Section II: Specialized Information

1. Introduction to Revised Application – Not Applicable

2. Letters of Reference:
Attached, please find letters of reference from Terry L. Jernigan, Raymond K. Mulhern, and Susan F. Tapert.

3. The Candidate

3a. Candidate's Background: Please see attached biographical sketch.

3b. Career Goals and Objectives: Scientific Biography
My interest in research began during my undergraduate education at Washington State University. Early exposure to scientific methods and theories in biology and psychology spawned my curiosity for studying the human brain and behavior. To explore my interest, I chose to become actively involved in Dr. Maureen Schmitter-Edgecombe's research laboratory studying cognitive functioning in aging adults. This research experience provided me with an introduction to research design and participant recruitment, and allowed for my first training in neuropsychological assessment. As I also had an interest in pediatric populations, I sought further research experience by working in Dr. Tristram Smith's autism laboratory. There, I gained additional research experience by participating in the writing of Institutional Review Board (IRB) proposals and quality control measures for behavioral therapy with autistic children. In addition to learning more formal research procedures, I was trained in pediatric neuropsychological assessment and behavioral therapy with autistic children. These initial research and clinical experiences prompted my decision to pursue a graduate education in clinical psychology and instilled interest in developmental issues in neurocognitive functioning. In 2003, I received my Ph.D. in clinical psychology with an emphasis in neuropsychology from the University of Memphis. Throughout my graduate school training, the majority of my clinical and research experience focused on human neurodevelopment and neuropathology. As a part of my curriculum, I completed intensive coursework in the biological bases of behavior, neuropsychological assessment, and human neuroanatomy. I initially spent three years studying some of the earliest developmental processes and deviance in those processes. Working under the umbrella of Dr. Sarah Raz's NIH-funded longitudinal project, I had the opportunity to study infants and young children who were born prematurely, suffered from birth complications, and whose mothers had pregnancy complications. While much of my role on these projects was focused on assessment, this experience provided insight into peri- and post-natal developmental processes as well as into disruption of normal neurodevelopment. This work resulted in a collaborative poster presentation and published abstract for the 2001 annual meeting of the International Neuropsychological Society (INS).

As a part of my clinical training, I spent several years conducting neuropsychological evaluations on pediatric populations and solidifying my focus on neurodevelopment. This clinical work provided exposure to many different types of neurodevelopmental abnormalities, as well to assessment tools that are sensitive to cognitive impairments in children. While patients were referred for a variety of neuropsychological impairments, deficits in the areas of learning and memory were frequently the issue of concern. This experience furthered my understanding of brain and behavior relationships and ways in which to assess those relationships both in clinical and research settings. In addition, these clinical experiences fostered my desire to begin studying memory dysfunction in pediatric populations.

Throughout graduate school, I collaborated on several projects examining memory functioning in individuals with traumatic brain injury and ways in which to estimate premorbid memory

ability. This work was presented at the 1999 through 2001 annual meetings of the National Academy of Neuropsychology, and provided me with further experience in research design, data collection, and analyses in the area of human memory research. Later in my graduate education, I began to study memory dysfunction in a specific pediatric population – pediatric brain tumor patients. This work became my primary dissertation project. While it had been demonstrated previously that these children evidence post-treatment memory impairments, the underlying neuropathology associated with these functional deficits had not been delineated. Based upon extensive work documenting the central role of the hippocampus in memory formation, I chose to focus on the hippocampus as a likely candidate for neuropathology in children treated for medulloblastoma (a brain tumor located within the posterior fossa region of the brain). Working with psychologists, engineers, radiologists, and oncologists in a highly collaborative, multi-disciplinary setting at St. Jude Children's Research Hospital, I was afforded the opportunity to learn new research skills that I have continued to use and build upon for studying neurodevelopment. I acquired experience with longitudinal study design and analyses, structural neuroimaging, and became proficient in a technique for measuring hippocampal volume. My dissertation results demonstrated that children treated for medulloblastoma evidence abnormal hippocampal volume development after diagnosis that may potentially be moderated by radiation tissue-dose exposure. These findings were presented at the INS annual conference in 2003, and have recently been published in the *American Journal of Neuroradiology* [1]. In addition to examining hippocampal morphology in this population, I am continuing collaborative efforts by working on a manuscript examining verbal memory functioning in children with medulloblastoma as compared to demographically similar control children.

Through my clinical and research experiences with pediatric populations, I became increasingly aware of the lack of normal developmental literature in the area of memory and its respective neuroanatomical underpinnings, despite the demonstration of memory dysfunction in a variety of types of pediatric neuropathology. The sparcity of normal developmental research is particularly true for the adolescent age range, and in part, may be due to the fact that until recently, it was considered appropriate to use adult models of brain and behavior relationships (e.g., neuroanatomical substrates of memory) to understand adolescents. However, it is now clear that the adolescent brain is actively developing, and adult findings cannot be generalized. This realization prompted my post-doctoral research endeavors.

In order to continue to pursue my research interests, I applied for and was accepted into the NIMH-sponsored Biological Psychiatry and Neuroscience fellowship program at the University of California San Diego (UCSD). During my post-doctoral fellowship years, I have investigated neurodevelopment and working memory functioning in normally developing youths under the primary guidance of Dr. Susan Tapert. In order to expand on the existing literature of normal neuromaturation and the neural bases of working memory functioning, I received training on utilizing a combination of neuroimaging techniques to provide greater depth in understanding of normal developmental processes. Specifically, I have been using structural neuroimaging to volumetrically examine the hippocampus and frontal lobe white matter in typically developing adolescents (ages 12 to 18 years). These structural brain findings are being correlated with verbal and spatial memory tasks to determine the relationship between regional volume and memory function. Using functional magnetic resonance imaging (fMRI), I have examined age-related patterns of spatial working memory brain activation as a function of adolescent age (Preliminary Studies). This work has resulted in a recent manuscript submission. Combining neuropsychological test data and fMRI data, I have also examined the relationship between neuropsychological performance and fMRI response to a spatial working memory task in normally developing adolescents. The findings from this study demonstrated that among

typically developing adolescents, those with better neuropsychological test performance (specifically working memory abilities) tend to have less brain activation to a spatial working memory task, suggesting that for those individuals, perhaps fewer neural resources are required to achieve the same level of performance as teens with poorer neuropsychological functioning. This work in currently In Press in the *Journal of Clinical and Experimental Neuropsychology [2]*. In addition, I have collaborated with members of Dr. Tapert's lab to examine several types of cognitive functioning, brain morphology, and fMRI response in alcohol use disordered and control teens. These collaborations have resulted in two additional manuscript submissions and several poster presentations at various scientific conferences. Combining structural and functional imaging techniques, I have begun to examine how prefrontal white matter volumes relate to changes in patterns of functional activation during a spatial working memory task across adolescent neurodevelopment (Preliminary Studies). This work will be presented at the 2005 annual INS conference. My fellowship program has allowed me to build on my existing skills in neuropsychological assessment and has furthered my knowledge of and experience with structural neuroimaging. In addition, I have been working in a resource-abundant environment where I have been able to begin to learn and use functional neuroimaging.

My primary career objective is to become an independent investigator of the neurobiological substrates underlying changes and improvements in cognitive functioning across the adolescent age range. By writing this application, I hope to further my existing knowledge of neuroimaging and neuromaturation by learning the novel technique of diffusion tensor imaging (DTI) which allows for *in-vivo* assessment of white matter microstructure and organization and by acquiring the necessary skills to conduct longitudinal behavioral and neuroimaging studies needed to investigate neurodevelopment.

In summary, with this Mentored Research Career Award, I hope to build on my graduate, dissertation, and postdoctoral work by acquiring the necessary skills to establish an independent program of research to study neuromaturation and cognition in developing youths. The resources of this award will allow me to pursue the answers to my own research questions, while providing me with the expertise and mentorship of a team of highly qualified sponsors and consultants. It is my hope that the answers to these research questions will lead to a better understanding of the neuroanatomical substrates underlying adolescent working memory, and that this, in turn, will provide a much-needed framework and information base for use in studying pediatric neuropathology and associated working memory dysfunction.

3c. Career Development/Training Activities During the Award Period

The primary goal of this training award is to acquire the necessary knowledge and skills for becoming an independent investigator of adolescent neurodevelopment. To that end, there are several skills that I need to develop and refine. The proposed training site provides a unique opportunity for me to receive excellent mentorship for all of my training needs. These training goals are designed to complement my desired research activities and aims.

Training Goal 1. In order to further scientific knowledge of white matter development, microstructure, and organization using the best *in vivo* tool available, the goal is to (a) learn the basic theoretical underpinnings of DTI and what information it can provide about white matter structure, (b) learn the experimental methods for DTI data collection, and (c) learn the methods for analyzing DTI data.

As technology is rapidly evolving, more tools are becoming available to investigate brain development. DTI is one of the newest techniques available for studying white matter structure and physiology during maturation. During adolescence, white matter is in an active state of

development, making this is a critical time to quantify its characteristics and changes. Co-sponsor Dr. Lawrence Frank is an Associate Professor of Radiology at the UCSD School of Medicine, Associate Director for Biomedical Applications at the UCSD Center for FMRI (CFMRI), and Director of Magnetic Resonance (MR) research at the VA San Diego Healthcare System. In addition to over 50 research publications in the area of MR physics, he has recently authored three papers on high angular resolution diffusion weighted imaging [3-5], and co-authored several published abstracts using DTI techniques for studying white matter in adolescent populations [6, 7]. In addition, Dr. Frank has been developing "plug-in" tools for the analyses of DTI data to be used in conjunction with the Analysis of Functional NeuroImages (AFNI) program [8].

To achieve this training goal, several activities are planned that will include structured coursework, regular meetings, and hands-on training. More specifically, I will attend a DTI course at the International Society for Magnetic Resonance in Medicine's (ISMRM) annual scientific meeting and exhibition. This course is designed to teach the underlying physics of DTI, explain how data are collected, and cover limitations and applications of the technique to clinical research. I will also take a directed readings course taught by Dr. Frank that will include more specifics on the physics of DTI, its application to studying neurodevelopment, and DTI data analysis. In addition to formal coursework, I will meet bi-monthly with Dr. Frank to discuss the application of DTI to my research questions and will participate in direct training in DTI analyses, using my own data, in his laboratory.

Training Goal 2. As fMRI is a technique that allows one to examine *in vivo* brain and behavior relationships in typically developing adolescents, I propose to continue to develop expertise in the design, collection, and analysis of fMRI data.

While I have had the opportunity to begin to learn and utilize fMRI techniques during my post-doctoral fellowship years, I seek to gain a level of expertise that will allow me to function as an independent investigator in the field of neuroimaging. To that end, under the tutelage of the proposed mentorship, I will be refining the fMRI task to be used in the current study, learning ways of collecting fMRI data that are amenable to longitudinal follow-up, and learning new ways of analyzing development trends in fMRI data.

My primary sponsor, Dr. Joan Stiles, is a Professor in the Department of Cognitive Science at UCSD and is an internationally recognized investigator in the area of developmental fMRI research. She has established a highly productive research laboratory investigating the relationships between brain development and the development of basic spatial cognitive abilities, and is currently the principal investigator of an R01 grant using fMRI techniques to examine the development of visuospatial processing. Dr. Stiles has an extensive record of training young investigators, and will be instrumental in mentoring me toward the above training objective.

