

Identification of Neural Correlates of Fear Over-generalization in Pathological Anxiety in Youth.

PUBLIC HEALTH RELEVANCE STATEMENT

Pediatric anxiety disorders are serious disorders not only because they are the most common mental illnesses, but also because it involves development of other debilitating diseases in their later lives, such as depression, substance abuse, and ADHD. This proposal will provide first data regarding relationships among fear overgeneralization, corticolimbic dysfunction and abnormal connectivity in pathological pediatric anxiety. These outcomes could lead a way to the development of new interventions, preventive strategies, and targets for developing therapeutics.

PROJECT SUMMARY

Anxiety disorders, as a group, are the most common mental illnesses in the US, affecting about 25% of adolescents and 18% of adults. Symptoms typically begin in childhood or adolescence. A crucial gap in studies of anxiety is the lack of empirical data linking pediatric anxiety to abnormal brain development. This K01 application presents a program for research and training that will support the applicant on a path towards becoming an NIH-funded independent investigator, focused on the application of a fear generalization paradigm and multimodal MRI to the study of neurobehavioral mechanisms of pediatric anxiety disorders. The activities proposed in this application build on the candidate's previous training and experience and are set in an environment that includes leading clinicians-researchers and affords vast availability of research resources that will foster his development of expertise in (1) the phenomenology of anxiety disorders; (2) patient-oriented, translational and developmental neuroscience to anxiety; (3) advanced computational neuroimaging; and (4) responsible and ethical conduct in scientific research with vulnerable populations. The current research proposal aims to (Aim #1) investigate fear generalization behavior and its relationship with vmPFC (ventromedial prefrontal cortex) function and corticolimbic connectivity in healthy youth, and (Aim #2) to investigate fear over-generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in youth with pathological anxiety. We hypothesize that childhood anxiety involves fear over-generalization and this fear over-generalization involves abnormal function and connectivity of the corticolimbic system. The present study will use a fear generalization fMRI task and multimodal MRI—diffusion and resting-state fMRI—in three groups of youth across the anxiety spectrum—healthy controls, those with subthreshold anxiety, and those with any DSM-5 anxiety disorders. Successful completion of this study will provide cross-sectional evidence of the influence of pediatric anxiety on fear generalization behavior, vmPFC function, and corticolimbic system connectivity. The multiple units of analysis will help elucidate the brain-behavior relationships underlying fear generalization. This demonstration along with the research expertise developed through this K01 award will support an R01, longitudinal study of youth to track developmental trajectories of fear generalization and pathological anxiety. The line of research will promote the development of neuroimaging markers for early detection and novel intervention for pathological anxiety (*NIMH Strategic Objectives 2 & 3*).

SPECIFIC AIMS. Anxiety disorders are collectively one of the most common mental illnesses, affecting about 25% of adolescents and 18% of adults³. Symptoms typically begin in childhood or adolescence. Delineating the neural substrates of anxiety in youth, and investigating how the developmental course of these substrates goes awry in the setting of pathological anxiety may lead to neuroimaging markers for early detection and intervention, and the development of novel treatments (*NIMH Strategic Objectives 2 & 3*). Prior studies using fear acquisition and extinction paradigms combined with functional neuroimaging have linked pathological anxiety to hyperactive amygdala⁴, hypoactive PFC (e.g., ventromedial PFC or vmPFC)⁵, and their interaction¹ in threat processing. However, mounting evidence implicates fear generalization, and specifically fear **over-generalization**, in pathological anxiety. Fear generalization refers to fear responses that extend to a range of stimuli resembling the original conditioned stimuli⁶. Normal fear generalization serves an adaptive function aiding survival, but in fear **over-generalization** individuals produce fear responses to a broader range of resembling stimuli. This **over-generalization** can contribute to pathological anxiety by proliferating anxiety cues. Recent studies in adults using laboratory paradigms of fear generalization (e.g., cued fear conditioning) implicate fear **over-generalization** in anxiety disorders, such as generalized anxiety disorder (GAD)⁷ and panic disorder⁸. Using a fear generalization and multimodal MRI the applicant demonstrated during his PhD training that the vmPFC activates more to safety vs threat cues⁹ in healthy adults. However, in adults with GAD, the vmPFC activation is less discriminating of safety vs threat cues¹⁰; in addition, this abnormal vmPFC function is associated with reduced vmPFC thickness and abnormal corticolimbic connectivity¹¹.

Significant questions remain: (1) Does fear generalization occur in youth, and if so, what neural systems are involved; and (2) Does fear **over-generalization** occur in youth with pathological anxiety, and if so, what neural abnormalities are involved. Addressing these questions will lead to subsequent longitudinal research to examine: (3) How does fear generalization and its neural correlates evolve across development; and (4) How does pathological anxiety affect these developmental processes. Answers to these questions will promote early detection, prevention, and novel therapeutics for pathological anxiety. The applicant aims to begin this line of research by addressing questions 1 & 2. A subsequent R01 funded study will address questions 3 & 4. The specific aims are as follows:

Aim 1: Investigate fear generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in healthy youth (n=25). We will recruit healthy youth from preadolescence to adolescence (ages 8-17 years). We will assess the degree to which they generalize conditioned fear by measuring vmPFC activation, self-reported fear, and skin-conductance resistance (SCR) using the fear generalization fMRI task adapted to youth (see Preliminary Data). We will assess vmPFC connectivity within the corticolimbic system using diffusion MRI (dMRI)-tractography and resting-state functional connectivity (rsFC).

