower than control or moderate in the encode phase ($p < 0.01$, $p = 0.03$).	NS	In the encode phase, in the right hemisphere, severe significantly lower than moderate in terms of average percent signal change ($p < 0.01$).
Inferior occipital gyrus	Severe significantly lower than moderate ($p < 0.001$) or control ($p = 0.02$).	NS	NS	In the encode phase, in the right hemisphere, severe significantly lower than moderate in terms of average percent signal change ($p < 0.01$).
Precentral gyrus	Severe significantly lower than control or moderate ($p <0.01$).	Severe significantly lower than control in the encode phase ($p <0.01$).	NS	In the retrieve phase, in the right hemisphere, severe significantly lower than moderate in terms of maximum percent signal change ($p = 0.04$).

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
Anterior cingulate	Moderate significantly higher than either control or severe ($p < 0.001$).	NS	NS	NS
Inferior temporal gyrus	Severe significantly lower than control or moderate ($p < 0.001$).	NS	NS	NS
Precuneus	Severe significantly lower than control ($p < 0.001$) or moderate ($p < 0.01$).	NS	NS	NS

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
Lingual gyrus	Control significantly higher than severe ($p < 0.001$).	NS	Control higher than severe ($p = 0.03$) in terms of mean percent signal change in the retrieve phase.	In the encode phase, in the left hemisphere, severe significantly lower than moderate in terms of average percent signal change ($p < 0.01$). In the encode phase, in the right hemisphere, severe significantly lower than moderate in terms of average percent signal change ($p = 0.02$).

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
Superior frontal gyrus	Moderate significantly higher than control ($p = 0.02$), and severe significantly lower than either moderate ($p < 0.001$) or control ($p = 0.001$).	Severe significantly lower than control or moderate in the encode phase ($p = 0.03$, $p = 0.02$).	NS	In the encode phase, in the left hemisphere, severe significantly lower than moderate in terms of average percent signal change ($p < 0.01$). In the retrieve phase, in the left hemisphere, severe significantly higher than moderate in terms of average percent signal change ($p < 0.01$). In the encode phase, in the right hemisphere, severe significantly lower than moderate in terms of average percent signal change ($p = 0.009$).

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
Superior occipital gyrus	Control significantly higher than either moderate or severe ($p < 0.001$ for each).	In the encode phase, control significantly higher than either moderate ($p = 0.001$) or severe ($p = 0.02$). Control significantly higher than moderate in the retrieve phase ($p = 0.01$).	NS	NS

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
Medial frontal gyrus	Moderate significantly higher than either control or severe ($p < 0.001$ for each).	Severe significantly higher than control in the retrieve phase ($p = 0.02$).	NS	In the encode phase, in the left hemisphere, severe significantly lower than moderate in terms of average percent signal change ($p = 0.02$). In the encode phase, in the right hemisphere, severe significantly lower than moderate in terms of average percent signal change ($p = 0.009$). In the retrieve phase, in the left hemisphere, control significantly higher than moderate in terms of average percent signal change ($p = 0.04$).
Fusiform gyrus	Severe significantly lower than moderate ($p < 0.001$) or control ($p < 0.01$).	NS	NS	NS

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
Middle frontal gyrus	Severe significantly lower than control or moderate ($p = 0.03$, $p = 0.04$).	Severe significantly lower than control in the encode phase ($p = 0.02$).	NS	In the left hemisphere, in the retrieve phase, moderate significantly lower than control or severe in terms of average percent signal change ($p = -0.04$, $p < 0.01$).
Superior temporal gyrus	Moderate significantly higher than either control or severe ($p <0.001$ for each).	NS	NS	NS
Inferior frontal gyrus	Moderate significantly higher than either control or severe ($p = 0.04$, $p <0.01$).	NS	NS	In the left hemisphere, in the retrieve phase, moderate significantly lower than severe in terms of average percent signal change ($p =-0.03$).

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
Middle occipital gyrus	Severe significantly lower than control or moderate ($p < 0.001$ for each).	NS	NS	In the encode phase, in the right hemisphere, severe significantly lower than control in terms of maximum percent signal change ($p < 0.05$).
Superior parietal	Control is higher than moderate and moderate is higher than severe (both $p < 0.001$).	Severe significantly lower than control ($p < 0.001$) or moderate ($p = 0.02$) in the encode phase. Severe significantly lower than control in the retrieve phase ($p = 0.04$).	Average percent signal change was significantly higher in control participants compared with patients with severe CKD ($p = 0.001$) or moderate CKD ($p = 0.007$) in the encode phase. Control higher than moderate ($p = 0.02$) in terms of mean percent signal change in the retrieve phase.	In the encode phase, in the left hemisphere, control significantly higher than either moderate or severe in terms of average percent signal change ($p = 0.04$). In the encode phase, in the right hemisphere, control significantly higher than severe in terms of average percent signal change ($p = 0.02$).
Insula	NS	Severe significantly lower than control in the encode phase ($p = 0.02$).	NS	NS

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
Posterior cingulate	Severe significantly lower than control ($p < 0.001$) or moderate ($p = 0.02$).	NS	NS	In the left hemisphere, in the encode phase, severe significantly lower than control or moderate in terms of average percent signal change ($p = 0.02$). In the left hemisphere, in the retrieve phase, moderate significantly lower than control in terms of average percent signal change ($p = 0.02$). In the right hemisphere, in the retrieve phase, moderate significantly lower than severe in terms of average percent signal change ($p = 0.03$).

Region	Eventstats analysis		FSL analysis	
	Average percent signal change over the whole course of the task	Peak signal change in encode and retrieve phases	Average percent signal change in encode and retrieve phases	Average percent signal change in each phase, differentiating left and right hemispheres
BA7	Severe significantly lower than either control or moderate ($p < 0.001$ for each).	Control significantly higher than either moderate or severe in the encode phase ($p < 0.001$).	Control higher than moderate ($p = 0.02$) in terms of mean percent signal change in the retrieve phase.	In the left hemisphere, in the encode phase, severe significantly lower than control or moderate in terms of average percent signal change ($p = 0.003$, $p = 0.048$). In the right hemisphere, in the encode phase, severe significantly lower than control in terms of average percent signal change ($p = 0.02$).
BA46+9	Severe significantly higher than control ($p = 0.02$).	NS	NS	In the left hemisphere, in the retrieve phase, moderate significantly lower than severe in terms of average percent signal change ($p = 0.03$).

CHAPTER V

DISCUSSION

Discussion of individual hypotheses

Cognitive outcomes are a concern in pediatric CKD. Cognitive impairments have been documented, particularly in children with severe CKD, and concerns have been raised about memory and executive functions in particular (Gerson et al., 2006; Gipson et al., 2006; Slickers et al., 2007). The goal of this experiment was to explore differences in brain activity during a visuospatial working memory task in children and adolescents with chronic kidney disease (CKD) compared with healthy same-age participants. Another question of interest was whether children and adolescents with more severe CKD showed differences from patients with less severe CKD.

It was hypothesized that activity in the brain areas associated with working memory would be lower in patients with CKD than in healthy participants, both while memory for object locations was encoded and while that information was retrieved. Secondary hypotheses posited that both patients with moderate CKD and patients with severe CKD would display reduced brain activity in relevant regions compared with controls. It was also hypothesized that performance on the visuospatial working memory task would differ among groups, with lower accuracy and longer reaction times in children and adolescents with CKD than in control participants. The fMRI results partially supported the hypotheses regarding brain activity.