In addition to primary sponsorship by and bi-monthly meetings with Dr. Stiles, I will have regular access to several authoritative consultants that specialize in various aspects of fMRI research. Dr. Greg Brown is a Professor in the Department of Psychiatry at the UCSD School of Medicine, Associate Director of Clinical Neuroscience at the UCSD Center for Functional MRI, and Co-Director of the Department of Psychiatry's Laboratory of Cognitive Imaging (LOCI). Dr. Brown has a wealth of experience in fMRI research design and analyses and has mentored many junior investigators toward independence. Dr. Susan Tapert is an Assistant Professor in the Department of Psychiatry at the UCSD School of Medicine and has published and obtained several NIH-funded grants on the study of adolescents with substance use disorders using

fMRI. She has provided excellent mentorship during my fellowship program and will continue to provide insight unique to the study of adolescents using fMRI techniques.

There are several training activities planned for the length of the award designed to assist me in meeting the above training goal. These activities involve a formal Cognitive Neuroimaging course taught by Dr. Ralph-Axel Mueller, an Assistant Professor of Psychology, at San Diego State University (SDSU). This course teaches the basic principals of both structural and functional neuroimaging techniques and discusses practical and analytical issues for the application of neuroimaging to a variety of populations and cognitive research areas. In addition to formal coursework, I will regularly attend both Greg Brown's weekly didactic lab meetings and the LOCI's bimonthly neuroimaging seminar where issues in MRI task design, data collection, and analyses are addressed and receive direct consultation by the above-mentioned sponsor and consultants.

Training Goal 3. In that longitudinal studies are the most accurate way to examine developmental change and processes, I will acquire the necessary knowledge and skills to design and implement future longitudinal neuroimaging studies of adolescents.

Although the currently proposed study is cross-sectional in nature, I ultimately hope to study development using longitudinal methods. To that end, I plan to design the current study and collect data so as to facilitate pilot longitudinal data collection to be used in a future R01 grant application and longitudinal study. Many of my sponsors and consultants have been involved in longitudinal study design and data collection and offer a wide breadth of knowledge regarding various aspects of such studies. Drs. Stiles, S. Brown, and Gamst have been involved in longitudinal neuroimaging studies and will be able to provide consultation and mentoring regarding a variety of issues relevant to such research. The training activities set forth in order for me to achieve this goal include regular and direct consultation with the above mentors, as well as formal coursework. During the first year of the training grant, I will attend the APA Advanced Training Institute in Longitudinal Research Methods – a week-long course in the fundamentals of longitudinal research methodology featuring lectures and hands-on computer laboratory time.

An important aspect to any study using human subjects is recruitment of participants. Recruitment becomes more complicated when studying children and adolescents, particularly when attempting to prepare for longitudinal follow-up. Dr. Sandra Brown has worked extensively with local schools and administrators to develop working relationships in the San Diego area. She has over 20 years of experience with a longitudinal study examining the developmental impact of adolescent and young adult substance involvement and has been the principal investigator of two longitudinal studies of youth (up to 20 year follow-up). She is currently the principal investigator on an R37 MERIT Award involving the longitudinal examination of adolescent development and substance use behavior, an R01 adolescent school-based intervention study, as well as Co-Director of a training grant for the development of new investigators in the area of alcohol research. While Dr. Sandra Brown's primary research area involves the study of adolescent substance use behaviors, her involvement in developmentally-focused longitudinal studies of adolescents, ties to the San Diego school and clinical services community, and extensive track record of training young investigators makes her an excellent mentor in the strategies for the recruitment of, research with, and follow-up of adolescent populations. I will meet bi-monthly with Dr. Sandra Brown to receive mentoring toward this training goal and attend her weekly research seminar where research designs and methods for longitudinal studies of youth are discussed.

Training Goal 4. In order to become an independent investigator of adolescent development with longitudinal models and to effectively combine multimodal neuroimaging data in analyses, I plan to develop competence in advanced statistical methods and analytical techniques.

In graduate school, I took courses in statistics and have received training in the use of general linear models, multiple regression analyses, and analysis of variance techniques. My dissertation was based on longitudinal data collection, and while I did not independently perform the statistical analyses, I did gain experience in the interpretation of this type of analysis. To build on this initial exposure to longitudinal data analysis, and to pursue the study of development in a longitudinal fashion, it is imperative that I receive training in the analysis of developmental trajectories. In addition, I need training on how to statistically analyze combined neuroimaging data from different neuroimaging modalities (e.g., fMRI and DTI data). One of my consultants, Dr. Gamst, is an Assistant Professor of statistics in the Departments of Neuroscience and Family and Preventative Medicine at the UCSD School of Medicine. He plays a critical role in statistical analyses of cross-sectional and longitudinal neuroimaging studies/grants. In addition, Dr. Gamst is a statistician for the UCSD Biostatistics and Informatics Laboratory (BIL), a program in which statistical training and assistance with analyses are available.

In order to achieve competence in more advanced statistical analyses, I have set forth a series of training activities including formal coursework, directed readings, and direct assistance from biostatisticians. During the first year of my training, I will take a directed readings course taught by Dr. Gamst to familiarize me with the various methods and techniques for statistically analyzing longitudinal data and combining multimodal neuroimaging data. During my third training year, and after I've had time to collect some of my own data, I will attend a summer course taught at Penn State entitled, "Summer Institute on Longitudinal Methods: Analyzing Developmental Trajectories." This course will prepare me to subsequently analyze developmental trajectories in longitudinal data. In addition to the more formal training activities, I will receive direct instruction and assistance with my own cross-sectional data from the UCSD Biostatistics and Informatics Laboratory as well as through monthly meetings with Dr. Gamst. I will also have the advantage of having regular contact with my sponsors and consultants, all of whom have a wealth of experience in various types of statistical analyses.

Training Goal 5. Continued training in the responsible and ethical conduct of scientific research with children and adolescents.

Ethical issues are important when conducting any type of research, and play a particularly important role in studies involving children. While I have received training in ethics throughout my scientific career, I will continue to receive mentoring and formal training in this critical area. The combination of well-seasoned sponsors and consultants involved with this grant will provide instruction in the proper methods of parent and youth consenting approaches, conducting subject interviews, ethical treatment of subjects, and subject confidentiality. In addition to the training provided by my sponsors and consultants, I will participate in formal coursework in the area of scientific ethics as well as monthly seminars to sustain a level of heightened awareness around these issues. Specially, during my first year of training, I will attend a Scientific Ethics course taught by Dr. Michael Kalichman at UCSD. This course is designed to engage trainees in reading, considering, and discussing the response conduct of science and review such issues as human subjects, data management, and conflicts of interest. Throughout my training, I will also attend a monthly Biomedical Ethics Seminar Series at UCSD that focus on a variety of topics related to ethics in scientific research.

Training Activities – Timeline

Training Goal:	Year 1	Year 2	Year 3	Year 4	Year 5
1. Learn DTI	Directed readings course taught by Dr. Larry Frank	Hands on instruction and assistance in Dr. Larry Frank's research lab			
		DTI course at the ISMRM Annual Scientific Meeting & Exhibition			
	Bi-monthly meetings with Dr. Larry Frank				
2. Develop expertise in fMRI	Cognitive Neuroimaging course at SDSU taught by Dr. Ralph-Axel Mueller (Psy 796)				
	Bi-monthly meetings with Dr. Joan Stiles				
	Monthly meetings with Drs. Susan Tapert and Greg Brown				
	Weekly attendance of Dr. Greg Brown's didactic lab meetings				
	Bi-weekly Neuroimaging Seminar – UCSD LOCI				
3. Longitudinal study design and recruitment	APA Advanced Training Institute in Longitudinal Research Methods				
	Bi-monthly meetings with Drs. Joan Stiles and Sandra Brown				
	Monthly meetings with Dr. Anthony Gamst				
	Weekly attendance of Dr. Sandra Brown's research seminar				
4. Advanced data analytic techniques	Directed readings course taught by Dr. Anthony Gamst		Summer Institute on Longitudinal Methods: Analyzing Developmental Trajectories – Course taught at Penn State		
		Monthly statistical consultation with Dr. Anthony Gamst			
	Directed assistance and instruction from the UCSD Biostatistics and Informatics Laboratory				
5. Ethical conduct of scientific research	Scientific Ethics course at UCSD taught by Dr. Michael Kalichman (SOMI 226)				
	Monthly UCSD Biomedical Ethics Seminar Series				
	Bi-monthly meetings with Drs. Joan Stiles and Sandra A. Brown				

Research Activities – Timeline

Year 1	Year 2	Year 3	Year 4	Year 5
Refine and pilot fMRI task				
Set-up database for study				
Recruit and test 10 teens	Recruit and test 20 teens	Recruit and test 20 teens	Recruit and test 20 teens	Recruit and test 20 teens
Draw prefrontal regions of interest to be used in DTI and fMRI analyses	Analyze neuropsychological, fMRI, and DTI data; write and submit manuscript/s			
	Submit work to scientific conferences (International Neuropsychological Society and Society for Neuroscience)			
		Write and submit R01 grant to fund longitudinal follow-up of study participants		Collect pilot longitudinal follow-up data

4. Statement by Sponsors and Consultants

The following individuals have agreed to serve as sponsors and consultants for the proposed award. These individuals comprise a group of nationally recognized experts who will be integral in implementing the proposed training and research activities. Following, please find statements from the following Sponsors and Consultants:

Joan Stiles, Ph.D., Primary Sponsor
Larry Frank, Ph.D., Co-sponsor
Sandra A. Brown, Ph.D., Co-sponsor
Anthony C. Gamst, Ph.D., Consultant
Greg G. Brown, Ph.D., Consultant
Susan F. Tapert, Ph.D., Consultant

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Joan Stiles, Ph.D.
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LA JOLLA, CALIFORNIA 92093

To Whom It May Concern:

This statement is to indicate my strong support and enthusiastic commitment to serve as the sponsor/mentor for Dr. Bonnie Nagel's proposed Mentored Clinical Scientist Development Award (K-08) on "Adolescent Working Memory and White Matter Development." I have had the pleasure of working closely with Dr. Nagel throughout the planning of this proposal and look forward to serving as her mentor throughout the duration of the award.

The core questions Dr. Nagel will pursue over the course of her K-award concern the development of verbal and spatial working memory, and their neurobiological underpinnings. The proposed work takes a novel and very sophisticated approach to addressing questions about this important aspect of neurocognitive development. Further, it focuses on an important, but comparatively understudied developmental period, adolescence. Behavioral studies have clearly shown that working memory continues to undergo significant developmental change well into adolescence. However, there is still debate over whether verbal and spatial working memory follow similar or divergent developmental trajectories. One important aspect of Dr. Nagel's project will be to implement a carefully designed and matched set of verbal and spatial working memory experiments that will allow her to explore this important question. Further, studies of neuranatomical development have shown that frontal lobe regions critical for working memory also undergo significant change during adolescence. However, few studies have attempted to systematically link brain changes in these areas with behavioral change, and thus little is known about the *relationship* between change in working memory and the development of the neural systems that mediate these important cognitive functions. Dr. Nagel's proposal takes a converging measures approach that combines carefully designed behavioral measures of verbal and spatial working memory, functional magnetic resonance imaging (fMRI) of neural activation obtained during these working memory tasks, and diffusion weighted imaging (DWI) measures of white matter organization within the critical frontal lobe systems. The data from each of these measures will make important, independent contributions to the study of brain and behavioral development in this age period. However, the strength of the proposal lies in the conjunction of measures; specifically, in the study design that will allow for the direct comparison of behavioral, neural activation, and neuroanatomical data. The combination of solid, traditional behavioral methodologies with cutting edge imaging technologies will allow Dr. Nagel to address questions about cognitive development that were inaccessible just a few years ago. This project is truly state-of-the art cognitive neuroscience that will make significant contributions to our understanding of the development of brain-behavior relations during the important transition from childhood to adulthood.