Hypothesis 1 (H1) Healthy youth will generalize conditioned fear: Self-reported fear and fear-potentiated SCR will increase, as cues resemble the ‘threat’ cue. Conversely, vmPFC activation will increase, as cues resemble the ‘safety’ cue. **(H2)** Steepness of the vmPFC activation gradient across threat vs safety cues will correlate with vmPFC connectivity patterns within the corticolimbic circuit as shown in healthy adults¹¹.

Aim 2: Investigate fear over-generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in youth with pathological anxiety (n=50). We will recruit youth with pathological anxiety, subdivided into a mild-to-moderate anxiety group (n=25) and a moderate-to-severe anxiety group (n=25). We will collect the same measures as Aim #1 (fear generalization fMRI: self-reported fear, SCR, vmPFC activation; multimodal MRI: dMRI and rsFC). This will allow examining the behavior and neural correlates across the spectrum of anxiety symptoms from the healthy (Aim #1) and to anxious youth (Aim #2).

(H3) As anxiety severity increases, generalization of conditioned fear will also increase (over-generalization of fear): Self-reported fear, fear-potentiated SCR, and vmPFC activation will show less discriminating responses to threat vs safety cues. **(H4)** Less discriminating vmPFC activation across threat vs safety cues (i.e., decreased steepness of the gradient) will correlate with decreased corticolimbic connectivity.

Exploratory Aim: Assess the impact of development (age) and the interaction of age-by-anxiety on fear generalization behavior, vmPFC function, and corticolimbic connectivity.

Expected Outcomes: Successful completion of this study will provide cross-sectional evidence of the influence of pediatric anxiety on fear generalization behavior, vmPFC function, and corticolimbic system connectivity. The multiple units of analysis will help elucidate the brain-behavior relationships underlying fear generalization. This demonstration along with the research expertise developed through this K01 award will support an R01, longitudinal study of youth to track developmental trajectories of fear generalization and pathological anxiety. The line of research will promote the development of neuroimaging markers for early detection and novel intervention for pathological anxiety (*NIMH Strategic Objectives 2 & 3*).

2. CANDIDATE'S BACKGROUND

I am committed to translational and developmental research focusing on the neural mechanisms of pathological anxiety. My prior training has focused on biological systems engineering, basic neurobiology, and human cognitive neuroimaging. This K01 will enable me to pursue translational neuroscience to delineate the biological and cognitive bases of pathological anxiety in youth to improve diagnosis and treatment strategies.

Undergraduate Training. As an undergraduate at Korea University I studied ecological and environmental engineering in the College of Life Sciences. There I learned a holistic, systems approach to understanding complex biological systems such as ecological systems from the microbial to forest level. In parallel, I was fascinated by human emotion, deeply inspired by Dostoevsky's literature dissecting human psychology, and I took additional courses on human emotion and cognition. These two experiences led me to decide to study how the human mind is engineered and to pursue master's degree in neurobiology.

Master's Training. During my Master's training in neurobiology in the Department of Anatomy at Catholic Medical College in Korea, I studied the structural underpinnings of diverse gap junctions (i.e., electrical synapses) crucial for extensive coupling and swift inter-cellular signaling in retinal interneurons. Using neuro-anatomical methodologies (e.g., immunohistochemistry and electron microscopy), I discovered novel morphological differences in the gap junctions potentially explaining functional diversities of the retinal interneurons¹². I won a travel award to present the results at the Association for Research in Vision and Ophthalmology. My master's degree training equipped me with a neurobiologist's eye leading me to focus on the structure of the nervous system as an avenue to understanding its function.

PhD Training. I then moved with my wife and children from Korea to the US to join the PhD program in Neuroscience at Stony Brook University. I conducted my thesis research and coursework both in the Departments of Neurobiology and of Biomedical Engineering. Under the mentorship of Dr. Lilianne Mujica-Parodi, I completed my dissertation, entitled "Neural underpinnings of anxiety: Multi-modal magnetic resonance imaging approach." In adults with and without generalized anxiety disorder (GAD), I aimed to identify neural substrates of two constructs of the negative valence system—'acute threat (fear)' and 'potential threat (anxiety)'—as defined in the NIMH Research Domain Criteria (RDoc). This was my first scientific work with humans. I learned basic neuroimaging methodologies, cognitive tasks, and multivariate statistical modeling. This research suggested corticolimbic correlates of maladaptive emotional processes in adults with GAD, such as fear generalization (published 4 first-authored papers). I also collaborated on studies of attentional bias in healthy adults (5 co-authored papers). I received two travel grants to present the findings at the Society for Neuroscience and Human Brain Mapping.