Hypothesis 1: Percent BOLD signal change over the encoding period will be significantly lower in VLPFC, DLPFC, right posterior parietal cortex, right premotor cortex, and IPS in patients with CKD as compared to controls.

Because regions of interest were chosen based on functional activation, post-hoc tests of significance were conducted using the Holm-Bonferroni procedure. Following these tests, significant differences remained in the superior parietal lobule, where patients with CKD displayed lower mean percent signal change than control participants during the encoding period ($p < 0.001$). This difference did not remain significant when the portions of the superior parietal lobule in the left and right hemispheres were considered separately. Patients with CKD showed nonsignificant trends toward lower mean percent signal change during the encode phase in BA7 and lower *peak* percent signal change in the superior parietal lobule and BA7. These differences suggest that reduced blood flow to bilateral posterior parietal cortex could contribute to difficulty maintaining spatial representations in working memory in children and adolescents with CKD. Although accuracy on the working memory task was not significantly affected, subclinical differences in visual-spatial working memory may be present and may contribute to the significant difference seen between patients with CKD and controls in terms of reaction time.

There were also trends toward lower peak signal change among patients with CKD in some brain regions involved in processing visual stimuli and transmitting information to the parietal cortex (superior occipital gyrus, precentral gyrus). No significant differences were observed in the encoding period between patients with CKD and controls in VLPFC or DLPFC apart from a nonsignificant trend in the precentral

gyrus. These results do not suggest differences between groups in attention or other executive functions mediated by frontal regions.

Hypothesis 1b: Percent signal change in these brain regions during the encoding period will be significantly lower in each group of patients with CKD as compared to healthy participants.

Again, differences between groups were evident in the posterior parietal cortex. Patients with severe CKD had significantly lower average percent signal change and peak signal change compared with controls ($p = 0.001$ for each) in the superior parietal lobule, and patients with moderate CKD showed a similar but nonsignificant difference in the same region. Both patient groups had significantly lower peak signal change than controls in BA7 ($p < 0.001$ for each). This indicates that patients with severe CKD experienced less blood flow to posterior parietal cortex while encoding visual-spatial information. Patients with moderate CKD showed less difference from healthy controls.

No other significant differences were evident in terms of average percent signal change among regions until the left and right hemispheres were considered separately. These comparisons resulted in some differences for which $p < 0.05$, but no differences that met more stringent post-hoc significance criteria. In the right hemisphere, patients with severe CKD had lower average percent signal change than those with moderate CKD in five regions encompassing parts of the DLPFC and secondary visual cortex as well as parietal cortex (right superior and medial frontal gyri, right angular gyrus, right lingual gyrus, right inferior occipital gyrus). Only in right posterior parietal cortex (right superior parietal lobule and right BA7) did patients with severe CKD show lower average percent signal change than controls ($p = 0.02$ for each). This suggests the possibility that

patients with severe CKD might allocate resources less efficiently to the right hemisphere, which is typically more active than the left during spatial working memory tasks (Belger et al., 1998; Smith & Jonides, 1999; Thomas et al., 1999; Smith et al., 1996).

However, results of left hemisphere analyses suggest that the differences in activation between patients with severe CKD and healthy controls were bilateral rather than reflecting higher left hemisphere activation in patients with CKD. The left hemisphere would be expected to be less activated by a visual-spatial task, and differences on this side of the brain were less numerous but were nonetheless in the direction of lower activation among patients with severe CKD. The severe group had lower average percent signal change compared with the moderate group in four regions (left superior and medial frontal gyri, left lingual gyrus, left BA7) and compared with controls in three regions (left superior parietal lobule, left BA7, left middle occipital gyrus). The moderate group resembled the control group, showing a difference of lower average percent signal change only in the left superior parietal lobule ($p = 0.04$).

A number of other differences among groups in terms of *peak* signal change resulted in $p < 0.05$ but did not meet criteria for significance based on post-hoc tests. Patients with severe CKD had lower peak signal change than controls in six functionally activated regions, including portions of DLPFC, secondary visual cortex, and parietal cortex (superior frontal and middle frontal gyri, insula, precentral gyrus, superior occipital gyrus, and angular gyrus). In two of those regions, the angular gyrus and superior frontal gyrus, peak signal change was also lower in patients with severe compared with moderate CKD. In only one region, the superior occipital gyrus, did

moderate patients have lower peak signal change than controls. That is, blood flow to relevant brain regions during the visual-spatial working memory task in patients with modified eGFR $\geq$ 30, who were classified as having moderate CKD, more closely resembled that in control participants than patients with modified eGFR < 30, who were classified as having severe CKD. Patients with severe CKD showed lower activation than controls in numerous regions, although the differences reached significance in only a few regions.

This suggests that the patients with severe CKD were more impaired than those with moderate CKD. Few studies of pediatric CKD have included patients with mild to moderate CKD, and therefore little is known about the cognitive and executive function capabilities of this group (Gerson et al., 2006; Slickers et al., 2007). One question of interest is whether declines in cognitive function precede the reduction in glomerular filtration that signals the need for dialysis or transplant. A recent large-scale study indicated that children with mild and moderate CKD have reduced health-related quality of life compared with controls, particularly in terms of school functioning, but this does not necessarily indicate cognitive impairments and may instead may result from interruptions in school attendance associated with disease management (Gerson, et al., 2010). Results of the current study support earlier findings that disease severity correlated with cognitive dysfunction (Slickers et al., 2007).

Hypothesis 2: Percent BOLD signal change over the retrieval period will be significantly lower in VLPFC, DLPFC, right posterior parietal cortex, right premotor cortex, and IPS in patients with CKD as compared to controls.

No differences in BOLD signal change in the retrieval period reached significance

based on post-hoc tests, but all trends between groups showed lower activation in patients with CKD than in controls. Patients with CKD had lower average percent signal change than controls in the superior parietal lobule and BA7, but to a lesser extent than in the encoding period ($p = 0.009$ and $p = 0.004$, respectively). Patients with CKD also had lower average percent signal change than controls in the lingual gyrus, posterior cingulate, and precuneus ($p < 0.05$). Considering only the right hemisphere, patients with CKD had lower average percent signal change compared with controls in the right lingual gyrus and right medial frontal gyrus. In the left hemisphere patients had lower average percent signal change in BA7. Analyses of peak signal change indicated no differences with $p < 0.05$.

Hypothesis 2b: Percent signal change in these brain regions during the retrieval period will be significantly lower in each group of patients with CKD as compared to healthy participants.

This hypothesis was not supported. No differences among the three participant groups in the retrieval period were significant based on post-hoc tests.

However, differences at a level of $p < 0.05$ suggested lower activation in patients with moderate CKD of left hemispheric frontal regions during the retrieval period compared with controls (middle and medial frontal gyri) and patients with severe CKD (superior, middle, and inferior frontal gyri). Patients with moderate CKD also had lower average percent signal change compared patients with severe CKD in DLPFC (BA46+9) and the right posterior cingulate, and compared with controls in parietal regions (superior parietal lobule, BA7) and left posterior cingulate. They had lower peak signal change than controls in the superior occipital gyrus. These results suggest that patients with

moderate CKD experienced lower activation of posterior parietal cortex throughout the task and lower activation than other groups in the retrieval period. The reduced activation in left but not right frontal regions might conceivably suggest efficient allocation of scarce resources by patients with moderate CKD. A larger sample size might enable examination of this question.