The multi-method approach of Dr. Nagel's project is challenging. However, Dr. Nagel's background and prior training have positioned her to meet the demands of this important project, and I am more than confident that she will succeed. Her background in the experimental design of behavioral studies is strong, and she will receive considerable additional support from my laboratory. Further, she has spent the past several years mastering both the theoretical and practical aspects of fMRI, and is well prepared to design and execute the study proposed here. My laboratory has been involved in pediatric functional neuroimaging for a number of years now, and has extensive personnel and facilities to assist Dr. Nagel in the successful completion of her project. Thus, the behavioral and fMRI portions of proposal will both build upon and extend Dr. Nagel's existing expertise and strengths. The most novel component of the proposal is the white matter imaging project. DWI uses MRI-based protocols to detect
the movement of water along white matter pathways. Dr. Nagel's experience with fMRI will provide excellent basic background for this new imaging project. The acquisition protocols for child data collection are similar across the fMRI and DWI projects, and analyses use related data processing and data analytic approaches. Statistical analysis of this multi-measure study present a number of challenges. However, Dr. Nagel has turned this challenge into a central goal of the proposal. She has assembled a very strong group of mentors in the area of statistical analysis. Thus, the analytic challenge presented by this project will become an opportunity for strong additional training in this area. The project consists of three basic studies; the behavioral and fMRI studies are very tightly yoked. While this is a large project, it is certainly one that can be completed in the five-year term of the award.

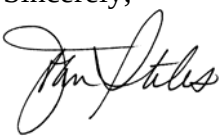
For the past 20 years, work my laboratory has focused on basic questions about the development of brain-behavior relations, in both typically developing children and in children with neurodevelopmental disorders. Until recently, techniques for mapping the brain bases of behavior in children have been extremely limited, relying on a small number of electrophysiological studies with typically developing children and studies of child patient populations. In the 1990's, advances in MRI technology provided, for the first time, a noninvasive method for measuring functional brain activation that could be used with children. UCSD offers a technologically sophisticated and intellectually rich community of functional brain imaging researchers. Seven years ago, my laboratory began to employ functional magnetic resonance imaging in our studies of children, and have now developed an extensive set of protocols for neuroimaging data collection and analysis. A year and a half ago I was awarded a $2 million grant from the NIH to do behavioral and fMRI studies of spatial cognitive processing in typically developing 7- to 16-year-olds. Dr. Nagel's work fits well within the context of studies ongoing in my laboratory. Dr. Nagel's training within my laboratory will include both behavior and functional imaging. My laboratory houses a full array of computers and data analytic tools necessary for experimental design and data analysis for both the behavioral and imaging components of the study. In addition, my laboratory has a suite of testing rooms that have been specifically designed for the kind of behavioral studies described in this proposal. There are currently four graduate students, two post-docs and one scientist in my laboratory. All are involved in pediatric functional imaging studies of spatial cognitive development. The laboratory group is very active and interactive, and will provide an excellent scientific community for Dr. Nagel.

My mentoring of Dr. Nagel will take several forms. As her primary sponsor, I will meet twice monthly with Dr. Nagel to oversee the development of her research program. The focus of these meetings will range from theoretical discussion to experimental design and data analysis.

An important goal of the meetings is to monitor the ongoing progress of the project, and to solve problems as they arise. As the project progresses, a particular focus of our discussions will be on the development of a competitive R01 application in her program area as this is the ultimate goal of this career development award. My more substantive contributions will be to help Dr. Nagel anchor her program of research within the emerging body of work on the developmental neuropsychology in typically developing children, and to provide her with the experimental tools and theoretical grounding she needs to launch an independent career in this field. By providing a solid foundation in basic theory and experimental design, my goal is to provide Dr. Nagel with background that will allow her to both continue her work with typically developing children and to extend her work to the study of populations with neurodevelopmental disorders.

In combination with my mentorship, Dr. Nagel has named a number of excellent co-sponsors and consultants who will mentor her in specific areas of substantive and methodological expertise. Dr. Frank is an internationally recognized expert in diffusion weighted imaging, and an excellent teacher and mentor. He is both highly qualified to serve as Dr. Nagel's mentor, and enthusiastic about the project she has proposed. Dr. Gamst is a statistician for the UCSD Biostatistics and Informatics Laboratory (BIL). He is highly qualified and will provide excellent guidance for the complicated statistical analyses required for this project. Dr. G. Brown is both an excellent statistician and first-rate neuroimager who will make significant contributions to Dr. Nagel's training. Dr. Tapert is a superb pediatric neuroimager, who knows Dr. Nagel and her work very well and is well positioned to advise and assist on all aspects of the behavioral and fMRI projects. Dr. S. Brown is an excellent neuropsychologist with extensive experience working with adolescent populations. Her work in the community and skill with clinical populations will make invaluable contributions to Dr. Nagel's training. In addition, the group of mentors Dr. Nagel has assembled know each other and have a history of working together effectively in a number of campus contexts. We all very strongly endorse Dr. Nagel's application for the Mentored Clinical Scientist Development Award, and look forward to working with her on this very well designed and important project. Dr. Nagel is a very promising young scientist. We are confident that she will be one of the rising stars in the field of developmental neuropsychology.

Sincerely,



Joan Stiles, Ph.D.
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September 4, 2004

I am pleased to write this statement in support of Dr. Bonnie Nagel's proposed Mentored Research Scientist Development Award (K-08) entitled, "Adolescent Working Memory and White Matter Development." Her proposal is focused on an important issue in development and her plan incorporates cutting edge methods for which intensive training is appropriate.

Dr. Nagel plans to integrate the novel technique of diffusion tensor imaging (DTI) into her research plan, and will participate in a variety of training activities to learn the physics, application, and interpretation of DTI data. Her education and training in my lab will consist of directed readings, regular meeting to discuss the readings and their application to the specifics of the project, and hands-on experience using the DTI acquisitions and analysis software. Dr. Nagel will also attend the DTI course at the ISMRM Annual Scientific Meeting & Exhibition, which is our primary research meeting each year. The goals outlined by Dr. Nagel are feasible within the 5 years of the grant.

The application of DTI to development is in it's very early stages, so Dr Nagel's proposed project and training will be an important contribution to the field of development by not only extending our understanding of white matter structure and physiology but also by training an individual in a novel and unique methodology in which few have been trained. DTI is my primary area of research and I have an active lab in which all aspects of DTI research (pulse sequence development, theory, data analysis) are ongoing. I was recently awarded an NIH R01 for the study of diffusion in complex neural tissues. I have an active group of students and post-doctoral fellows working on various aspects of DTI with whom Dr. Nagel will fit in well. The UCSD Center for Functional MRI provides a unique environment for research applications in DTI. There are two 3T human scanners available for research, as well as significant computational resources.

I am enthusiastic about Dr. Nagel's training and research plan, and have confidence that she will appropriately utilize the award to establish herself as an independent investigator in an innovative and integrated program of research.

Sincerely,

Lawrence R. Frank, Ph.D.
Associate Professor of Radiology

Statement by Co-Sponsor Dr. Sandra Brown:

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September 10, 2004

Bonnie Nagel, Ph.D.
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San Diego, CA 92093-0603

Dear Dr. Nagel,

This letter confirms my commitment to serve as a consultant for your Mentored Career Development Award (K08) application entitled, "Adolescent Working Memory and White Matter Development." I am pleased that you have asked me to serve as a statistical consultant and mentor and look forward to overseeing the statistical training and research components of the project.

As we have discussed, I am available to meet with you on a regular monthly basis to discuss ongoing analysis of the data collected as a part of your research plan. In addition you will have access to direct assistance from the UCSD Biostatistics and Informatics Laboratory, of which I am a part. From a more theoretical perspective, I agree to provide you with a directed readings course on methods for statistically analyzing longitudinal data.

I look forward to our work together on this important project.

Sincerely,

Anthony C. Gamst, Ph.D.
Assistant Professor of Biostatistics
UCSD School of Medicine

UNIVERSITY OF CALIFORNIA, SAN DIEGO UCSD

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September 10, 2004

Bonnie Nagel, Ph.D.
Postdoctoral Fellow
University of California San Diego
9500 Gilman Drive
San Diego, CA 92093-0603

Dear Dr. Nagel,

I am pleased serve as a consultant on your proposed project: Adolescent Working Memory and White Matter Development. I have had some experience over the years in the design, analysis, and interpretation of fMRI projects and data. I would be pleased to provide you with the necessary mentorship and guidance toward achieving your training and research goals in this area.

In my role as a consultant, we will meet regularly to discuss issues relating to the fMRI task design and analysis for your proposed project. It is also expected that you will attend my weekly didactic lab meetings, where you will benefit not only from collaborative discussions of your own fMRI methodology and data, but will also learn from others in the discussion of different issues and topics related to fMRI research.

I am enthusiastic about the importance of this project. Your career development and accomplishments thus far have well prepared you for this award. You are the type of exceptional young scientist that the K-Award program was designed for. I have no doubt that implementing the proposed research and training plan will launch your career as an independent investigator within the field of neuroimaging.

I look forward to participating in your research and training efforts and providing whatever resources and expertise I have to facilitate your goals.

Sincerely,

Gregory G. Brown, Ph.D.
Co-Chair, Statistics Group, Functional BIRN Program
Professor of Psychiatry
UCSD School of Medicine

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September 9, 2004

Bonnie Nagel, Ph.D.
Postdoctoral Fellow
University of California San Diego
9500 Gilman Drive
San Diego, CA 92093-0603

RE: "Adolescent Working Memory and White Matter Development"

Dear Bonnie,

I am very pleased to serve as a consultant for your K08 on the role of prefrontal white matter development in adolescent working memory skills. As part of your training award, I will look forward to meeting with you weekly on your progress, as well as providing you with access to our database of adolescents who have received neuroimaging and have consented to being contacted about additional research opportunities. In our meetings, we will continue to have ongoing discussion, guided readings, and consultation on neuropsychological assessment, functional, volumetric, and diffusion brain imaging with adolescents, as well as the integration of these sources of data.

I think your project is very important and interesting, and your sample appears appropriate for addressing a range of critical neuromaturational issues that are likely to lead to an active and productive career of studying adolescent neurodevelopment.

As one of your mentors in our Department, you are assured of office space and access to the logistical resources needed to make this project happen.

I look forward to working with you on this critical project!

Sincerely,

Susan F. Tapert, Ph.D.
Assistant Professor-In-Residence

5. Environment and Institutional Commitment to Candidate

5a. Description of the Institutional Environment

The UCSD School of Medicine is ranked among the top twenty U.S. medical schools in biomedical research (2005 U.S. News and World Report). Over 400 faculty members are involved in laboratory or clinical research, representing more than $130 million in sponsored research funding. Within the School of Medicine, the Department of Psychiatry is nationally known for the breadth and depth of its research and strong support of K-Award applicants and recipients. The department has an established K-Award committee that oversees the planning and writing of such grant proposals to ensure that the applicant is appropriately prepared by the time of submission. The members of this committee each take the time to meet individually with all K-applicants in order to review his/her grant proposal and provide constructive feedback regarding the training and research plans as well as to provide mentorship in the grant-writing and application process.