In addition to my thesis research, I assisted a pediatric neuroimaging study (ages 4-6 years) in the same laboratory. This study used an affective task and fNIRS (functional Near-Infrared Spectroscopy) and was designed to develop a neuroimaging marker of risk for developmental psychopathology. The study showed that network efficiency of toddlers' PFC during negative affect processing correlates child's capability for effortful control¹³. In this project, I helped to recruit toddlers and collect neuroimaging and eye tracking data. Through this research experience, I learned the importance of developmental neuroscience research in public mental health, and began to develop research skills in working with children. Based on this experience, I decided to pursue pediatric neuroimaging as a method for understanding psychopathology in youth.

Postdoctoral Training. I started my postdoctoral training under the mentorship of Jonathan Posner, MD in the Division of Child & Adolescent Psychiatry in the Department of Psychiatry at Columbia University Medical Center (CUMC) and the New York State Psychiatric Institute (NYSPI). I have been investigating abnormal brain connectivity that can reliably predict symptoms in youth with various psychiatric disorders, including ADHD and anorexia nervosa. During this training, I have been acquiring additional skills in the acquisition of multimodal MRI data in youth and large-scale data analysis using a super-computing system. I published a first-authored article about childhood ADHD reporting on the relationship between aggression and brain connectivity within the reward circuit (Cha et al., *Neuropsychopharmacology*, in press¹⁴). This work led me to win an Outstanding Postdoctoral Researcher Award from the Association of Korean Neuroscientists at Society for Neuroscience. I completed two other first-authored and two co-authored manuscripts (currently in revision or submission). I received two additional travel grants to present my multimodal MRI work (ADAA (Anxiety and Depression Association of America) and Wisconsin Symposium on Emotion), a Career Development Leadership Program award (ADAA), and a pilot research grant (at CU/University of Glasgow) to develop a novel fMRI paradigm to study abnormal cortical feedback in affective disorders in youth.

Transition to Independent Career. My ultimate career goal is to become an independent research scientist to develop brain measures predicting the development of pathological anxiety and guide novel treatment strategies. I will focus on elucidating fear processing behaviors and their underlying neural circuits across

development and across the spectrum of anxiety from normal to pathological. My PhD training offered me the first opportunity to learn how to study anxiety in adults, the fundamentals of task-fMRI experimentation, and the analysis of diffusion MRI and rsFC. In my postdoctoral training, I began understanding the fundamentals of pediatric neuroimaging research in children with ADHD. Now my goal is to successfully transition to research independence and compete for an R01-level longitudinal study to examine developmental trajectories of fear generalization. To achieve these goals, I need to develop (i) **a sophisticated understanding of the phenomenology of anxiety disorders across the life span from children to young adults** (*Training Goal #1–phenomenology of anxiety disorders*), (ii) **expertise in translating developmental, affective neuroscience to patient-oriented research** (*Training Goal #2–patient-oriented translational neuroscience to anxiety*), (iii) **competency in cutting-edge MRI data analysis strategies to improve both sensitivity and specificity of neuroimaging-based predictive modeling towards precision medicine** (*Training Goal #3–advanced computational neuroimaging*). These additional experiences will help me to effectively link neuroscience and neuroimaging results to clinical populations, to facilitate developing neurobehavioral markers to promote a biologically-derived nosology of anxiety, and to develop neurobehavioral markers predictive of the development of psychopathology and treatment response. Lastly, as English is not my first language, my training will include mentorship to improve my skills in the writing of manuscripts, grants, and oral presentations. In sum, receiving this K01 award will allow me to fully benefit from the didactics and hands-on mentored training essential for the attainment of my ultimate goal of becoming an innovative, NIH-funded, independent scientist.

3. CAREER GOALS AND OBJECTIVES

My long-term career goal is to develop objective, reliable neuroimaging measures having clinical utility in the diagnosis and treatment of affective disorders. Towards this, my short-term goal is to learn the phenomenology of anxiety disorders, to acquire expertise in trans-diagnostic neuroimaging experimentation in youth with anxiety disorders, and to learn up-to-date imaging data analysis technologies. Since I have no formal training in these domains, I have primarily relied on collaborators for my work. To enrich my skills and ensure my research independence, I have therefore developed three training objectives for the K01 award:

- (1) **Phenomenology of anxiety disorders from youth to young adults**
- (2) **Patient-oriented, translational and developmental neuroscience to anxiety**
- (3) **Advanced computational neuroimaging.**

These newly developed skills and expertise along with the data from the proposed study described below will lead to a R01-level, longitudinal study across the developmental stages, from children to young adults, to delineate developmental trajectories of fear generalization and pathological anxiety. This future study will aim to identify behavioral and neuroimaging biomarkers that predict developmental changes of fear processing behavior and of brain circuits, and thus facilitate early detection and intervention of pathological anxiety.

4. CANDIDATE'S PLAN FOR CAREER DEVELOPMENT

During the period of this K01 Award, I will focus my training on three areas essential to my successful transition to research independence and responsible conduct of research. The mentoring team consists of experienced, leading scientists in anxiety disorders across the life span, translational and developmental neuroscience, and computational data analysis. Through this mentoring team, this K01 award will enable me to develop productive collaborations with leading researchers worldwide, which will contribute to my independent research career. The training plan will be implemented and overseen by two mentors, Drs. Jonathan Posner and Helen Blair Simpson, with weekly meetings throughout the K01 period to ensure that timely progress is made towards (1) publication of research articles for each training/research aim, and (2) preparation of an R01 application to NIMH. Drs. Posner and Simpson will organize a quarterly meeting with the advisors via conference call to review progress, to identify any roadblocks to success, and to ensure that training and research goals are met.