Differences between patients with severe CKD and other groups were muted compared with those of the encoding period, but some trends remained toward lower activation in patients with severe CKD. Patients with severe CKD had lower peak signal change than controls or patients with moderate CKD in a few regions (superior parietal lobule, precentral gyrus) and lower average percent signal change than controls in the lingual gyrus. Unusually, controls had lower peak signal change compared with patients with severe CKD in the medial frontal gyrus.

Hypothesis 3: Percent BOLD signal change over the course of the task will be significantly lower in the VLPFC, DLPFC, right posterior parietal cortex, right premotor cortex, and IPS in patients with CKD as compared to controls.

This question was explored using eventstats, which tracks percent signal change over the course of the task, including the period over which information must be maintained following encoding. In terms of percent signal change over the entire time course, the groups differed significantly in numerous regions. Patients with CKD had significantly lower average percent signal change than controls ($p < 0.001$) in posterior parietal cortex (angular gyrus, superior parietal lobule, BA7), posterior cingulate, insula, and secondary visual cortex (superior and middle occipital gyri, lingual gyrus). A difference in the same direction in the precuneus approached significance ($p = 0.009$).

These results indicate less activation among patients with CKD of the dorsal visual processing stream and the parietal regions active in maintaining spatial information. The facts that differences between patients and controls were evident primarily in the analyses of activation over the entire time course and that they included regions normally activated during delays suggest that patients with CKD may show less activation related to maintenance of visual-spatial information.

In the medial frontal gyrus and superior temporal gyrus, patients showed trends toward *higher* average percent signal change than controls ($p = 0.002$ and $p = 0.006$, respectively). It is possible that higher activation may represent more effort, that for instance higher signal change in the medial frontal gyrus in the retrieval period (seen in patients with severe CKD, illustrated in Figure 15) could indicate more effort required for decision making.

Hypothesis 3b: Percent signal change in relevant brain regions during the retrieval period will be significantly lower in each group of patients with CKD as compared to healthy participants.

As in the two-group comparison, the measure that yielded the highest number of significant results was average percent signal change over the entire course of the task. In ten regions of interest, patients with severe CKD had significantly lower average percent signal change than controls ($p < 0.001$): superior frontal gyrus, lingual gyrus, inferior temporal gyrus, precuneus, posterior cingulate, superior and middle occipital gyri, angular gyrus, superior parietal lobule, and BA7. These include DLPFC, though not VLPFC, along with posterior parietal cortex, regions associated with attention, and visual processing regions. Four additional regions showed nonsignificant trends with $p < 0.05$ in

the same direction (middle frontal gyrus, inferior occipital gyrus, fusiform gyrus, precentral gyrus). These results indicate lower activation among patients with severe CKD compared with healthy controls in numerous regions active in working memory, substantially supporting Hypothesis 3b.

Patients with moderate CKD had patterns of brain activation more closely resembling controls. In only a few regions did patients with moderate CKD have significantly lower average percent signal change over the course of the task ($p < 0.001$) than controls (superior parietal lobule, superior occipital gyrus). However, in three regions associated with decision making and target detection, the moderate group showed significantly higher average percent signal change over the course of the task than either controls or patients with severe CKD ($p < 0.001$ for the anterior cingulate, medial frontal gyrus, superior temporal gyrus). Also, in eight regions, the moderate group showed significantly higher activation than the severe CKD group ($p < 0.001$ for the superior frontal gyrus, inferior and middle occipital gyri, angular gyrus, fusiform gyrus, precuneus, inferior temporal gyrus, and superior parietal lobule). The posterior cingulate showed a nonsignificant trend ($p = 0.02$) in the same direction.

That is, grouping all the CKD participants together for the initial analyses masked some differences between the severe and moderate groups. Patients with moderate CKD (modified eGFR $\leq$ 30 ml/min/1.73m^2) more closely resembled controls. In many cases they showed more blood flow to regions associated with working memory than controls did. This may suggest that these patients were working harder or less efficiently to generate answers of comparable accuracy. In contrast, patients with severe CKD (modified eGFR $\leq$ 30 ml/min/1.73m^2 or dialysis dependence) showed lower activation in

a number of regions of interest compared with controls and patients with moderate CKD. The burden of disease load on patients with moderate CKD may not yet be heavy enough to significantly affect blood flow within the brain.

Hypothesis 4: Accuracy will be higher in controls as compared to patients with CKD.

This hypothesis was not supported. Accuracy on the task was similar among controls and CKD patients. Averaging the results of all four runs of data (that is, 40 questions), control participants got 6.6 answers out of 10 right, while patients with CKD got 6.0 answers right. A three-group comparison was also conducted. Accuracy did not differ significantly between the two patient groups or between controls and either patient group. Cohen's d tests were conducted as a measure of effect size on the results for accuracy, reaction time, and mean percent BOLD signal change in selected regions of interest for which the difference between patients with CKD and healthy controls reached or approached statistical significance. Effect-size calculation for the difference in accuracy between patients with CKD and controls generated a Cohen's *d* of 0.372, which is classified as a small effect.

These results contradict earlier studies that found memory deficits in children with CKD, including research that found deterioration or impaired development of memory over time in children with CKD (Fennell et al., 1990b), associations between better memory and markers of better renal function in children with CKD (Fennell et al., 1990a; Slickers et al., 2007), and post-transplantation improvements in immediate recall (Rasbury et al., 1983) and working memory (Mendley & Zelko, 1999). However, not all studies of memory in children with CKD have found impairments, either compared with

standardized scores (Qvist et al., 2002) or compared with controls (Rasbury et al., 1986; Bawden et al., 2004).

Attention is an important part of working memory, according to both emergent process (Baddeley & Hitch, 1974) and buffer system models (Andersen, 1983; Cowan, 1988; Postle, 2006). Some studies have found sustained attention to be impaired in children with CKD (Gipson et al., 2006; Fennel et al., 1990a) or ESRD (Slickers et al., 2007) or to improve post-transplant (Mendley et al., 1990). These data suggest that working memory would be impaired in patients with CKD compared with healthy controls. Other studies have found no differences in attention between controls and patients with CKD (Rasbury et al., 1983) or have found no differences between the groups' mean scores but considerable variability in scores, with many patients' scores falling well below the mean (Slickers et al., 2007; Qvist et al., 2002).

The current study did not assess attention specifically but would be expected to assess attention as part of working memory. No significant differences between controls and patients with CKD were found in terms of average working memory as assessed by performance on the visual-spatial task. Variability was substantial but similar in all groups and was lower among patients than among control participants.

It is notable that previous studies of memory in children and adolescents with CKD almost universally enrolled only patients with ESRD (Rasbury et al., 1986; Bawden et al., 2004) or kidney transplant recipients (Rasbury et al., 1983; Mendley & Zelko, 1999; Qvist et al., 2002). Fennell et al. enrolled a minority of patients with "moderate" CKD (5 patients out of 56 in the longitudinal study) but enrolled primarily patients with ESRD and those who were dialysis- or transplant-dependent. The studies by Gipson et al.