This Mentored Career Development Award will be pursued within the context of the Laboratory of Cognitive Imaging (LOCI) – seeded within the UCSD Department of Psychiatry (https://loci.ucsd.edu). This laboratory houses the research of a number of UCSD faculty and supports regular didactic seminars and collaborative laboratory meetings to discuss issues in neuroimaging data collection and analyses, providing an optimal learning environment.

In addition to the proposed mentors and sponsors, UCSD employs a number of other full-time faculty members who have active research programs in the areas of development, memory, and neuroimaging. Also, there are several relevant faculty from San Diego State University (SDSU) that collaborate with UCSD faculty to engage in research. Please see the table below for names of these faculty and descriptions of his/her relevant interests and research.

Name	Position/Department	Relevant Interests/Research
Doris Trauner, M.D.	Chief Pediatric Neurologist and Professor/UCSD Department of Neuroscience	The development of specific cognitive skills in children after early brain damage, as well as the impact on cognitive development of early metabolic, genetic, and structural insults to the human nervous system
Dean Delis, Ph.D.	Professor/UCSD Department of Psychiatry and Director of the Psychological Assessment Unit at the VA San Diego Healthcare System	Cognitive deficits in children and adults with various neurological or psychiatric disorders; cerebral organization of spatial cognition, memory, and executive functions; neuropychological test development
Terry Jernigan, Ph.D.	Professor/UCSD Department of Psychiatry and Co-Director of LOCI	Brain mapping of memory and neurodegeneration, cognitive dysfunction of major psychiatric disorders, and brain development across the lifespan

Judy Reilly, Ph.D.	Professor/SDSU Department of Psychology	Developmental trajectories of language and emotion in both typically and atypically developing children, children with early unilateral brain damage, children with language impairment, and children with Williams or Down Syndrome
Ralph-Axel Mueller, Ph.D.	Assistant Professor/SDSU Department of Psychology	Functional neuroimaging of language, auditory processing, motor control and learning; development of neurofunctional organization in healthy children and in children with developmental disorders (especially autism); neural plasticity in children and adults following brain damage

Along with providing a collaborative environment in which to learn about and engage in neuroimaging research, the LOCI will provide the necessary office space, telephones, computer equipment, data processing and analysis programs, technical support, and locked data storage space for conducting the proposed study. I will also have access to the UCSD Center for Functional Imaging (CFMRI), an entirely research-devoted imaging center which houses two 3-Tesla (3T) imaging systems and will be able to make use of all technical advances made by Dr. Larry Frank in the area of DTI acquisition and analyses. UCSD also provides excellent libraries and related resources including several online database search engines as well as access to a wide range of electronic journals and an efficient intra-library loan system.

5b. Institutional Commitment to the Candidate's Research Career Development

6. RESEARCH PLAN

6a. STATEMENT OF HYPOTHESES AND SPECIFIC AIMS

It has been demonstrated that working memory abilities improve from childhood through adolescence [9]. It has also been established that working memory skills are crucial to intact functioning in a variety of cognitive domains [10], and that a number of child and adolescent populations suffer from working memory dysfunction [11-14]. While much is known about the behavioral manifestations associated with the normal development of working memory abilities, the neurodevelopment underlying these behavioral changes is less understood. Because the frontal lobes are actively developing during adolescence [15], it is reasonable to expect that this neuromaturation facilitates increases in performance. Advances in technology now allow us to take the next step in understanding the neurodevelopmental analogs to these behavioral improvements as well as to the explore the neurobiological underpinnings of working memory in developing adolescents. The goals of the proposed study are designed to integrate relatively novel neuroimaging techniques available for providing a comprehensive picture of neurodevelopment. The objectives of this research are to characterize adolescent spatial and verbal working memory functioning, examine neural activation patterns in the brain that may account for observed age-related changes in working memory abilities, examine the microstructure and organization of prefrontal white matter across adolescence, and relate those neuroanatomical findings to the behavioral and functional changes in working memory abilities. By combining functional and diffusion tensor imaging data, as well as neuropsychological test data collected on normally developing adolescents, I propose to examine specific relationships between normal white matter development and working memory functioning that can subsequently serve as an anchor from which to study a variety of adolescent pathological populations.

Aim 1: Using neuropsychological assessment and an out-of-scanner version of the fMRI task, I will assess verbal and spatial working memory abilities across adolescence. By utilizing a stratified age sample (across ages 12 to 17 years), I will be able to provide a preliminary cross-sectional characterization of the development of verbal and spatial working memory functioning, and I hypothesize that each will improve as a function of age.

Aim 2: Using functional neuroimaging, I will examine the extent to which there is a dissociation between patterns of prefrontal activation during the spatial versus the verbal working memory task among adolescents. I hypothesize that adolescents will demonstrate a hemispheric dissociation between the verbal and spatial working memory tasks, with greater left hemisphere neural activity during verbal working memory and greater right hemisphere activation during spatial working memory. Further, I hypothesize that this dissociation will become more pronounced with increasing age.

Aim 3: Using DTI, I will assess the microstructure and organization of prefrontal white matter in normally developing adolescents using two measures of anisotropy (fractional anisotropy and generalized fractional anisotropy). These measures of white matter integrity will then be correlated with age and working memory measures to determine the relationship between prefrontal white matter development and working memory functioning across adolescent neurodevelopment. I hypothesize that prefrontal white matter integrity and organization will increase as a function of age and that this development will mediate the relationship between age and working memory performance.

Aim 4: Combining fMRI and DTI techniques, I will examine how prefrontal white matter integrity and organization relate to patterns of functional activation within prefrontal regions, during

verbal and spatial working memory tasks. I hypothesize that indices of prefrontal white matter development will be positively associated with fMRI response to the working memory tasks in nearby cortical regions.

While four separate aims were presented, it is critical to understand that each aim builds upon another in order to provide an integrated examination of both verbal and spatial working memory during the adolescent years. Thus, while more specific hypotheses are presented for each of the above aims (see the Research Design and Methods section; section 6d. – part 5), the ultimate goal is the assimilation of data from multiple modalities.

6b. BACKGROUND, SIGNIFICANCE, AND RATIONALE

1) Development of working memory. Working memory refers to the ability to actively maintain and manipulate information in the brain for a short period of time [16]. It has been posited that the working memory system is comprised of a central executive control system and two subordinate (slave) systems responsible for the manipulation, rehearsal, and retention of domain-specific information – the phonological loop and the visuospatial sketchpad [17]. More recent modifications to the working memory model include the notion of an episodic buffer capable of integrating information in working memory with information from other cognitive systems [18]. Research suggests that these slave systems, specific to the processing of verbal and visuospatial information, are functionally distinct by the time children reach school-age [9, 19]. However, it has been demonstrated that working memory abilities continue to improve through adolescence. Specifically, working memory accuracy increases and reaction times decrease as a function of age [20, 21]. These improvements are thought to be a function of better executive strategies and improved processing speed that occur with adolescent neuromaturation [22]; however, the direct link between improved functioning and neurodevelopment has not been clearly demonstrated. The advent of functional neuroimaging has made essential contributions to the current understanding of the neuroanatomical substrates underlying human working memory functioning. Functional neuroimaging studies of adults have consistently revealed activation of the prefrontal and parietal cortices during adequate performance on working memory tasks [for review, see23, 24]. However, few studies have utilized functional neuroimaging in normally developing youth populations to explore the brain/behavior relationships of working memory at different stages of development.

2) Adolescent brain response to working memory tasks. Despite the limited research examining brain activation during working memory tasks among developing youths, several developmental patterns are beginning to emerge. Two studies have demonstrated that, overall, children and adolescents exhibit greater activation in bilateral frontal and parietal brain regions, with increasing age, to spatial working memory tasks [20, 25]. Another study of visuospatial working memory, comparing children to adults, demonstrated similar patterns of right hemisphere frontal and parietal activation between both age groups [26]. A study that examined working memory for locations revealed that children activated more bilaterally than adults, who tended to show a more right-lateralized pattern of parietal activation [27]. The only examination of verbal working memory in children demonstrated that, compared to activation by adults, children activate similar brain regions, but in a more widely distributed fashion [28]. In addition, children demonstrated a trend toward left hemisphere lateralization in frontal brain regions. While these are the only studies to examine child and adolescent age-related patterns of functional activation during working memory tasks, a number of studies of visuospatial and language processing have demonstrated increased lateralized patterns and shifted patterns of activation in adults as compared to children [for review, see29].

Taken together, these data suggest that, brain activation patterns change with age, and

developing youths display more diffuse activation while adults exhibit more focal and lateralized patterns in response to cognitive tasks. These differences in brain activation patterns with age may be a function of increasing functional specialization within developing brain regions. However, little research has examined the contributions of different brain regions to specific aspects or types of working memory during development. There is also no indication of how prefrontal white matter integrity, something that is actively changing during the adolescent years, may help to explain age-related developmental differences in functional activation patterns during working memory tasks. This lack of information regarding normal functional and structural brain development forces researchers studying pediatric neuropathology to rely heavily on literature from adult populations. While the adult literature provides an important framework for studying development, it is clear from the known functional and neuroanatomical differences between children and adults, that generalizations between the two should be made with extreme caution.

3) Dissociation between verbal and spatial working memory. In the adult literature, there has been a focus on identifying specific brain regions responsible for subserving various components and types of working memory functioning. To that end, there is considerable controversy as to whether or not verbal and spatial working memory functions are subserved by different hemispheres in the brain. The idea for this material-specific dissociation originally grew out of the lesion literature suggesting that the left hemisphere was more specialized in processing verbal information, while the right hemisphere was more apt to process spatial stimuli [30, 31]. Since then, a number of adult studies have examined whether or not such a hemispheric dissociation exists for working memory functions. Findings from these studies, although disparate, suggest that there may be a hemispheric dissociation for verbal and spatial working memory [32]; however, this functional distinction may be restricted to the ventrolateral prefrontal cortex [33]. Studies that have failed to demonstrate this dissociation may have utilized stimuli that were more amenable to verbal encoding strategies than those who were able to show this hemispheric distinction or may not have had sufficient power to detect such lateralization effects. In addition, most studies addressing this question have done so by contrasting neural activation patterns in one cognitive condition versus another, and lateralization is examined by means of visual inspection. This type of voxel-based examination is insufficient to adequately address issues of lateralization in a statistically driven manner [34]. To date, no study has examined this potential material specific dissociation in developing adolescent populations. While another equally controversial and interesting dorsal/ventral dissociation exists in the literature between spatial and non-spatial information processing [35], it is beyond the scope of the current proposal and will not be discussed.