Mentoring Team. **Dr. Jonathan Posner** (*primary mentor*) is a clinician-researcher in child psychiatry and pediatric neuroimaging. His research focuses on the use of MRI to understand the neural bases of emotional processing and regulation in a range of psychiatric disorders affecting children. He has been a principal investigator on 3 NIH grants including NIMH K23, R01, and R21 awards, and is the project leader for a Multi-modal Imaging Core within a Conte Center examining the effects of serotonergic modulation on brain development. I have a successful history of research with Dr. Posner having written 2 first-authored manuscripts (one published¹⁴, the other in review). Dr. Posner is an ideal choice for the primary mentor of this application because his expertise in child psychiatry and developmental neuroimaging is central to this proposed research.

Dr. Blair H. Simpson (*co-mentor*) is an authority on translational research in psychiatry to transform the current diagnosis and treatments for anxiety, obsessive-compulsive disorder (OCD), and related disorders. She uses clinical trial methodology to test new treatments in patients with anxiety disorders and to determine how to combine and sequence current treatments to maximize outcome. Dr. Simpson participates in the RDoC project at the National Institutes of Mental Health, first as a member of the Negative Valence Systems Workshop, and now as the Principal Investigator of an NIMH-grant (R01) consistent with the RDoC approach. Dr. Simpson also was awarded a NIMH midcareer investigator award in patient-oriented research (K24), recognized her commitment to mentoring and training in patient-oriented research in OCD and related anxiety disorders. Dr. Simpson and I are currently writing two manuscripts. The first reports on neural correlates of comorbid anxiety and depression in the hippocampus. The second reports on abnormal brain connectivity in anorexia nervosa. Dr. Simpson is an ideal mentor for this proposal because of her clinical experiences in adulthood anxiety disorders, her track record of patient-oriented translational research, and her experience of successful mentoring for research independence.

Advisors. **Dr. Moira Rynn** (advisor #1) is Chief of the Division of Child and Adolescent Psychiatry. Her research is focused on psychopharmacological studies of pediatric anxiety disorders. Dr. Rynn has been the co-principal investigator on the Child Child/Adolescent Anxiety Multimodal Treatment Study (CAMS¹⁵). Dr. Rynn is an ideal advisor for this proposal because of her expertise in the phenomenology, treatment, and characterization of pediatric anxiety disorders. **Dr. Daniel S. Pine** (advisor #2) is Chief of Section of Development and Affective Neuroscience at NIMH, whose research is focused on translational neuroscience including biological aspects of mood, anxiety, and behavioral disorders in children, as well as classification of psychopathology across the lifespan. Dr. Pine is an ideal advisor for this proposal because of his leading role of applying affective neuroscience, psychobiological and neuroimaging experimentation to studies of pediatric anxiety. **Dr. DuBois Bowman** (advisor #3) is Chair of the Department of Biostatistics at CUMC/NYSPI, whose research is focused on the development of predictive modeling integrating fMRI and diffusion MRI to establish markers of depression, schizophrenia, Parkinson's disease, and cocaine addiction. His research focus and expertise makes him an ideal advisor for Training Goal #3—advanced computational neuroimaging. **Dr. Klaas E. Stephan** (advisor #4) is founding Director of Translational Neuromodeling Unit at ETH Zurich, whose research is focused on development of computational tools to delineate maladaptive emotion/cognitive processes using neuroimaging. He is an ideal advisor for the Training Goal #3 because of his research focus and his recent development of novel Bayesian learning modeling (e.g., Hierarchical Gaussian Filtering) to infer learning mechanisms underlying maladaptive emotion processes. **Dr. Shinjae Yoo** (advisor #5) is a data scientist at Brookhaven National Laboratory, who has developed and applied state-of-the-art machine learning algorithms to data-intensive fields, such as nuclear physics, climate science, and social network analysis. He is an ideal advisor because he will teach me the cutting edge big data science approach that has yet to be applied to neuroimaging and psychiatric research.

Goal 1: Phenomenology of anxiety disorders from youth to young adults

Rationale. I seek to obtain proficiency in the etiology, phenomenology, and assessments of pathological anxiety across the lifespan from children to young adults. Given my research focus on youth with pathological anxiety, I need to be fluent not only in formal diagnoses, but also with a spectrum of the disorders and symptomatology of the childhood anxiety disorders. This training is an essential prerequisite for me to become a fully independent investigator conducting translational research in anxiety disorders. **Drs. Simpson and Rynn** will supervise my training in this area.

Coursework.

1. Anxiety Disorders (young adults): Directed by **Dr. Simpson**, this is a 10-session course in the psychiatry residency Program at CUMC. It covers the phenomenology, psychobiology of the anxiety disorders.
2. Psychopathology (children and adolescents): Directed by **Dr. Laurence Greenhill**, a course in the child and adolescent psychiatry residency program at CUMC. Recommended by Dr. Rynn, this will cover clinical assessments and diagnosis of anxiety disorders and offer firsthand experiences of patients.