(2006) and Slickers et al. (2007) were unusual in enrolling patients with moderate as well as severe CKD.

In a sample of 20 patients, Gipson et al. enrolled 8 patients with CKD who had not progressed to ESRD, and eGFR for these patients ranged from 22.7 to 72.9 mL/min/1.73m2. Slickers et al. assessed 29 patients with CKD, of whom half had mild to moderate CKD, with estimated creatinine clearance of 31–89 mL/min per 1.73 m^2 body surface area. Creatinine clearance was estimated using the Schwartz formula (Schwartz et al., 1976). In the current study, mean modified eGFR was 54.7 for patients in the moderate group, and the range was 43.4 to 67.1. Given that creatinine clearance and GFR calculations provide higher estimates than the modified eGFR formula used in this study (Schwartz et al., 2009), these patient populations may have had disease severity similar to the patients with moderate CKD in the current study.

Granted, in the current study no differences in accuracy were apparent between the patients with moderate CKD and those with severe CKD, and in fact the patients with severe CKD had marginally better accuracy on the working memory task. On the dimension of task accuracy, data from patients with moderate CKD could not have masked differences between patients with severe CKD and controls. However, it is possible that the differences in blood flow to some relevant brain regions signal a subclinical difference in processes relevant to working memory. It is also possible that a larger study would have had the statistical power to reveal more significant differences.

It may be that the task was too difficult for these participants to adequately discriminate among the groups, although mean accuracy for each group was above chance. An earlier study used the same task with the addition of distracters between the

stimulus and response, and adults in that study responded accurately at a rate of 86.22% (SD = 0.08) (Hart, 2007), considerably higher than any group in the current study—although the control participants during the first data set of the current study did have a mean accuracy of 80% (SD = 1.9). Accuracy was lower on later runs ($p < 0.01$ for run 1 vs. run 4), suggesting that fatigue may have impaired participants' overall performance rather than learning improving performance over time. Additional training on the task, beyond the printed example and verbal explanation provided before each participant entered the scanner, might have improved group accuracy on the first run.

It is also possible that this fMRI task, which was not a standardized neuropsychological instrument, was not well suited to assessing working memory in these participants. Previous studies that found impairments in memory or attention in patients with CKD have often used standardized tests or subtests, such as the Gordon Diagnostic System (Gipson et al., 2006; Slickers et al., 2007), Connors Continuous Performance Test (Mendley et al., 1999) and NEPSY (Qvist et al., 2002) to assess attention and the Wide Range Assessment of Memory and Learning (Slickers et al., 2007; Bawden et al., 2004) to assess memory. However, the task in the current study had previously been used in a working memory study at UNC (Hart, 2007) and was similar to other tasks used to assess working memory at other sites (Thomas et al., 1999; Klingberg et al., 2002)

Hypothesis 5: Reaction time will be lower—that is, performance will be faster—in controls as compared to patients with CKD.

This hypothesis was supported. Control participants exhibited significantly lower mean reaction time—that is, responded more rapidly to the test questions—than the

patients with CKD, particularly the patients with severe CKD ($p = 0.001$). Effect-size calculation for the difference between patients with CKD and controls generated a Cohen's *d* of -0.841, which is classified as a medium effect size (Cohen, 1988). The number is negative because controls' average reaction time was shorter than that of patients with CKD. The difference between control participants and patients may reflect either more rapid decision-making or more rapid movement, since the responses consisted of pressing a button.

An fMRI study assessing performance on a spatial working memory task in patients ages 9 to 19 years (Klingberg et al., 2002) found that reaction time did not vary significantly across the age span, suggesting that the age makeup of the patient and control groups should not have affected this measure. Participant groups in the current study did not differ significantly in terms of age, but participants did range in age from 9 to 19 years.

The finding that patients with CKD and particularly patients with severe CKD responded more slowly to the working memory task than healthy controls is consistent with the executive function study conducted by Gipson et al. (2006), which found impairment of initiation (starting a task) in children and adolescents with CKD compared with healthy controls. Similar to the current study, the 2006 Gipson et al. study enrolled participants ages 7 to 19 years and patients whose CKD was being managed with conservative therapy as well as patients who were on dialysis. An earlier study by Mendley et al. (1999) found that within-subject reaction time on the CPT, which also requires button-pushing in response to stimuli, improved following renal transplant. Like the study under discussion, this result supports the idea that current renal functioning

affects reaction time.

Conclusions

In conclusion, the results of this experiment suggest that children and adolescents with chronic kidney disease experience reduced activity in certain brain regions involved in visual-spatial working memory, notably in bilateral posterior parietal cortex. Reduced activation in secondary visual cortex suggests potential disruption of the dorsal visual processing stream that transmits spatial information to parietal regions. Although these differences did not correlate with performance deficits as assessed by accuracy on a visual-spatial memory task, they were associated with slower response time compared with healthy controls.

Differences from controls were more pronounced in patients with more severe CKD. Patients with moderate CKD more closely resembled controls in terms of their activation patterns, but in some regions associated with executive functions, patients with moderate CKD showed increased activation compared with controls. This may suggest that the task was more difficult for them.

Interpretation of these results should take into account the medical care patients in this study were receiving. All of the patients enrolled in the study were under the care of pediatric nephrologists, and many were receiving medications to reduce hypertension and anemia. In the whole U.S. population of children and adolescents with CKD, it is estimated that at least 25% do not see a nephrologist until they progress to end-stage renal disease (United States Renal Data System, 2008b). Hypertension is often undertreated (Flynn et al., 2008) and appropriate treatment for anemia remains a concern (United States Renal Data System, 2008b). Although significant differences between

patients with CKD and controls in this study were not numerous, it is notable that even after treatment to normalize blood pressure and hemoglobin levels, some significant differences remained.

Future directions

The current study is limited by its small size and the heterogeneous nature of the patient groups. Given the participants available through the UNC Kidney Center, the decision was made to include transplant-dependent patients in either the moderate or severe CKD group depending on their modified eGFR at the time of the fMRI scan. Post-transplant patients, who are more likely than others to have had longstanding CKD and to take particular medications, may have unique cognitive profiles or trajectories that were not analyzed in the current study. Several studies following individual patients over time have suggested that transplantation improves IQ (Davis, Chang & Nevins, 1990), sustained attention, working memory (Mendley & Zelko, 1999), and other cognitive measures (Rasbury et al., 1983; Mendley & Zelko, 1999). Other studies have failed to find improved cognitive performance following transplantation (Rasbury, Fennell, Fennell & Morris, 1986; Brouhard et al., 2000). Future studies may wish to examine post-transplant patients as a separate group.

A larger sample size will be required to confirm results of the current study and explore possible moderators and mediators. The current study lends support to earlier research indicating that current disease severity, of which modified eGFR is one measure, influences the extent of cognitive impairment in CKD (Slickers et al., 2007; Fennell et al., 1990b; Hulstijn-Dirkmaat et al.,1995). Other important contributing factors suggested by the Slickers et al. study were longer duration of disease and younger age at disease

onset. Additional factors that may contribute to cognitive deficits in children with CKD include uremia (accumulation of waste products in the blood), hypoxia, modality of renal therapy (peritoneal dialysis, hemodialysis, or conservative therapy), structural abnormalities, hypoxia, and common complications including anemia, hypertension, cardiovascular disease, and malnutrition (Gerson et al., 2006). Another question of interest is whether cognitive difficulties observed in children with CKD represent permanent impairment or delayed development that may normalize following appropriate medical care. Following the same patients over time rather than assessing a cross-sectional sample would reduce confounding factors such as disparate etiologies and would permit examination of patients' neurodevelopmental trajectories.