4) Adolescent neuromaturation. Recent advances in neuroimaging have enabled researchers to more precisely examine brain development and functioning. Structural neuroimaging studies have provided a wealth of information regarding regional brain development throughout the periods of childhood and adolescence [36, 37]. It has been consistently demonstrated that the frontal lobes, and in particular frontal lobe white matter [38], increase in volume until at least the third decade of life [39], while frontal cortical gray matter volume decreases [36, 40]. These volumetric changes are a function of the concomitant processes of dendritic arborization, synaptic pruning, and myelination that occur during adolescent development [41, 42]. Whereas the peak number of synaptic connections are present at an early age [43], the refinement of neural connectivity via synaptic pruning allows for more mature patterns of neural activity. Myelination, a process that contributes to the efficiency of neural activity, occurs in subcortical pathways well before cortical association pathways [44]. These neuromaturational processes appear to underlie changes in adolescent behavior and emotion, as well as improvements in cognitive ability. The instability and evolution of the

developing adolescent brain, provide further impetus for using adolescent, as opposed to adult, models for understanding brain and behavior relationships.

5) Diffusion tensor imaging (DTI) and development. Given that many of the neuromaturational changes occurring during adolescence involve white matter, it is important to be able to accurately assess the development of this brain tissue. DTI provides the only known *in vivo* assessment of white matter microstructure and organization. Using the same hardware as traditional MRI, DTI measures water diffusion anisotropy, or the restriction of water movement, within each image voxel of the brain. Anisotropy changes are associated with structural changes in the white matter that affect water motion. Increases in diffusion anisotropy can result from increased myelination, increased axonal thickness, and/or parallel axonal organization [45]. While few studies have applied DTI methods to adolescent populations, the existing research suggests that, during adolescence, the diffusion anisotropy of white matter pathways increases with age [42, 46]. Again, these increases in white matter anisotropy are likely a function of increased axonal myelination and organization, thus suggesting greater neuromaturation. The only study to date to integrate DTI with fMRI in adolescents, demonstrated a positive relationship between measures of white matter anisotropy and blood oxygen level dependent (BOLD) response to a spatial working memory task in nearby cortical gray matter [47]. This finding suggests that DTI measures may be helpful in explaining adolescent patterns of brain activation during fMRI, and in particular, working memory tasks.

6) Summary of proposed research plan. It is well established that working memory abilities are crucial to intact performance in a number of cognitive domains, including arithmetic skills, complex problem solving, reading and language comprehension, and overall academic achievement [for review, see 10], and that these abilities continue to improve through adolescence [9]. However, the neurobiological substrates underlying working memory functioning and improvements in performance are not established. Working memory impairments have been documented in a variety of developing adolescent populations, including those with a history of substance use disorders [11, 48], head injury [12, 49], schizophrenia and related genetic predisposition [13], spina bifida [50], sickle cell disease [51], attention deficit/hyperactivity disorder [14, 52], and learning disabilities [53]. Further, researchers have demonstrated frontal lobe white matter abnormalities in many of the same pediatric populations that demonstrate working memory impairments, including those with attention deficit/hyperactivity disorder [54] and depression [55]. However, understanding of normally developing brain and behavior relationships, as well as the links between white matter abnormalities and functional deficits during development, remain unclear. In order to elucidate the role of prefrontal white matter development in working memory functioning and neural activation among normally developing adolescents, I plan to combine neuroimaging techniques to provide greater depth in understanding normally developing brain and behavior relationships. While there has been a good deal of behavioral work documenting developmental change in working memory systems, comparatively few studies have attempted to address developmental changes in the neural substrates that mediate these behavioral changes. Even fewer studies have adopted a converging measures approach incorporating measures of behavior, functional brain activation, and white matter anatomy to provide a comprehensive picture of neurodevelopment as it relates to functional outcome. This study proposes to begin to fill in these critical gaps.

6c. PRELIMINARY STUDIES

My recent research has focused on utilizing the methods of structural and functional neuroimaging, as well as the integration of the two, to better characterize adolescent neurodevelopment and working memory functioning. Here, I will briefly present two such studies

demonstrating that functional activation patterns during working memory tasks change across adolescent development, and that prefrontal white matter volumes directly impact those patterns. In addition, I will present some preliminary data suggesting that working memory abilities do improve with age across our adolescent age sample. The efficacy of using this converging measures approach to study the development of working memory is demonstrated by this preliminary work.

Study 1: fMRI of spatial working memory across adolescence. In collaboration with Dr. Susan Tapert's studies of substance abusing and at-risk youths, 49 typically developing adolescents were examined using a spatial working memory task. Teens were all right-handed and free from neurological and psychiatric disorders, head injury, learning disorder, and immediate family history of psychotic disorder. In that preliminary past research has suggested that spatial working memory fMRI response patterns change as a function of age [20, 25], this study attempted to elucidate developmental age effects on BOLD response during a spatial working memory task. The fMRI task design was as follows:

Spatial Working Memory task. The spatial working memory task [56, 57] was adapted from McCarthy [58], and consisted of 18 20-second (sec) blocks alternating between experimental and baseline conditions and included blocks of rest in the beginning, middle, and end of the task, during which a fixation cross appears in the center of the screen. In the experimental condition (spatial working memory), abstract line drawings (Kimura figures) were back-projected one at a time in one of eight locations in a circular array onto a screen placed at the foot of the MRI cradle. Stimuli and locations were chosen to minimize verbal labeling (e.g., stimuli were not presented in the four compass points). Participants were asked to press a button when a design appeared in a location that had already been occupied during that block. On average, three of the 10 stimuli in each block were repeat locations, and, unbeknownst to participants, repeats were 2-back (e.g., stimuli presented in the same spatial location as the stimuli presented 2 prior). In the active baseline condition (vigilance), the same stimuli were presented one at a time in the same eight locations, but a dot appeared above figures on 30% of trials. Participants were asked to press a button when they saw a design with a dot. The purpose of the baseline condition was to control for the simple motor and visual attention processes involved in the working memory condition. In both conditions, stimuli were presented for 1000 milliseconds (ms), and each interstimulus interval was1000 ms (20 sec/block; total task time=7 minutes 48 sec).

Our results suggest that, while teens demonstrate the expected SWM neural response in bilateral prefrontal and parietal brain regions, BOLD response patterns to a SWM task change across the adolescent age range. Specifically, among 12 to 17-year-old adolescents, there was increasing BOLD response with age to the spatial working memory task (when compared to the vigilance condition) in left dorsolateral prefrontal cortices and bilateral inferior parietal regions ($p < .05$). Increasing adolescent age was associated with significantly decreased BOLD response in bilateral superior parietal regions ($p < .05$). The findings suggest that perhaps older teens use greater verbal strategies to encode and recall information in this task, and that there is a superior to inferior parietal activation shift with age, which may be explained by maturing attentional networks utilized in the task. This work has recently been submitted for publication.

Study 2: The role of prefrontal white matter volume on fMRI response to a spatial working memory task across adolescent development. This study was designed to examine how morphological measurements of prefrontal brain development impact fMRI response patterns during spatial working memory across adolescent development and utilized the integration of structural and functional neuroimaging methods. In order to examine the contribution of

structural neuromaturation on adolescent brain response to the above-described spatial working memory task, prefrontal white matter measurements were performed and regressed on spatial working memory BOLD response for 54 typically developing adolescents (ages 12 to 18). The prefrontal region of interest (ROI) was defined and white matter volume was quantified by the methods outlined below.

Prefrontal ROI drawing. The delineation of the prefrontal region was performed in AFNI [8] using all three available image orientations (coronal, axial, and sagittal). The posterior boundary was demarcated by the sagittal and coronal slices that most clearly display the anterior commissure. From this level forward, the frontal cortex was isolated from nearby temporal cortex, subcortical structures, and insula, but included the corpus callosum, cingulate, and orbitofrontal cortex. On the coronal slice that runs perpendicular to the most anterior portion of the genu, dorsal and ventral prefrontal cortex were separated by a stereotactic line that runs horizontally through the genu. This line was followed through to the most anterior slice of the brain to distinguish dorsal from ventral prefrontal cortex. Using these methods, high levels of inter- and intra-rater reliability were attained (intraclass correlation coefficients >.90).

Quantification of prefrontal white matter. Fully skull-stripped T1 images were processed using the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain's (FMRIB) automated segmentation tool (FAST) [59]. This program segments the brain into different tissue types (e.g., white and gray matter), while correcting for spatial intensity variations. The resulting segmented white matter was then applied to the prefrontal ROI, which allowed for the determination of each teen's prefrontal white matter volume.

Results from this study suggest that prefrontal white matter volumes were not significantly associated with fMRI task performance. However, prefrontal white matter volumes were associated with SWM neural response patterns. Specifically, teens with greater prefrontal white matter volumes demonstrated significantly greater brain activation during SWM in left inferior frontal regions (Broca's area) and right hippocampus and parahippocampal gyrus ($p < .05$). In addition, teens with greater prefrontal white matter volumes evidenced less neural response in the right supplementary motor cortex and bilateral primary visual and association cortices during SWM ($p < .05$). These results suggest that, with greater frontal maturation, teens utilize changing neural networks and strategies to adequately perform the SWM task. Specifically, adolescents with greater prefrontal white matter, and thus greater prefrontal neuromaturation, may utilize greater subvocal rehearsal processes to remember the spatial information. Also, it appears that those teens with greater prefrontal white matter development may utilize greater hippocampal encoding networks to encode and recall information. This work has been submitted for presentation at the 2005 International Neuropsychological Society conference and is being written up for publication.

Preliminary data: working memory in adolescents. While the neuropsychological batteries used in these studies were not designed to comprehensively examine working memory functioning, all participants were administered a version of the Wechsler Digit Span subtest (either the Wechsler Intelligence Scale for Children-Third Edition for teens age 12 to 16 [60] or the Wechsler Adult Intelligence Scale-Third Edition or Revised version [61, 62] for 17-year-olds). Examination of raw digit span scores (both forward and backward) in 48 youth (ages 12 to 17) confirms that working memory abilities (even assessed in this relatively crude way) do significantly improve with age across adolescence (digits forward: $r = .33$, $p < .05$; digits backward: $r = .54$, $p < .001$).

The above findings demonstrate the feasibility of examining adolescents using neuroimaging and neuropsychological assessment techniques, confirm that age and morphologic indices of brain development impact fMRI response patterns, and demonstrate the likelihood of detecting working memory improvements across the proposed adolescent age range.

6d. RESEARCH DESIGN AND METHODS

1. Study Design. The proposed study will be an age and gender stratified cross-sectional investigation examing a) neuropsychological performance, b) BOLD response during spatial and verbal working memory tasks, and c) diffusion anisotropy of prefrontal white matter at various ages across adolescence. Additionally, as longitudinal data provides a more precise depiction of neurodevelopment, this data will be collected in a manner so as to facilitate collection of longitudinal pilot data for a future R01-funded study.

2. Participants. The proposed study will include 90 normally developing adolescents (age range 12 to 17 years). The goal will be to recruit and study 10 teens in year 1 and 20 teens per year for years 2 through 5, resulting in 15 participants at each age across the study range. Exclusionary criteria will be: lifetime history of a *DSM-IV* psychiatric or substance disorder, neurological illness or significant head trauma (loss of consciousness > 2 minutes), serious medical problems, mental retardation, or learning disability; current use of medications that could affect the central nervous system; smoking more than 4 cigarettes per day; significant maternal use of alcohol (≥4 drinks per occasion or ≥7 drinks per week) or other drugs during pregnancy; reported history of bipolar I or psychotic disorders in biological parents; inadequate English skills (e.g., not fluent in English); sensory problems; left-handedness; and irremovable metal on the body. These exclusion criteria have been set forth to remove participants who may have abnormal neuropsychological performance, BOLD response, and/or white matter abnormalities or who are unable to undergo the MRI procedure for safety reasons.