Supervision and Hands-On Practicum.

1. Anxiety Disorders in Young Adults: I will meet **Dr. Simpson** weekly at CUMC/NYSPI to learn to screen range of anxiety disorders and relevant clinical measures in young adults.
2. Anxiety Disorders in Children and Adolescents: (1) I will attend weekly Friday research meetings in Pediatric Anxiety and Mood Research clinic led by Dr. Rynn to learn about clinical cases, therapeutics, and clinical research. (2) I will also meet Dr. **Rynn** one-on-one monthly at CUMC/NYSPI to review the progress of the training and observership of clinical assessments, psychotherapy, and research at the Pediatric Anxiety and Mood Research Clinic.

Goal 2: Patient-oriented, translational, developmental neuroscience to anxiety disorders

Rationale. I seek to obtain expertise in translating developmental neuroscience research to patient-oriented research in anxiety disorders. This is crucial to harness basic neuroscience knowledge, and the neuroimaging approach to achieve better understanding, treatment, and prevention of anxiety disorders. Successful completion of this training goal will help me design and conduct neuroscience studies using a dimensional, not categorical, approach and a trans-diagnostic approach, and translate the research outcomes to inform diagnosis and treatment of anxiety disorders. **Drs. Posner, Simpson, and Pine** will supervise this training.

Coursework.

1. Development and Psychopathology (HUDK 5040, Developmental Psychology Program, Psychology Dept. at CU) will focus on the interface between classical developmental psychology theories and atypical development patterns.
2. Seminar in Risk, Resilience and Development Psychology (HUDK 6529, Developmental Psychology Program, Psychology Dept. at CU) will cover developmental models for understanding risk and resilience.

Supervision and Hands-On Practicum.

1. Translational Pediatric Neuroimaging (1) I will meet **Dr. Posner** weekly at CUMC/NYSPI to discuss study design, recruitment, MRI data collection in youth with or without anxiety disorders. (2) I will attend a weekly meeting of Dr. Posner's lab at CUMC/NYSPI to learn about translational neuroimaging study in youth.
2. Translational Neuroscience to Clinical Anxiety: (1) I will meet **Dr. Simpson** weekly at CUMC/NYSPI to discuss how to identify neurocognitive and neuroimaging biomarkers for pathological anxiety, and based on the identified measures, how to establish a new way to classify pathological anxiety; (2) I will attend the weekly Science Meeting at Anxiety Disorders Clinic, led by **Dr. Simpson**, where experts of patient-oriented, translational research in anxiety-related disorders present on-going research.
3. Translational, Affective, Developmental Neuroscience: I will meet **Dr. Pine** bimonthly via conference call to discuss how to use insights from neuroscience to inform understandings of risk, diagnosis, and treatment of anxiety disorders. I will also visit **Dr. Pine**'s lab at NIMH to learn psychobiological and functional neuroimaging experiments in youth with anxiety disorders (~5d/yr).

Seminars and Conferences. I will attend Columbia Translational Neuroscience Initiative (CTNI) Symposium. CTNI represents disease-focused neuroscience research centers at CUMC to enhance basic and translational neuroscience research. I will also attend the annual meeting of Biological Psychiatry and Society for Neuroscience to present on-going research and to meet **Dr. Pine**.

Goal 3: Advanced computational neuroimaging

Rationale. My long-term goal is to develop neuroimaging measurements having such sensitivity and specificity to be used for diagnosis of mental disorders, essential is robust computational methods to neuroimaging. I have used structural equation modeling and multivariate analyses in prior neuroimaging studies, but these were not originally designed for single-subject level prediction. Thus I seek additional training in cutting-edge

Table 2. Short-term K01 goals linked to training, research, mentorship, and R01 grant proposal