Age is likely to have influenced individual participants' working performance, since working memory is known to improve over the course of childhood and adolescence (Bayliss, Jarrold, Baddeley & Leigh, 2005; Brocki & Bohlin, 2004; Demetriou, Christou, Spanoudis & Platsidou, 2002; Gathercole, Pickering, Ambridge & Wearing, 2004; De Luca et al., 2003; Luciana, Conklin, Hooper & Yarger, 2005; Luna, Garver, Urban, Lazar & Sweeney, 2004; Siegel & Ryan, 1989; Swanson, 1999). Participants' socioeconomic status is also likely to influence their neurodevelopmental outcomes (Warady et al., 1999). A larger sample size would permit age and socioeconomic status of participants to be examined as possible covariates for working memory performance.

One large-scale effort to provide information regarding neurocognitive outcomes and risk factors for neurocognitive impairment in pediatric CKD is the Chronic Kidney Disease in Children Prospective Cohort Study (CKiD), a longitudinal cohort study funded

by the National Institutes of Health (NIH) and enrolling children and adolescents with mild to moderate kidney disease from 48 pediatric nephrology centers (Gerson et al., 2006; Gerson et al., 2010). A small number of patients enrolled in the current study are also enrolled in CkiD, which began in 2006. This study will provide substantial information about memory, attention, and executive function in pediatric CKD, which may be of use in designing future brain imaging studies for this population.

It is possible that structural differences in the brain associated with CKD, such as arteriosclerosis, swelling, atrophy, or reduced or delayed myelination, might influence imaging results or task performance. Cardiovascular complications are the leading cause of death in pediatric ESRD patients (United States Renal Data System, 2008b), and even in childhood patients with CKD are at risk for arteriosclerosis (Sozeri, Mir, Kara, & Levent, 2010). Stiffness and narrowing of blood vessels may limit blood flow to the brain. ESRD in adults has been associated with brain swelling (Fennell et al., 1990a), and high rates of cerebral atrophy have been identified in children with certain types of CKD (Qvist, et al., 2002; Nichols, et al., 1990). Such structural differences could reduce MR signals as well as potentially affecting cognitive function. Atrophy in a study population can be investigated using structural MRI. Arterial spin labeling uses magnetic labeling of arterial blood water to non-invasively assess cerebral blood flow. Data from arterial spin labeling can be used in conjunction with MRI to account for differences among study participants in delivery of blood to the brain (Wolf & Detre, 2007).

Myelination normally continues through adolescence and is thought to subserve development of working memory (Klingberg, Vaidya, Gabrieli, Moseley & Hedehus, 1999; Paus et al., 1999; Thomas et al., 1999). Some causes of CKD are associated with

reduced myelination (Pueschel, Brem & Nittoli, 1992 Nichols, et al., 1990), and CKD commonly retards growth and maturation (United States Renal Data System, 2008b). Diffusion tensor imaging (DTI) can be used to investigate white matter structure. In combination with fMRI data, DTI can inform theories regarding connectivity of brain regions and development of functional networks (Olesen et al., 2003). Future studies may benefit from using DTI in conjunction with fMRI.

It is possible that a greater difference in performance might be evident in a larger participant population or perhaps with a task on which participants performed better or performance varied less widely. In a future study the task could be made simpler, for example by reducing the number of stimuli to be remembered in addition to eliminating distracters. One study assessing development of spatial working memory over childhood varied the number of stimuli for different participants based on pretesting outside the scanner to ensure performance of 75%–95% accuracy for all participants (Thomas et al., 1999). Alternatively, participants could be given time to practice the task with feedback to improve performance until they reached a threshold of accuracy. However, some studies that have shown improvements in visual-spatial working memory have utilized daily practice over several weeks, which might not be practical (Klingberg et al., 2002; Klingberg et al., 2005). Of course, it would also be possible to assess verbal working memory instead of visual-spatial working memory, which is arguably more crucial to school performance. This has often been done in fMRI studies of other groups (Smith & Jonides, 1997; Nystrom et al., 2000).

Interpretation would also be enriched by assessing working memory and attention outside the scanner based on standardized neuropsychological measures, such as the

CVLT-C; Digit Span subtest of the WISC-IV; List Memory, Memory for Designs, and Auditory Attention subtests of the NEPSY-II (Davis & Matthews, 2010); or a continuous performance test such as the Gordon Diagnostic System. Additional assessment measures could verify participants' working memory performance as assessed during the fMRI scan or indicate deficits in attention or working memory not identified by the fMRI task.

Conducting analyses both in FSL and eventstats was fruitful. It permitted comparison of results, so that the significance of results found in the FSL analyses, e.g. reduced activation among patients with severe CKD in posterior parietal cortex, could be confirmed by similar results in the eventstats analyses. The strengths of both programs were utilized. FSL simplified assessment of the hemodynamic responses to initial stimuli and target stimuli presentation, while eventstats permitted analysis of activation over the course of the task and generated graphic comparisons of percent signal change over time. These graphs confirmed the presence of clear peaks in activation timed to the encoding and retrieval events and permitted visual comparison of the three participant groups. Future fMRI studies may benefit from using software such as eventstats that graphically presents activation over the course of a task.

Visual examination of the time course graphs suggested differences among groups during the maintenance phase, the time between encoding and retrieval when participants must retain encoded information before being prompted to answer a question. This period has been studied by many other researchers, particularly in primate studies (Goldman-Rakic, 1987; D'Esposito, 2007). In a future analysis it would be interesting to examine differences between patients with chronic kidney disease and controls.

APPENDIX 1

Tables detailing Holm-Bonferroni tests of significance

The initial adjusted critical significance level was set at 0.05/40 = 0.00125 to account for the consideration of 20 functionally activated regions of the brain with, in some analyses, the left and right sides of those regions considered separately.

Table 11

FSL t-tests (control > patient in all cases of significant difference).

Region	Phase	Control, mean % signal change	Patient, mean % signal change	Adjusted critical *p*	*p*, Control vs. Patient
Superior parietal lobule	Encode	0.800577	0.49355	0.00125	0.000099
BA7	Retrieve	0.371198	0.210627	0.00128	0.003656
BA7	Encode	0.500895	0.327964		0.004788
Superior parietal lobule	Retrieve	0.442359	0.253853		0.008783
Posterior cingulate	Retrieve	0.195003	0.020410		0.015973
Lingual gyrus	Retrieve	0.423941	0.188823		0.024432
Medial frontal gyrus	Retrieve	0.015805	-0.048546		0.03522
Precuneus	Retrieve	0.326799	0.181158		0.046496
Superior temporal gyrus	Retrieve	0.138462	0.040323		0.051866
Anterior cingulate	Retrieve	0.140471	0.034444		0.101699