Power Analysis. After reviewing relevant behavioral, fMRI, and DTI studies [20, 42, 47, 63, 64], power estimates were based on a previous behavioral and fMRI study of working memory [20] with the smallest estimated effect size (r = 0.33) across the studies. Using this conservative estimate of an effect, with the proposed sample of N = 90, there will be power of approximately 0.90 to detect significant effects with a 2-sided alpha of 0.05.

Recruitment. Participants will be recruited from among normal control participants in Dr. Susan Tapert's studies on substance use disorders in youth, as well as via flyers distributed at demographically representative local schools from Dr. Sandra Brown's studies. Participants recruited from Dr. Susan Tapert's studies will have already received comprehensive screening through parent and youth interviews, and will undergo a brief update interview to evaluate for any changes since last study involvement. Teens not already involved with Dr. Tapert's studies and recruited from local schools will undergo the same extensive screening procedures. Teens will be informed that participation in the study will take approximately 4-5 hours, and they will be compensated $100 for completing the study. Parents will be compensated $20 for completing the parent interview.

Screening. All participants will be screened for inclusion into the study. For teens not previously involved in other studies, screening will first entail a short telephone interview that assesses for basic exclusionary criteria (e.g., age, handedness, unremovable metal, sensory problems). If the teen is deemed eligible on the basis of these brief screening questions, consent/assent forms will be mailed to both the parent/legal guardian and the teen. Once the signed consent/assent forms have been returned, detailed screening interviews with both the teen and the parent/guardian will be scheduled, including a structured clinical interview specifically

designed for use with adolescents [65] and the computer assisted version of the NIMH Diagnostic Interview Schedule for Children – Version IV (C-DISC4) [66]. The structured clinical interview will be used to assess health history, childhood behavior, psychosocial functioning, family characteristics, and socioeconomic status. The C-DISC4 will assess for the current presence or lifetime history of Axis I psychiatric disorders.

3. Measures

Neuropsychological Assessment. All participants will undergo a 2-hour neuropsychological assessment performed by a trained research assistant (hired for this grant). Testing will take place in the neuropsychological testing suite at the San Diego Veteran's Administration Healthcare System. The assessment procedures were chosen to evaluate a variety of verbal and spatial memory skills, complex problem solving and strategy formation, and processing speed, as well as to gain an assessment of overall intellectual functioning. The specific standardized tests were chosen because they will 1) facilitate hypotheses testing, 2) assess working memory and other neurocognitive functions supported by prefrontal brain development, 3) provide an estimate of full-scale IQ, and 4) each has normative data spanning childhood through adulthood, which will ultimately facilitate long-term follow-up without having to modify test versions or protocols. The assessment procedures will include: the Wide Range Assessment of Memory and Learning – 2nd Edition (WRAML-2) [67], the Tower, Color-Word Interference, Verbal Fluency, and Design Fluency subtests from the Delis-Kaplan Executive Functioning System (D-KEFS) [68], and the Wechsler Abbreviated Scale of Intelligence (WASI) [69]. In addition to standardized neuropsychological test administration, all participants will be administered a version of the spatial and verbal working memory fMRI task (details below) for collection of out-of-scanner behavioral data.

Mood Assessment. At the time of imaging, all participants will be given state measures assessing current levels of depression, anxiety, and overall alertness – all variables which may affect task performance. Current levels of depression will be assessed using the Beck Depression Inventory-2nd Edition (BDI-2) [70], anxiety will be assessed using the Spielberger Stait-Trait Anxiety Inventory (STAI) [71], and alertness will be assessed using the Karolinska Sleepiness Scale [72].

Pubertal Assessment. In order to provide an estimate of pubertal development in addition to chronological age, the Pubertal Development Scale (PDS) [73] will be administered to each teen. The PDS is a 5-item self-report measure of pubertal status, with demonstrated reliability and validity. The PDS correlates significantly (0.61 – 0.67) with both physician ratings and Sexual Maturation Scale (Tanner) self-ratings [74] of pubertal maturation [75].

Exit Interview. Upon completion of the scanner tasks, all teens will be administered a series of questions assessing the type of strategies employed during the spatial and verbal working memory tasks. This subjective report will be used as a variable of interest when examining different cognitive strategies as a potential source of variation in fMRI response patterns.

4. Procedures

Structural Magnetic Resonance Imaging Acquisition. Participants will be scanned using a 3.0 Tesla General Electric scanner at the UCSD Center for Functional Magnetic Resonance Imaging. All imaging will be performed by a trained and certified scanning technician and will be reviewed by a neuroradiologist for anatomical abnormalities. The scanning protocol will consist of two high-resolution, whole-brain structural image series collected in the sagittal plane using T1-weighted 3D fast spoiled gradient recalled echo (3dspgr) sequence (TI = 450ms, Flip

Angle = 12 degrees, TE = 3.1ms, TR = 8ms, bandwidth = 31.25, 256x192 matrix, FOV = 250mm, slice thickness = 1mm, NEX = 1, total scan time ~ 7:24 min).

DTI Acquisition. Using the same hardware as the structural MRI, whole-brain DTI (~36 slices) will be performed using a high-angular resolution diffusion sequence with EPI acquisition (TE = 103ms, 128x128 matrix, slice thickness = 2mm, FOV = 240mm, TR = 10000ms). Diffusion encoding gradient pulses will be employed in 42 directions (determined by the icosahedral tessellations of a sphere) with a b-value of ~3000 s/mm^2. The pulse sequence utilizes a double spin echo acquisition in order to significantly reduce eddy current effects due to the large diffusion gradients [76]. These effects can distort the images and cause errors in the parameters derived from the diffusion tensor (e.g., FA).

Functional Magnetic Resonance Imaging (fMRI) Acquisition. Once supine in the scanner, a soft cloth will be placed on the participant's forehead then taped to the head coil to minimize head motion. In addition a piece of tape will be place across the participant's chin and to the head coil to further minimize movement. All participants will be instructed on the importance of remaining as still as possible throughout the entire scan session. Earplugs will be given to teens to use during scanning in effort to reduce participants' exposure to scanner noise. A 4-button opto-isolated button box designed for use in an MRI will be placed in the subject's right hand and tested. Each button press will trigger an indicator light so that responding can be monitored throughout the experiment. Task stimuli will be presented from a laptop computer through a data projector to a screen in the MRI room near the foot of the scanner bed. Participants will view stimuli through a mirror mounted on the head coil and make responses using the button box. Blood-oxygen level dependent (BOLD)-weighted functional imaging will be performed in the axial plane using T2*-weighted echo-planar gradient recall echo imaging (TR=3000ms, TE=40ms, flip angle=90°, FOV=240mm, 19-21 slices covering the whole brain, slice thickness=7mm, in-plane resolution=1.875 x 1.875mm, 156 repetitions). Imaging parameters may be modified after completion of the fMRI task development.

Spatial and verbal working memory fMRI task. The spatial working memory component of the fMRI task will be a modified version of the task described in the Preliminary Studies section. This task will be modified in several ways, with the goal being a well-designed task that includes verbal and spatial working memory components of matched-difficulty. It has been suggested that the ideal way in which to test for dissociations in working memory processes is to design a task that includes ways to directly compare all task conditions, thus avoiding false-positive conclusions [64]. The task to be used in this study will be similar to that used by Nystrom and colleagues [64], in that it will be a spatial and verbal working memory n-back task that differentiates between the two conditions by way of task instruction. As with the spatial working memory task described in the Preliminary Studies section, during the spatial working memory component, stimuli will be arranged in various spatial locations on the screen so as to minimize the likelihood of verbal encoding strategies. Instead of using abstract line drawings for stimuli, this task will utilize phonemically similar letters (e.g., d, g, p, etc.) during both the spatial and verbal working memory conditions. During the spatial working memory condition, the task will be to respond by a button press each time a stimulus appears in a repeat location, regardless of the stimulus content. During the verbal working memory condition, the respondent will press a button each time a letter repeats, regardless of spatial location.

Pilot testing of the task will be performed so that the spatial and verbal working memory conditions will be behaviorally matched in terms of accuracy and reaction time for the oldest aged teens in the sample, and significantly above-chance responding will be ensured for the target age population. In addition, the spatial or verbal processing difficulty as well as the

memory load will be systematically varied (e.g., increase spatial locations, increased n-back, directed versus implicit n-back, increase letter string) to provide matched-performance comparisons across the age-range. Each working memory block type will include two working memory load levels (high and low) that will be determined by the n-back. This will allow for the comparison of activation during easier versus more difficult working memory trials. As with the spatial working memory task described earlier (Preliminary Studies), this task will be presented in a blocked design, and will be interspersed with blocks controlling for visual fixation and vigilance. Response time and accuracy data will be collected for all blocks requiring a participant response. Previous research has suggested that, for the original spatial working memory task, practice/learning effects (as indicated by improvements in performance) can be effectively eliminated by allowing participants to practice one block of the task just prior to scanning (unpublished work; personal communication with Dr. Sean Drummond at UCSD). Thus, teens in this study will practice one block of each task type just prior to the scanning procedure. The out-of-scanner version of the task will include more blocks of the spatial and verbal working memory conditions (to increase the amount of data collected and statistical power) without blocks controlling for visual fixation and vigilance. Task programming and presentation will be preformed using E-Prime [77]. The design and piloting of this task will be performed during my first training year.

5. Data Analysis

Functional Image Processing. Data will be processed and analyzed using Analysis of Functional NeuroImages (AFNI) [8]. For each participant, the following steps will be applied. First, motion in the time series data will be corrected by registering each acquisition to a selected repetition with an iterated least squares algorithm [78] to estimate three rotational and three displacement parameters for each participant. An output file specifying adjustments made will be used in subsequent analyses as a covariate to control for spin history effects [79], and applied adjustments will be correlated with age to see if motion indices will need to be corrected in subsequent analyses. Next, the time series data will be deconvolved with a reference vector that codes the hypothesized BOLD signal across the time series of the task while covarying for linear trends and the degree of motion correction previously applied [80]. The reference vectors (for each task condition) will be convolved with a gamma variate function to account for delays in the hemodynamic response [81]. BOLD signal will be contrasted between each working memory condition versus the visual attention baseline condition and versus the fixation condition (as described in Preliminary Studies). In addition, the within subject design of the task will allow for direct contrasts between the verbal and spatial working memory conditions. Then, each participant's high-resolution anatomical images will be transformed into standard Talairach coordinates [82]. The fit coefficient will be the basis for statistical analyses rather than percent signal change due to favorable distribution properties. To evaluate for stimulus-correlated motion, the reference vector will be correlated with each of the six motion parameter vectors for each functional dataset. Datasets with statistically significant stimulus-correlated movement will be excluded from subsequent analyses. Other bulk motion will be visually inspected by the P.I. and a decision will be made as to whether the task repetition containing movement will be removed or if the entire dataset must be discarded.

DTI processing. Diffusion data will be processed and analyzed using AFNI tools developed by Dr. Larry Frank. Using diffusion data collected from 42 gradient directions, the diffusion tensor will be calculated, from which will be determined the principal eigenvectors and their associated eigenvalues. From these the Fractional Anisotropy (FA), or the fraction of the diffusion tensor that is associated with anisotropic diffusion [83], will be calculated. Spherical harmonics decomposition (SHD) is a technique designed by Dr. Frank to allow for more precise detection local anisotropy [3]. From the SHD can be calculated a generalized form of the Fractional

Anisotropy, called the Generalized Fractional Anisotropy, which is an extension of the FA to more complex local diffusion characteristics. SHD can characterize diffusion in multifiber voxels, but can also reproduce DTI results in single fiber voxels, and reduce imaging artifact unrelated to diffusion.