Formal Didactic Course		Seminars & Conferences		Tutorials & Hands-on Practicum			
Training Aim	Year 1	Year 2	Year 3	Year 4			
1. Phenomenology of anxiety disorders	<ul style="list-style-type: none"> Anxiety Disorders (2hr/wk; fall) Psychopathology (2hr/wk; spring) 						
	<ul style="list-style-type: none"> Anxiety Disorders in Young Adults: Dr. Simpson (one-on-one meeting–1hr/wk) Anxiety Disorders in Children and Adolescents: Dr. Rynn (research meeting in Pediatric Anxiety & Depression Clinic–1hr/wk; 1-on-1 meeting–1hr/mo) 						
2. Patient-oriented, translational, neuroscience to anxiety disorders			<ul style="list-style-type: none"> Development and Psycho-Pathology (4hr/wk; fall) 	<ul style="list-style-type: none"> Seminar in risk, resilience and development psychology (1hr/wk; fall) 			
	<ul style="list-style-type: none"> Columbia Translational Neuroscience Initiative (CTNI) symposium (1hr/wk) 		<ul style="list-style-type: none"> Society of Biological Psychiatry (3d/yr) 	<ul style="list-style-type: none"> Society for Neuroscience (5d/yr) 			
	<ul style="list-style-type: none"> Translational Pediatric Neuroimaging: Dr. Posner (research meeting in Laboratory for Pediatric Neuroimaging–1hr/wk; 1-on-1 meeting–1hr/wk) Patient-oriented, Translational Neuroscience: Dr. Simpson (Anxiety Disorders Clinic science meeting–1hr/wk; 1-on-1 meeting–1hr/wk) Translational, Affective, Developmental Neuroscience: Dr. Pine (meeting via conference call–1hr/2mo; visit Dr. Pine's lab to learn psychobiological experiments–5d/yr) 						
	<ul style="list-style-type: none"> Computational Psychiatry Course (London, 2d/yr) Human Brain Project Workshop (Switzerland, 3d/yr) 		<ul style="list-style-type: none"> Machine learning for data science (4hr/wk; spring) 	<ul style="list-style-type: none"> Bayesian Statistics (4hr/wk; spring) 			
3. Advanced computational neuroimaging	<ul style="list-style-type: none"> Data Science Institute Colloquium (1hr/wk) Biostatistics Seminar (1hr/wk) 		<ul style="list-style-type: none"> Data Science Institute Bi-annual symposium (1d/yr) 				
			<ul style="list-style-type: none"> Computational Psychiatry (2d/yr) 				
	<ul style="list-style-type: none"> Integration of Multi-Modal MRI for Biomarkers of Anxiety: Dr. Bowman (1-on-1 meeting–1hr/mo) Advanced Bayesian Modeling: Dr. Stephan (visit Dr. Stephan's Translational Modeling Unit in Zurich to learn Hierarchical Gaussian Filtering–2w/yr) Big-data science approach to neuroimaging: Dr. Yoo (1-on-1 meeting–1hr/mo) 						
4. Career development for independence.	<ul style="list-style-type: none"> Writing a Successful NIH Grant Application (1hr/wk; fall) 		<ul style="list-style-type: none"> Funding for Research (1hr/wk; fall) 				
Ethics: Responsible Conduct of Research	<ul style="list-style-type: none"> Psychiatric Ethics (1hr/wk; fall) Responsible Conduct of Research (1hr/wk; spring) 		<ul style="list-style-type: none"> Annual Ethnics Symposium (1hr/wk; spring) 				
	<ul style="list-style-type: none"> 1-on-1 meetings with Drs. Posner (advisor; 1hr/wk), Simpson (co-mentor; 1hr/wk), Rynn (advisor; 1hr/mo) 						

computational methods including predictive modeling integrating structural and functional connectivity, model-based fMRI analysis, state-of-the-art big data science approach that has been successfully applied to computation-intensive fields, but has yet to be applied to neuroimaging (e.g., social network analysis, physics, bioinformatics and climate science). Dr. Bowman will supervise the training goal and Drs. Stephan and Yoo will provide additional consultation.

Coursework.

1. Machine Learning for Data Science (W4721, Dept. of Computer Science at CU) will provide an introduction to the field of machine learning and hands-on experience in the application to various data, including fMRI time series or MR-based connectivity data.
2. Bayesian Statistics (W4640, Dept. of Statistics at CU) will provide a foundation of Bayesian inferences and modeling including Bayesian hierarchical models. This course will help successfully apply Gaussian Hierarchical Filtering (HGF) to fear generalization fMRI time series, developed by **Dr. Stephan**'s group. I will use HGF to infer cognitive learning mechanisms underlying maladaptive fear processing in pathological anxiety in this research proposal (see Research Strategy-Approach-Research Plan-Data Analysis 2)
3. Computational Psychiatry Course (UK, 2d/y) will cover Model-based approaches at multiple levels of analysis (behavioral, neurophysiological, fMRI).
4. Human Brain Project (HBP) School on Future Computational Medicine (Switzerland, 3d/yr) will cover novel computational methods developed by HBP led by European Research Council.

Supervision and Hands-On Practicum.

1. Integration of Multi-Modal MRI for Biomarkers of Anxiety: I will meet **Dr. Bowman** monthly at CUMC/NYSPI to discuss how to integrate multimodal MRI (e.g., structural and functional connectivity) to search for a reliable biomarker of pathological anxiety. During the meetings, Dr. Bowman will provide hands-on training in analyzing the task-fMRI and multimodal MRI data collected in Aim #1 and #2.

2. *Advanced Bayesian Modeling*: I will visit **Dr. Stephan**'s lab (Translational Neuromodeling Unit) at Zurich, Switzerland to learn Hierarchical Gaussian Filtering (research Aim#1 and 2) (14d/yr).
3. *Big-Data Science Approach to Neuroimaging and Psychiatric Research*: I will meet **Dr. Yoo** *monthly* at Stony Brook University (an hour and a half's drive from CUMC) or via Google video chat to discuss how to employ big-data science approach (e.g., data mining techniques using machine learning algorithms and scalable computational methods). I have already got an access to the core computational facility run by **Dr. Yoo**'s group for advanced computational analyses.

Seminars and Conferences. I will attend *Data Science Institute Colloquium* (Data Science Institute, CU; 1hr/w), *Data Science Institute Bi-annual symposium* (1d/yr), and *Biostatistics Seminar* (CUMC/NYSPI; 1hr/wk), and to the annual meeting of Computational Psychiatry to present on-going results and to meet Dr. **Stephan**.