Region	Phase	Control, mean % signal change	Patient, mean % signal change	Adjusted critical *p*	*p*, Control vs. Patient
Precuneus	Encode	0.360045	0.255219		0.138409
Posterior cingulate	Encode	0.093299	-0.012195		0.179067
Middle occipital gyrus	Encode	0.297085	0.220507		0.182590
Middle occipital gyrus	Retrieve	0.238168	0.167280		0.237133
Inferior frontal gyrus	Retrieve	0.197435	0.133338		0.310071
Angular gyrus	Encode	0.264966	0.010762		0.328931
Angular gyrus	Retrieve	0.098587	0.034978		0.365704
Superior frontal gyrus	Retrieve	0.075610	0.053041		0.448005
Precentral gyrus	Encode	0.065771	0.084723		0.453610
Medial frontal gyrus	Encode	-0.023073	-0.004482		0.461416
Inferior temporal gyrus	Retrieve	0.044103	0.0083165		0.471050
Inferior frontal gyrus	Encode	0.135280	0.097882		0.488871
Inferior occipital gyrus	Encode	0.228259	0.179081		0.584553

Region	Phase	Control, mean % signal change	Patient, mean % signal change	Adjusted critical *p*	*p*, Control vs. Patient
Middle frontal gyrus	Retrieve	0.185626	0.159028		0.593917
Anterior cingulate	Encode	-0.009673	0.010539		0.600032
Precentral gyrus	Retrieve	0.028475	0.009268		0.616455
Middle frontal gyrus	Encode	0.121832	0.137377		0.723926
Fusiform gyrus	Encode	0.158475	0.146221		0.775949
Inferior occipital gyrus	Retrieve	0.275363	0.294650		0.792970
Inferior temporal gyrus	Encode	0.081854	0.072834		0.845407
Superior frontal gyrus	Encode	0.009842	0.005893		0.891913
BA9+46	Encode	0.165788	0.169348		0.957876
Superior temporal gyrus	Encode	0.050658	0.052052		0.972455
BA9+46	Retrieve	0.206386	0.207862		0.986333
Lingual gyrus	Encode	0.361919	0.361793		0.998409

Table 12

FSL t-tests with left-right differentiation, comparing mean percent signal change for patients with CKD and healthy controls.

Region	Side	Phase	Adjusted critical *p*	*p*, Control vs. Patient
Superior parietal	Left	Encode	0.00125	0.003731
Superior parietal	Right	Encode	0.00128	0.006616
BA7	Left	Encode	0.00132	0.017081
BA7	Left	Retrieve		0.020647
Lingual gyrus	Left	Retrieve		0.026769
BA7	Right	Encode		0.031891
Superior temporal gyrus	Left	Retrieve		0.052707
Superior parietal	Left	Retrieve		0.053850
BA7	Right	Retrieve		0.079372
Superior parietal	Right	Retrieve		0.084315
Medial frontal gyrus	Left	Retrieve		0.093580
Inferior frontal gyrus	Left	Encode		0.161708
Medial frontal gyrus	Right	Retrieve		0.185644
Middle frontal gyrus	Right	Encode		0.224701
Inferior temporal gyrus	Left	Retrieve		0.225714
Inferior frontal gyrus	Left	Retrieve		0.256126
Precentral gyrus	Left	Retrieve		0.300738
Angular gyrus	Left	Encode		0.303758
Angular gyrus	Left	Retrieve		0.304547
Middle occipital gyrus	Right	Encode		0.312098
BA9+46	Right	Retrieve		0.332928

Region	Side	Phase	Adjusted critical *p*	*p*, Control vs. Patient
Middle frontal gyrus	Left	Retrieve		0.333765
Lingual gyrus	Right	Retrieve		0.336851
BA9+46	Right	Encode		0.336982
Middle occipital gyrus	Right	Retrieve		0.369889
Fusiform gyrus	Left	Retrieve		0.371172
Superior temporal gyrus	Right	Retrieve		0.381637
Middle occipital gyrus	Left	Encode		0.406840
Medial frontal gyrus	Right	Encode		0.408264
Precentral gyrus	Right	Encode		0.433158
Superior frontal gyrus	Left	Retrieve		0.453166
Middle occipital gyrus	Left	Retrieve		0.463638
BA9+46	Left	Retrieve		0.506478
Superior temporal gyrus	Right	Encode		0.533168
Fusiform gyrus	Left	Encode		0.542354
Superior temporal gyrus	Left	Encode		0.546415
Angular gyrus	Right	Encode		0.570229
Fusiform gyrus	Right	Encode		0.602751
Inferior frontal gyrus	Right	Encode		0.609650
Inferior temporal gyrus	Left	Encode		0.640879
Inferior occipital gyrus	Left	Encode		0.661325
BA9+46	Left	Encode		0.663627
Middle frontal gyrus	Right	Retrieve		0.706760
Angular gyrus	Right	Retrieve		0.720637
Middle frontal gyrus	Left	Encode		0.735581

Region	Side	Phase	Adjusted critical *p*	*p*, Control vs. Patient
Inferior temporal gyrus	Right	Encode		0.746259
Lingual gyrus	Left	Encode		0.746259
Inferior occipital gyrus	Right	Encode		0.754435
Superior frontal gyrus	Right	Retrieve		0.766230
Inferior occipital gyrus	Left	Retrieve		0.772802
Precentral gyrus	Left	Encode		0.808344
Medial frontal gyrus	Left	Encode		0.810052
Inferior frontal gyrus	Right	Retrieve		0.821050
Precentral gyrus	Right	Retrieve		0.823111
Superior frontal gyrus	Left	Encode		0.836794
Fusiform gyrus	Right	Retrieve		0.866342
Inferior temporal gyrus	Right	Retrieve		0.880018
Lingual gyrus	Right	Encode		0.934530
Inferior occipital gyrus	Right	Retrieve		0.938122
Superior frontal gyrus	Right	Encode		0.996022

Table 13

FSL ANOVA comparing mean percent signal change for patients with moderate CKD, patients with severe CKD, and healthy controls.

Region	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Superior parietal lobule	Encode	0.001	0.00125	0.007	0.001	1.000
BA7	Retrieve	0.012	0.00128	0.019	0.062	1.000

Region	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Superior parietal lobule	Retrieve	0.019		0.019	0.178	0.874
BA7	Encode	0.032		0.111	0.051	1.000
Lingual gyrus	Retrieve	0.037		0.743	0.032	0.646
Posterior cingulate	Retrieve	0.056				
Anterior cingulate	Encode	0.079				
Medial frontal gyrus	Retrieve	0.100				
Precuneus	Retrieve	0.135				
Superior temporal gyrus	Retrieve	0.153				
Medial frontal gyrus	Encode	0.164				
Anterior cingulate	Retrieve	0.169				
Superior temporal gyrus	Encode	0.172				
Inferior occipital gyrus	Retrieve	0.188				
Precuneus	Encode	0.230				

Region	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Inferior frontal gyrus	Encode	0.238				
Superior frontal gyrus	Encode	0.251				
Middle occipital gyrus	Encode	0.328				
Precentral gyrus	Encode	0.340				
Middle frontal gyrus	Encode	0.404				
Posterior cingulate	Encode	0.412				
Middle occipital gyrus	Retrieve	0.489				
BA46+9	Encode	0.496				
Angular gyrus	Encode	0.499				
Inferior frontal gyrus	Retrieve	0.589				
Superior frontal gyrus	Retrieve	0.618				
Angular gyrus	Retrieve	0.644				

Region	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Inferior temporal gyrus	Retrieve	0.766				
Inferior temporal gyrus	Encode	0.791				
Fusiform gyrus	Retrieve	0.813				
Precentral gyrus	Retrieve	0.819				
Fusiform gyrus	Encode	0.841				
Middle frontal gyrus	Retrieve	0.855				
BA46+9	Retrieve	0.860				
Lingual gyrus	Encode	0.987				

Table 14

FSL ANOVA separately considering left and right hemispheres for each brain region, comparing mean percent signal change for patients with moderate CKD, patients with severe CKD, and healthy controls.