Examination of Specific Aims and Hypothesis Testing.

Aim 1: Using neuropsychological assessment and an out-of-scanner version of the working memory fMRI tasks, I will assess verbal and spatial working memory abilities across adolescence.

Hypothesis 1: Verbal and spatial working memory abilities will improve as a function of increasing age during adolescence. Specifically, reaction times will decrease and accurate responding will increase.

- Rationale: As presented previously (Background and Rationale), this hypothesis is strongly supported by previous developmental literature suggesting that working memory abilities improve during adolescence [20, 21].
- Methods: This will be tested using regression analyses predicting working memory performance with chronological age. Specifically, age will be correlated with performance on the WRAML2 indices of verbal and spatial working memory, as well as with an out-of-scanner version of the verbal and spatial working memory task, while statistically controlling for demographic variables that are significantly associated with performance.
- Anticipated Results: As hypothesized, it is expected that age will show a significant positive correlation with working memory accuracy and response time. This may be the case particularly for the out-of-scanner version of the working memory task, where the working memory load can be varied so as to avoid a ceiling-effect for performance. It is also possible, based on other scientific findings [9], that working memory skills across this age-range will demonstrate a nonlinear pattern of change. Specifically, age-related performance increases may occur during the earlier periods of adolescence and level off later during adolescence. To examine this possibility, regression model diagnostics and fit will be assessed. If the data do not fit the linear model, a piecewise linear model will be tried and/or a linear transformation of the data will be employed.
- Potential Problems: It is possible that the neuropsychological measures chosen to assess working memory performance may be insensitive to age-related improvements during adolescence. For this reason, the out-of-scanner working memory task will be designed with a high ceiling so as to detect improvements in this age range.

Aim 2: Using functional neuroimaging, I will examine the dissociation between patterns of prefrontal activation during a spatial versus a verbal working memory task across adolescent development.

Hypothesis 2a: Adolescents will show a hemispheric dissociation between verbal and spatial working memory. Specifically, fMRI response to the verbal working memory task will show a more left prefrontal hemispheric distribution, while response to the spatial working memory task will show a more right prefrontal hemispheric pattern of response.

- Rationale: This hypothesis is based evidence of hemispheric functional specialization, such that the left hemisphere is more responsible for verbal tasks, and the right hemisphere shows functional specificity for visuospatial processing [84], as well as preliminary neuroimaging evidence suggesting that there may be the same hemispheric dissociation for verbal and spatial working memory functioning [32].
- Methods: To examine this hypothesis, repeated-measures, two-way ANOVAs will be performed on a voxel by voxel basis, in which subject will be treated as a random factor, and processing type (verbal or spatial) will be entered as a within-subject factor. According to Nystrom and colleagues [78], "… A main effect of stimulus type will identify

stimulus-specific brain regions...” While previous studies have not included hemisphere (or location) in this model in order to statistically examine hemispheric lateralization, the current study will also employ right and left prefrontal regions of interest to more carefully test for significant lateralization effects.

- Anticipated Results: As hypothesized, it is expected that fMRI response to the spatial and verbal working memory tasks will show a right/left prefrontal hemispheric dissociation. The possibility of detecting a processing-specific dissociation will be increased by the task design - increasing both the spatial (more difficult spatial judgments) and verbal processing (using phonologically similar letters).
- Potential Problems: Given that the evidence supporting a processing-specific hemispheric dissociation in the adult literature is mixed, it is possible that one will not be evident during adolescent development. In that event, this study will still provide important information regarding the different neuroanatomical networks involved in verbal and spatial working memory processes during adolescent development. It is also possible that the a-priori specified prefrontal regions of interest will not capture the lateralization effects. In that event, exploratory analyses of other brain regions will be performed (e.g., parietal regions of interest).

Hypothesis 2b: It is further hypothesized that this dissociation will become more evident with increasing age, such that younger adolescents will show a more bilaterally distributed pattern of activation to both types of tasks.

- Rationale: As explained in the Background and Rationale section, this hypothesis is based on previous developmental fMRI literature suggesting that patterns of brain activation patterns tend to localize and lateralize with increasing age [29].
- Methods: As in the above analyses (2a), repeated measures multi-factorial ANOVAs will be used with subject treated as a random factor, and processing type (verbal or spatial) entered as a within-subject factor. To examine the current hypothesis, age will be entered into the model as a between subject factor, and the interaction between age and processing type will be used to determine the influence of age on activation in the prefrontal regions of interest.
- Anticipated Results: It is anticipated that younger adolescents will activate bilaterally in prefrontal regions to both spatial working memory and verbal working memory, and with increasing age, activation will become more lateralized to the right prefrontal cortex for spatial working memory and to the left for verbal working memory.
- Potential Problems: As this hypothesis is dependent upon the support of Hypothesis 2a, it is possible that this particular hypothesis will be unable to be explored. In that event, and perhaps even if Hypothesis 2a is supported, other developmental trends in the functional data will be examined, such as overall changes in activation patterns with increasing age and potential dorsal/ventral prefrontal dissociative patterns.

Aim 3: Using DTI, I will assess the microstructure and organization of prefrontal white matter in normally developing adolescents. These measures of white matter integrity will then be correlated with age and working memory measures to determine the relationship between white matter development and working memory functioning across adolescent neurodevelopment.

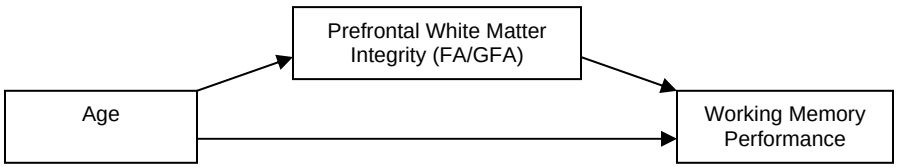
Hypothesis 3a: Anisotropy of prefrontal white matter will show a significant positive correlation with age.

- Rationale: This hypothesis is in line with previous DTI findings suggesting increasing white matter anisotropy with age during development [47].
- Methods: FA and GFA will be measured within each teen’s prefrontal ROI (see Preliminary Studies). The correlation between age and DTI measures of white matter integrity will be tested using regression analyses, in which demographic variables associated with FA and GFA are statistically controlled.

- Anticipated Results: It is expected that overall measures of prefrontal FA and GFA will increase as a function of age in this population. To more carefully examine this relationship, further analyses will be performed in which sub-regions within the prefrontal ROI will be examined (e.g., dorsal and ventral prefrontal regions), as these two sub-regions may be developing at different rates.
- Potential Problems: If the white matter fibers within the prefrontal regions are heavily crossed (instead of parallel), it is possible that the current measures available to examine anisotropy will lead to misleading results. Using SHD, we may be able to more accurately detect fiber orientation, thus providing greater awareness into any such measurement limitations [3].

Hypothesis 3b: Measures of anisotropy of prefrontal white matter will mediate age-related improvements in verbal and spatial working memory performance.

- Rationale: Based on the notion that greater FA is indicative of more mature white matter, and the idea (above) that the nervous system matures as a function of age, this hypothesis suggests that prefrontal white matter development may underlie behavioral improvements in working memory.
- Methods: Regression analyses will be performed in which prefrontal measures of FA and GFA will be used to predict verbal and spatial working memory performance, while covarying for the effects of variables also associated with behavioral performance. If support is found for Hypothesis 1 and 3a, a mediational model will be tested to determine whether prefrontal white matter integrity mediates the relationship between age and working memory performance.



- Anticipated Results: A significant positive relationship between measures of white matter integrity and working memory performance is expected, and it is expected that white matter integrity/maturation will mediate the relationship between age and working memory performance.
- Potential Problems: Similar to the limitation noted in the above hypothesis, this hypothesis depends upon the ability to accurately assess prefrontal white matter anisotropy. The same techniques (see Hypothesis 3a) will be employed to collect the most accurate data possible.

Aim 4: Combining functional and diffusion tensor imaging techniques, I will examine how prefrontal white matter integrity and organization relate to patterns of functional activation within prefrontal regions, during verbal and spatial working memory tasks.

Hypothesis 4a: Greater left prefrontal white matter anisotropy will predict activation within left ventrolateral prefrontal cortex during the verbal working memory task.

- Rationale: This hypothesis is based on a study suggesting that measures of white matter anisotropy are strongly correlated with blood-oxygen level dependent (BOLD) responding in nearby cortical gray matter [47], and research supporting the hemispheric dissociation between verbal and spatial working memory functions specific to ventrolateral prefrontal regions [33].
- Methods: Measures of left and right prefrontal white matter anisotropy will be hierarchically regressed on BOLD response to the verbal working memory task with ventrolateral prefrontal regions (using a ventrolateral prefrontal ROI). Regression coefficients will be examined to determine which anisotropic measurement best predicts verbal working memory activation.

- Anticipated Results: It is expected that increased left prefrontal white matter integrity (increased FA and GFA) will be associated with ipsilateral BOLD response during the verbal working memory task.
- Potential Problems: As this hypothesis is dependent upon both the ability to accurately measure FA in prefrontal white matter and there being a hemispheric dissociation between verbal working memory and spatial working memory in the ventrolateral prefrontal regions, a number of problems could potentially arise. Assuming that FA is able to be accurately examined (using the measures explained above), this analysis is dependent on there being ipsilateral ventrolateral prefrontal activation. If this is not the case, FA could be examined to predict left prefrontal response more broadly (e.g., not restricted to the ventrolateral region) and could even be extended to bilateral prefrontal activation patterns. While these analyses would not support the current hypothesis, they would provide information regarding the relationship between prefrontal white matter integrity and BOLD response during verbal working memory.

Hypothesis 4b: Greater right prefrontal white matter anisotropy will predict activation within the right ventrolateral prefrontal cortex during a spatial working memory task.

- Rationale: As with the previous hypothesis, this hypothesis is also based on the notion that white matter anisotropy will predict brain activation in neighboring cortical regions [47, 67] and that there will be a ventrolateral prefrontal hemispheric lateralization for spatial and verbal working memory [33].
- Methods: Measures of left and right prefrontal white matter anisotropy will be hierarchically regressed on BOLD response to the spatial working memory task. Regression coefficients will be examined to determine which anisotropic measurement best predicts spatial working memory activation.
- Anticipated Results: Similar the previous hypothesis, it is expected that increased right prefrontal white matter integrity (increased FA and GFA) will be associated with ipsilateral BOLD response during the spatial working memory task.
- Potential Problems: Just as with the previous hypothesis, this hypothesis is dependent upon the success of other analyses and is thus subject to similar problems. These potential difficulties will be handled in the same way as Hypothesis 4a, where the relationship between right prefrontal white matter FA and BOLD response during spatial working memory will be examined more broadly if the specific hypothesis is unsupported or un-testable.

Follow-up analyses. The above hypotheses and analyses will be replicated using an estimate of pubertal development [73] in place of age. These analyses will be conducted in effort to examine the differential effects of age and pubertal development. In addition, after sufficient data have been collected, gender effects in the above hypotheses will be explored.