A. SIGNIFICANCE

Overview. Anxiety disorders are extremely common affecting about 25% of adolescents and 18% of adults³. Anxiety disorders are associated with substantial functional impairment and economic costs related to lost productivity and treatment. These disorders in children, convey a host of negative outcomes including school refusal, poor academic performance¹⁶, or increased risks for other mental disorders, such as depression^{17, 18}, substance abuse^{19, 20}, and ADHD²¹. Though treatments are available, they are often inadequate with response rates typically ranging between 35%²² and 81%¹⁵ (compared to 24% those of placebo). Moreover, we have no means of determining which of the available treatments is most likely to be helpful to a particular patient. Lastly, anxiety disorders are typically chronic conditions that either emerge in youth or have developmental antecedents. In youth who display symptoms or risk factors for an anxiety disorder, we are scarcely able to predict which children go on to develop a chronic disorder vs those for whom the symptoms resolve. We therefore maintain that it is critical to understand the neural mechanisms underlying anxiety in youth. This will not only foster the development of new interventions and preventive strategies, but will also aid in discovering new targets for developing therapeutics.

This proposal is significant for the following reasons:

- 1) ***Pathological anxiety in youth*** is extremely common and is associated with a range of negative outcomes. Understanding the neurobiological substrates underlying pediatric anxiety disorders is critical to developing better interventions (*NIMH Strategic Objective #1–Mechanisms of Complex Behaviors*).
- 2) ***Fear over-generalization*** is thought to be an important mechanism underlying pathological anxiety in adults; it has never been studied in anxious youth. Understanding fear over-generalization in pediatric anxiety disorders is important in understanding what causes anxiety, how to better diagnose pathological anxiety, and to promote new treatments (*NIMH Strategic Objectives #3–Strive for prevention and cures*).
- 3) ***Implementing a dimensional approach to the study of anxiety*** is critical to discovering neurobehavioral mechanisms that cut across anxiety disorders; this knowledge will ultimately contribute to a biologically informed psychiatric nosology, eschewing dependence on symptom-based classification (*NIMH Strategic Objective #1–Mechanisms of Complex Behaviors*).
- 4) ***Charting the developmental course of anxiety and its neural correlates*** is critical for early detection and intervention (*NIMH Strategic Objectives #2–Charting trajectories*). Though this K award is cross-sectional in nature, it will provide data and skills/expertise to inform an R01 proposal for a large-scale longitudinal study tracking changes of fear generalization and the underlying corticolimbic circuit.

WHY CHILDHOOD ANXIETY DISORDERS? Roughly 25% Americans will meet lifetime criteria for an anxiety disorder²³. Anxiety disorders are the earliest psychiatric conditions to manifest, with a median age of onset of 11 years²⁴. Since transient fears and anxieties are considered normal development, this developmental expectation often serves to mask the presence of an emerging or existing anxiety disorder²⁵. Indeed 80% of kids with a diagnosable anxiety disorder do not receive treatment²⁶. Untreated childhood anxiety disorders are associated with considerable functional impairment, such as poor academic performance and school refusal^{16, 27}. Childhood anxiety disorders also have adverse long-term outcomes such as increased risks for other negative mental health outcomes over time, such as depression¹⁸, substance abuse^{19, 20}, and ADHD²¹.

WHY STUDY FEAR GENERALIZATION? Generalization of fear is a normal adaptive behavior. Humans interpret the perceptual or conceptual details of a learning episode and generalize learned behavior (e.g., fear responses) to different, similar stimuli. In this way generalization of fear can serve as a normal adaptive function that aids survival. However, an excessive form of it, ***fear over-generalization, can contributes to anxiety pathology, because it proliferates anxiety cues and therefore increases or sustains anxiety symptoms***. Despite its clinical relevance, compared with other associative learning processes linked to pathological anxiety (e.g., extinction/recall²⁸⁻³⁰ and fear inhibition^{31, 32}), fear generalization has been relatively understudied.

Psychobiological studies in adults demonstrate that fear over-generalization occurs in various anxiety disorders, such as generalized anxiety disorder (GAD)⁷ and panic disorder⁸. These studies used cued fear conditioning to test generalization effects (**Figure 1**). In this paradigm, participants are typically shown a cue signaling a threat (CS+) as well as a range of cues that are similar but not identical to the threat cue and are never paired with a threat. One can then test a differential response to threat vs a continuum of safety cues. This study will employ a similar fear generalization paradigm adapted to youth (see **Figure 5** for preliminary data and **Figure 6** for the study design).

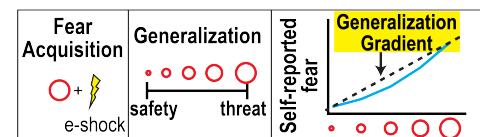


Figure 1. Pavlovian conditioning paradigm testing generalization of conditioned fear. After fear acquisition, a generalization effect can be determined by measuring generalization gradients of fear-associated responses (e.g., self-reported fear; see **Figure 3**).