Region	Side	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Superior frontal gyrus	Right	Encode	0.002	0.00125	0.185	0.137	0.002

Region	Side	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
BA7	Left	Encode	0.003		0.806	0.003	0.048
Angular gyrus	Right	Encode	0.004		0.706	0.052	0.003
Lingual gyrus	Left	Encode	0.006		0.245	0.230	0.005
Middle frontal gyrus	Left	Retrieve	0.007		0.043	1.000	0.009
Superior frontal gyrus	Left	Retrieve	0.007		0.067	0.923	0.008
Medial frontal gyrus	Right	Encode	0.008		0.059	1.000	0.009
Posterior cingulate	Left	Encode	0.009		1.000	0.021	0.018
Inferior occipital gyrus	Right	Encode	0.011		0.607	0.147	0.009
Superior frontal gyrus	Left	Encode	0.011		0.510	0.185	0.009
Superior parietal lobule	Left	Encode	0.016		0.039	0.039	1.000
Inferior temporal gyrus	Left	Retrieve	0.017		0.055	1.000	0.029
Posterior cingulate	Left	Retrieve	0.018		0.015	0.544	0.383

Region	Side	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Medial frontal gyrus	Left	Encode	0.020		0.284	0.535	0.017
Superior parietal lobule	Right	Encode	0.020		0.122	0.024	1.000
BA7	Right	Encode	0.022		0.807	0.019	0.237
Lingual gyrus	Right	Encode	0.024		0.539	0.329	0.021
BA46+9	Left	Retrieve	0.027		0.155	1.000	0.029
Medial frontal gyrus	Left	Retrieve	0.028		0.036	1.000	0.115
Inferior temporal gyrus	Left	Retrieve	0.034		0.075	1.000	0.067
Medial frontal gyrus	Right	Retrieve	0.040		0.071	1.000	0.093
Middle occipital gyrus	Right	Encode	0.052				
Lingual gyrus	Left	Retrieve	0.067				
BA7	Left	Retrieve	0.071				
Inferior temporal gyrus	Left	Encode	0.073				

Region	Side	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Superior temporal gyrus	Left	Retrieve	0.075				
Superior temporal gyrus	Right	Encode	0.077				
Superior temporal gyrus	Left	Encode	0.080				
Superior parietal lobule	Left	Retrieve	0.097				
Inferior occipital gyrus	Left	Encode	0.120				
Middle occipital gyrus	Left	Encode	0.152				
Fusiform gyrus	Left	Retrieve	0.194				
Fusiform gyrus	Right	Retrieve	0.204				
BA46+9	Right	Retrieve	0.208				
BA7	Right	Retrieve	0.210				
Fusiform gyrus	Left	Encode	0.215				
Superior parietal lobule	Right	Retrieve	0.232				

Region	Side	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Fusiform gyrus	Right	Encode	0.263				
Precentral gyrus	Left	Retrieve	0.263				
Angular gyrus	Left	Encode	0.264				
Inferior temporal gyrus	Left	Encode	0.277				
Precentral gyrus	Right	Retrieve	0.374				
Superior frontal gyrus	Right	Retrieve	0.374				
Middle frontal gyrus	Left	Encode	0.447				
Angular gyrus	Left	Retrieve	0.486				
Precentral gyrus	Left	Encode	0.501				
BA46+9	Right	Encode	0.587				
Inferior temporal gyrus	Right	Encode	0.607				
Posterior cingulate	Right	Encode	0.608				
BA46+9	Left	Encode	0.608				

Region	Side	Phase	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Lingual gyrus	Right	Retrieve	0.614				
Middle occipital gyrus	Right	Retrieve	0.626				
Precentral gyrus	Right	Encode	0.724				
Angular gyrus	Right	Retrieve	0.740				
Middle occipital gyrus	Left	Retrieve	0.745				
Superior temporal gyrus	Right	Retrieve	0.759				
Inferior temporal gyrus	Right	Encode	0.828				
Inferior occipital gyrus	Left	Retrieve	0.945				
Inferior temporal gyrus	Right	Retrieve	0.951				
Inferior occipital gyrus	Right	Retrieve	0.984				
Inferior temporal gyrus	Right	Retrieve	0.987				

Table 15

Eventstats t-tests comparing mean percent signal change over entire course of working memory task in patients with CKD vs. controls.

Region	Control, mean % signal change over task	Patient, mean % signal change over task	Adjusted critical *p*	*p*, Control vs. Patient
Angular gyrus	0.001445	0.000709	0.00125	< 0.001
Insula	0.000984	0.002173	0.00128	< 0.001
Lingual gyrus	0.001244	0.000810	0.00132	< 0.001
Middle occipital gyrus	0.000866	0.000521	0.00135	< 0.001
Posterior cingulate	0.000684	0.000283	0.00139	< 0.001
Superior occipital gyrus	0.001535	0.000749	0.00143	< 0.001
Superior parietal lobule	0.003483	0.002173	0.00147	< 0.001
BA7	0.002337	0.001750	0.00152	< 0.001
Medial frontal gyrus	0.000632	0.000877	0.00156	0.002
Superior temporal gyrus	0.000704	0.000947	0.00161	0.006
Precuneus	0.001661	0.001329	0.00167	0.009
Precentral gyrus	0.001165	0.000994	0.00172	0.047
BA 46+9	0.001168	0.001386		0.047
Inferior occipital gyrus	0.000557	0.000413		0.165
Superior frontal gyrus	0.000988	0.000861		0.299

Region	Control, mean % signal change over task	Patient, mean % signal change over task	Adjusted critical *p*	*p*, Control vs. Patient
Inferior temporal gyrus	0.000949	0.000855		0.300
Inferior frontal gyrus	0.000963	0.001018		0.547
Anterior cingulate	0.000709	0.000765		0.554
Fusiform gyrus	0.000645	0.00597		0.569
Middle frontal gyrus	0.001356	0.001195		0.090

Table 16

Eventstats t-tests comparing individual peak signal change in patients with CKD and controls for each phase of the task.