6. Consideration of Study Limitations and Caveats

The proposed study is designed to investigate the relationships between neuropsychological functioning, BOLD response, and prefrontal white matter anisotropy among normally developing adolescents. The strengths of this proposal include a sizeable and well-characterized sample and the application of the best techniques and methods available for assessment and hypotheses testing; however, several limitations and caveats should be noted.

First, this study proposes to examine “normal” adolescent neurodevelopment and working memory functioning. While the importance of understanding these processes in typically developing adolescents is evident, the logistics of such an endeavor are more complicated. For purposes of this study, “normal” participants will be recruited on the basis of no known

neurological or psychological pathology that may impact the variables of interest. While this is the accepted and widely used way of examining normal development, it must be noted that this well-screened sample is not truly representative of typical adolescents. However, it does provide important baseline information from which to compare any subtle or more overt pathological process.

A second caveat in the design of this study involves the current state and limitations of the described neuroimaging techniques and analytical procedures. For example, DTI involves a static measure that is not actually measuring the active process of diffusion, but rather estimates the distribution of the final position of water molecules. The actual diffusion signal has a very complicated relationship with the diffusing molecules, which are interacting with local tissue geometry. Therefore, we can only make gross estimates of local diffusion characteristics such as anisotropy [85]. There are a number of other limitations with DTI, which will all be addressed using the most appropriate and best methods available, including 1) susceptibility to movement artifact – this will be reduced by using a single-shot acquisition in the current study, 2) poor resolution – this will be improved by using a 128x128 instead of a 64x64 matrix, 3) difficulty assessing anisotropy in crossed fiber voxels – the assessment is better using SHD, and 4) susceptibility to Eddy currents – the most major source of artifact and will be reduced using the methods described by Reese and colleagues [76]. There are also several limitations involving the use of fMRI. One such limitation of fMRI is that we do not directly measure neuronal activity, but rely on changes in BOLD response which is detected as relative changes in MR signal as a function of changes in cerebral blood flow to different cognitive conditions. There are also several developmental issues that impact fMRI data including differences in synaptic density and connectivity, degree of myelination, resting cerebral blood volume and flow, and glucose metabolism. These issues may confound observed developmental changes in the BOLD response, and will be addressed both experimentally and statistically whenever possible. As mentioned earlier, there are also issues of statistical inference with fMRI data. Aside from using region of interest analyses, currently there is no way to “localize” neuronal activity as we are unable to statistically compare activation levels of nearby voxels [34]. Despite these imaging limitations, these methods are still the best and most sensitive methods for the in-vivo assessment of brain-behavior relationships and white matter microstructure and development.

7. Future Directions

In addition to following the proposed sample in a longitudinal manner, there are several other directions that I hope to pursue with future grant funding. While the current study will assess pubertal development with a self-report measure, a more objective assessment of pubertal staging would be beneficial for exploring the impact of pubertal development. Through future collaboration with a neuroendocrinologist, I hope to ultimately assess pubertal development using hormone assays. Also, as previously mentioned, there are likely significant gender influences on both neural and cognitive development that will need to be systematically explored, and in future studies, I hope to examine these gender differences. Finally, once I have a good understanding of DTI, I hope to use more sophisticated measures to examine not only regional white matter integrity, but to use fiber-track mapping techniques to explore the development of white matter tract pathways in relation to behavioral and fMRI developmental change.

6e. HUMAN SUBJECTS

1. Risks to the Subjects

a. Human Subjects Involvement and Characteristics. The participant sample for the proposed study will include approximately 90 typically developing, 12 to 17-year-old

adolescents. Efforts will be made to recruit a well-distributed age, gender, and ethnically representative sample.

Inclusion Criteria: Male and female adolescents, between the ages of 12 and 17 years and of any ethnic background will be eligible.

Exclusion Criteria: Adolescents will be excluded for the following reasons:

i. History or presence of: a DSM-IV psychiatric or substance disorder
ii. History of a neurological illness or significant head trauma (loss of consciousness > 2 minutes), serious medical problems, or learning disability
iii. Current use of medications that could affect the central nervous system
iv. Smoking more than 4 cigarettes per day
v. Significant maternal use of alcohol (4 drinks per occasion or 7 drinks per week) or other drugs during pregnancy
vi. History of bipolar I or psychotic disorders in biological parents
vii. Inadequate English skills
viii. Sensory problems
ix. Left-handedness
x. Irremovable metal on the body

b. Sources of Materials. Data will be collected from teen participants and their parents. Informed consent and assent for screening will be obtained from both the parent and youth. Parents will be informed that all data collected about the teen is confidential and that no information will be shared with the parent. For teens who meet preliminary eligibility criteria, structured diagnostic interviews will be collected from both teen and parent. General interviews of the teen and parent will provide social, academic, and medical functioning, and family history of psychiatric disorders data. Mood state questionnaires will be collected from the adolescent. Neuropsychological assessment, fMRI brain activation, structural MRI, and cognitive task performance measures will also be collected from the teen. No personally identifying information will be collected on the questionnaires, interviews, or other scoring sheets thus assuring confidentiality. Subject identification numbers will be assigned to each participant. All data are collected for research purposes and will be stored in locked file cabinets in a locked office.

c. Potential Risks. The cognitive tasks involved in the protocol entail no foreseeable risk, besides perhaps fatigue or mild to moderate demands on attention and cognition. These are not seen as significant risks. Risks associated with fMRI are minimal for individuals meeting the inclusion/exclusion criteria discussed above. Some discomfort may result from lying supine in the scanner for approximately 60 minutes without full mobility. Finally, there is some psychological risk inherent in screening participants for psychiatric symptomatology, especially if results are contrary to participants' expectations. One potential risk is breach of confidentiality related to collection of sensitive information. Confidentiality issues are significant for the protocol development portion since this study collects a variety of sensitive data. Procedures described below minimize the possible breach of confidentiality. Relatedly, since personal information is gathered, there exists the risk of possible invasion of privacy. However, since informed consent is obtained, the likelihood of invasion of privacy is minimal. Another concern involves the issue of coercion, which consists of the ability of adolescents to provide informed consent. However, attempts will be made to minimize perceived coercion to participate.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent. Prospective participants will be recruited using a normal control database from Dr. Susan Tapert’s ongoing studies of substance using and at-risk youth, as well as by flyers that will be distributed at local schools from Dr. Sandra Brown’s studies. Parental/legal guardian consent to include minor adolescents in

the study will be obtained by calling parents of youth who indicated interest in participating and mailing consent forms. Subjects with signed parental informed consent forms will be asked to provide written informed consent prior to their participation. It will be made clear to parents and subjects that all information collected will be confidential. Only participants with both self and parental/legal guardian consent will be included in the study.

b. Protection Against Risk. Participants will be told about the potential discomforts associated with lying in the scanner, and their heads and necks will be supported for maximum comfort. Participants will be assured that all records will be kept confidential in research files located in a locked office and entered into a password-protected computer located behind a secure and maintained firewall. Breach of confidentiality is highly unlikely because all personally identifying information will be kept separate from data collected, and will be linked only by a master subject identification list maintained by the P.I. All data are collected for research purposes and will be stored in locked file cabinets in a locked office. In order to address any concerns regarding coercion, subjects will be informed that they are free to choose not to participate and may withdraw at any time. Since this study involves minors, particular caution will be exercised in obtaining informed consent separately and independently from parental consent. To this end, the initial step in subject recruitment will involve obtaining parental/legal guardian permission for participation by the adolescent. Once parental consent is secured, subjects will be recruited by a research assistant who will approach teens for separate and independent informed consent (i.e., parental consent will not be used to persuade teens to participate). This approach is considered very effective in minimizing coercion to participate.

3. Potential Benefits of the Proposed Research to the Subjects and Others

Participation in this fMRI study involves minimal risk for participants, yet has the potential for providing substantial long-term benefits for understanding how neuromaturation subserves working memory development and better understanding of how these processes become dysfunctional. Typically developing adolescents are an understudied population. All adolescent participants who complete the entire protocol will receive $100 (prorated for those who do not complete the scanning session). In addition, all participants will be provided with options of free transportation to and from the research site, *snacks* after NP testing, and snacks following scanning. Parents will receive *$20* for providing interviews. The only anticipated expense to participants is the time spent conducting the experiment. Scanning appointments will be flexible to accommodate participant schedules, including evening and weekend times as needed. Given the minimal risks to participating and the amount of financial compensation, we believe the risk/benefit ratio is acceptable.

4. Importance of the Knowledge to be Gained

The results of the proposed research will provide critically needed information toward an understanding of the neurological underpinnings of adolescent working memory development. This information will allow for better understanding of populations impacted not only by working memory dysfunction, but also those populations that demonstrate impaired cognitive abilities that are dependent upon intact working memory skills. In addition, the findings from the research may be extended to those developing populations who suffer white matter neuropathology, by better explaining expected patterns of functional impairment.

Inclusion of Women and Minorities in Clinical Research

The proposed study will not apply any gender or minority exclusion criteria for participant selection. All efforts will be made to insure that the number of female and minority participants will be representative of the local San Diego population. Below is a

demographic breakdown of San Diego County as provided by the U.S. Census Bureau, 2000:

Race/Ethnicity:
African American: 7%
American Indian: 2%
Asian: 6%
Hispanic or Latino: 26%
Native Hawaiian or Other Pacific Islander: 4%
White: 55%

Gender:
Male: 50%
Female: 50%

Inclusion of Children

All participants in the proposed study will be children (ages 12 to 17 years). This age range was chosen to study the pubertal years of adolescent development – ages during which functional and neuroanatomical development, relevant to the study questions, is actively occurring. The principal investigator and several of the sponsors and consultants on the project have extensive expertise in working with this age range, and will ensure the safety and proper treatment of all participants.

Finally, all procedures will be approved by the UCSD Institutional Review Board (IRB) prior to the commencement of the project. The candidate, sponsors, and all consultants have completed coursework in the protection of human subjects.

6f. VERTEBRATE ANIMALS

Not applicable

6g. LITERATURE CITED

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6h. CONSORTIUM/CONTRACTUAL AGREEMENTS
Not applicable

6i. CONSULTANTS
Greg G. Brown, Ph.D., UCSD Department of Psychiatry. Dr. Greg Brown is an expert in fMRI research design and analysis. Dr. Greg Brown will meet regularly with Dr. Nagel through the duration of the proposed study to discuss issues related to fMRI task design and analytical techniques. Dr. Nagel will also attend Dr. Greg Brown's weekly didactic lab meetings where issues in fMRI task design, data collection, and analyses are addressed.

Anthony C. Gamst, Ph.D., UCSD Departments of Neuroscience and Family and Preventative Medicine. Dr. Gamst is an expert statistician that has been involved with numerous neuroimaging studies. During the first year of the proposed project, Dr. Gamst will offer a directed readings course to familiarize Dr. Nagel with the various methods and techniques for statistically analyzing longitudinal data and combining multimodal neuroimaging data. He will also oversee and supervise all statistical and analytic aspects of the proposed project.

Susan F. Tapert, Ph.D., UCSD Department of Psychiatry. Dr. Tapert is an expert in fMRI studies of adolescent populations. Throughout the duration of the award, Dr. Tapert will meet regularly with Dr. Nagel to mentor her on issues related to adolescent neuroimaging, will provide collaboration on recruitment efforts, and will provide the necessary office space and resources needed to complete the proposed study.