Region	Phase	Control	Patient	Adjusted critical *p*	*p*, Control vs. Patient
BA7	Encode	0.008542	0.006194	0.00125	0.0314
Precentral gyrus	Encode	0.005605	0.003911		0.0315
Superior occipital gyrus	Encode	0.008899	0.005845		0.0343
Superior parietal lobule	Encode	0.004438	0.004800		0.047
Superior parietal lobule	Retrieve	0.006265	0.006626		0.1411
Posterior cingulate	Retrieve	0.005341	0.004328		0.1491
Superior frontal gyrus	Encode	0.003378	0.004455		0.1971
Fusiform gyrus	Encode	0.004172	0.006554		0.2142

Region	Phase	Control	Patient	Adjusted critical *p*	*p*, Control vs. Patient
Fusiform gyrus	Retrieve	0.005196	0.006910		0.2546
Insula	Encode	0.003900	0.003299		0.2737
Medial frontal gyrus	Retrieve	0.005944	0.007326		0.2932
Lingual gyrus	Encode	0.005363	0.006592		0.31721
Angular gyrus	Encode	0.004887	0.004108		0.3303
Precuneus	Encode	0.005322	0.004459		0.3368
Superior occipital gyrus	Retrieve	0.007777	0.006870		0.3501
Inferior occipital gyrus	Retrieve	0.006052	0.007609		0.3591
BA46+9	Encode	0.005791	0.005265		0.3693
Inferior frontal gyrus	Retrieve	0.006608	0.008065		0.3783
Anterior cingulate	Retrieve	0.004864	0.005554		0.4111
Medial frontal gyrus	Encode	0.003036	0.003471		0.4692
Anterior cingulate	Encode	0.002566	0.002903		0.5120
Inferior occipital gyrus	Encode	0.005338	0.006665		0.5389
Middle occipital gyrus	Retrieve	0.005068	0.005785		0.5448
Inferior temporal gyrus	Encode	0.005421	0.004999		0.5757
BA46+9	Retrieve	0.008403	0.009295		0.5913
Superior frontal gyrus	Retrieve	0.008149	0.009171		0.5913

Region	Phase	Control	Patient	Adjusted critical *p*	*p*, Control vs. Patient
Superior temporal gyrus	Encode	0.012418	0.008227		0.5998
Inferior frontal gyrus	Encode	0.004168	0.003923		0.6370
Inferior temporal gyrus	Retrieve	0.005683	0.006392		0.6626
BA7	Retrieve	0.008542	0.006194		0.7212
Lingual gyrus	Retrieve	0.006209	0.006566		0.7443
Middle frontal gyrus	Retrieve	0.007582	0.008027		0.7445
Posterior cingulate	Encode	0.003396	0.003166		0.7459
Angular gyrus	Retrieve	0.006060	0.005799		0.7527
Superior temporal gyrus	Retrieve	0.010271	0.008243		0.7544
Precentral gyrus	Retrieve	0.006062	0.005814		0.7813
Insula	Retrieve	0.005675	0.005565		0.8673
Middle frontal gyrus	Encode	0.004617	0.004723		0.8763
Middle occipital gyrus	Encode	0.005296	0.005373		0.9415
Precuneus	Retrieve	0.006545	0.006599		0.9535

Table 17

Eventstats ANOVA comparing average percent signal change over entire course of task for patients with moderate CKD, patients with severe CKD, and healthy controls.

Region	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Angular gyrus	< 0.001	0.00125	0.096	< 0.001	< 0.001
Middle occipital gyrus	< 0.001	0.00128	1.000	< 0.001	< 0.001
Superior parietal lobule	< 0.001	0.00132	< 0.001	< 0.001	< 0.001
Superior frontal gyrus	< 0.001	0.00135	0.021	0.001	< 0.001
Inferior temporal gyrus	< 0.001	0.00139	0.110	0.005	< 0.001
Inferior occipital gyrus	< 0.001	0.00143	0.667	0.016	< 0.001
Anterior cingulate	< 0.001	0.00147	0.003	0.280	< 0.001
Medial frontal gyrus	< 0.001	0.00152	< 0.001	1.000	< 0.001
Superior temporal gyrus	< 0.001	0.00156	< 0.001	1.000	< 0.001
Fusiform gyrus	< 0.001	0.00161	0.246	0.113	0.001
Precuneus	< 0.001	0.00167	1.000	0.001	0.008
Posterior cingulate	< 0.001	0.00172	0.486	< 0.001	0.022
Middle frontal gyrus	< 0.001	0.00179	1.000	0.025	0.041
Lingual gyrus	< 0.001	0.00185	0.014	< 0.001	0.299

Region	*p*, ANOVA	Adjusted critical *p*	*p*, Control vs. Moderate	*p*, Control vs. Severe	*p*, Moderate vs. Severe
Superior occipital gyrus	< 0.001	0.00192	< 0.001	< 0.001	1.000
Precentral gyrus	0.001	0.00200	1.000	0.003	0.005
Inferior frontal gyrus	0.002		0.035	0.843	0.001
BA7	0.005		0.769	0.004	0.210
BA46	0.141				
Insula	0.449				

APPENDIX 2

IRB approval letter

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

OFFICE OF HUMAN RESEARCH ETHICS
Medical School Building 52
Mason Farm Road
CB #7097
Chapel Hill, NC 27599-7097
(919) 966-3113
Web site: ohre.unc.edu
https://my.research.unc.edu for IRB status
Federalwide Assurance (FWA) #4801

To: Stephen Hooper
Psychiatry
CB: 7255

From: Biomedical IRB

Authorized signature on behalf of IRB

Approval Date: 6/10/2010
Expiration Date of Approval: 6/09/2011

RE: Notice of IRB Approval by Expedited Review (under 45 CFR 46.110)
Submission Type: Renewal
Expedited Category: 8(c) Continuing Review - Data Analysis Only
Study #: 03-0830 (Former IRB Number 03-MED-33)

Study Title: Functional Neuroimaging and Chronic Kidney Disease in Children
Sponsors: Renal Research Institute

This submission has been approved by the above IRB for the period indicated.

Study Description:
The purpose of this research is to conduct a pilot study to evaluate the changes in functional neuroimaging with a working memory task between children with chronic kidney disease (CKD) and unaffected controls. This is a case control pilot study that consists of a single fMRI assessment (without sedation) and specific tasks in the area of working memory. Enrolled subjects with mild to end-stage renal disease and unaffected controls between 9 and 19 years old will be enrolled.

Regulatory and other findings:
This research, which involves children, meets criteria at 45 CFR 46.404 (research involving no greater than minimal risk). Permission of one parent or guardian is sufficient.

This research, which was originally approved by the Full Board, is being renewed by the IRB under Expedited Review, Category 9. The Board agreed that it involves no more than minimal risk and future reviews may be done on an expedited basis.

This research, which was originally approved by the Full Board, is being renewed by the IRB under Expedited Review, Category 8c. The research has been closed to the accrual of new subjects and all subjects have completed intervention/interaction. Renewal is granted for data analysis only.

Investigator's Responsibilities:
Federal regulations require that all research be reviewed at least annually. It is the Principal Investigator's responsibility to submit for renewal and obtain approval before the expiration date.

You may not continue any research activity beyond the expiration date without IRB approval. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.

When applicable, enclosed are stamped copies of approved consent documents and other recruitment materials. You must copy the stamped consent forms for use with subjects unless you have approval to do otherwise.

You are required to obtain IRB approval for any changes to any aspect of this study before they can be implemented (use the modification form at ohre.unc.edu/forms). Any unanticipated problem involving risks to subjects or others (including adverse events reportable under UNC-Chapel Hill policy) should be reported to the IRB using the web portal at https://irbis.unc.edu/irb.

This study was reviewed in accordance with federal regulations governing human subjects research, including those found at 45 CFR 46 (Common Rule), 45 CFR 164 (HIPAA), 21 CFR 50 & 56 (FDA), and 40CFR 26 (EPA), where applicable.

CC: Debbie Gipson, Medicine

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