WHY FOCUS ON VMPFC IN FEAR GENERALIZATION? *Studies show that in adults with GAD,*

vmPFC activation is less discriminating of safety vs threat cues compared to healthy adults. The applicant's previous fMRI study in healthy adults demonstrate that vmPFC activation during fear generalization shows a steep generalization curve in HC, such that it activates more towards cues similar to a safety cue, suggesting that in healthy adults the vmPFC tracks safety signals (see Preliminary Studies—Figure 3)⁹. In contrast, in adults with GAD, vmPFC activation shows a *less* steep generalization curve (hence less discriminating) across the continuum of threat and safety¹⁰. A less steep vmPFC generalization gradient in adults with GAD correlates with a greater anxiety symptom severity. This suggests that fear over-generalization in adults with GAD is associated with the inability of the brain (i.e., vmPFC) to discriminate threat vs safety cues. Based on this finding, this study will examine the same system in youths with pathological anxiety for the first time (Aim #2).

BRAIN CORRELATES OF FEAR GENERALIZATION. The vmPFC is considered as an integral hub of the corticolimbic system that mediates emotion and cognitive processes. Within this system *interactions between the excitatory subcortical areas (ventral affective system) and inhibitory PFC areas (PFC regulatory system) are essential in generating affective meaning in the vmPFC*^{33, 34}. The vmPFC *integrates* extensive corticolimbic afferents, *computes* an affective value, and *mediates* an adaptive response. During threat processing, the vmPFC orchestrates several regions within the corticolimbic system: the amygdala^{35, 36}, the hippocampus³⁶⁻³⁸, the thalamus^{39, 40}, and the prefrontal attentional network^{41, 42}. VmPFC function thus may depend upon how it is connected to the corticolimbic circuit.

The applicant's recent study supports this view. In a multimodal MRI study, impaired vmPFC threat-safety discrimination in adults with GAD correlates with multiple factors of the corticolimbic system: (i) decreased vmPFC grey matter thickness, (ii) less positive vmPFC intrinsic functional coupling with the dorsal PFC and more positive coupling with the amygdala, and (iii) decreased structural connectivity of the major white matter tracts encompassing the vmPFC. Moreover, these corticolimbic circuit measures have significant impact on a diagnosis of GAD (see Figure 4 for preliminary data). These support vmPFC's integral role as a hub node of the corticolimbic system in assessment of threat vs safety³³. *Therefore examining vmPFC fear generalization may offer a useful proxy of how multiple related regions/systems are orchestrated to generate affective meaning (e.g., threat or safety).* This study will use this multimodal MRI approach for the first time to study neural correlates of pathological anxiety in youths.

WHY STUDY FEAR GENERALIZATION IN YOUTH? A few studies have examined other fear associative learning processes, such as fear conditioning or extinction. One psychobiological study found that healthy youth (mean age=11.5 ± 2.57_{sd}) showed comparable fear learning and extinction to adults⁴³. An fMRI study found that preadolescents with anxiety disorders (mean age=9.8 ± 21.2_{sd}) showed greater amygdala activation to fear compared with neutral faces, similar to anxious adults⁴⁴. Based on these, it is likely that healthy youth may be capable of generalizing fear; however, this has yet to be directly tested (see Figure 5, preliminary data). A related question is how pathological anxiety affects fear generalization behavior and its neural correlates, such as the vmPFC and the corticolimbic system. *Addressing these question may contribute to public health, in that they may present potential neural markers of pathological anxiety in youth.*

ANXIETY DISORDERS AS DEVELOPMENTAL DISORDERS. Pathological anxiety has been previously been primarily defined by symptoms. A recent conceptualization suggests that *only after disruptions of brain circuits do noticeable anxiety symptoms occur*. Essential is early detection of atypical brain development, as well as the relationship between atypical brain development and abnormal behavior linked to anxiety symptoms.

Brain development is a nonlinear process (Figure 2). Rates of development are different across regions. For example, the ventral affective system (including the amygdala) in youth shows comparable activity to that of adults, whereas the PFC regulatory system including (the ventral and dorsal PFC) in youth is still reaching maturity¹. These unparalleled developmental rates between the two systems may contribute to changes in typical fear/threat processing across developmental stages. Pathological anxiety may involve abnormal brain developmental processes that contribute to abnormal fear/threat processing. Yet there exists little empirical research linking developmental changes in youths' brains (function, structure, or circuit connectivity) to developmental changes in behavior, cognition, or affect^{45, 46}. In this study, we will explore the effect of age on fear generalization and its underlying neural sys-

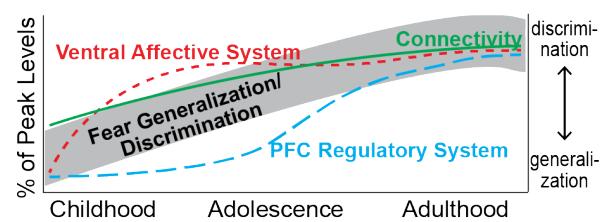


Figure 2. Nonlinear neurodevelopmental processes. The developmental chart shows nonlinear, multiple processes across brain systems: the ventral affective system and PFC regulatory system. *Of note, the trajectory of fear generalization (grey bend) may depend on development of both systems and inter-system connectivity.* Adapted from Hartley and Lee¹.

tem. Due to the limited scope of a K01 award, we cannot create a comprehensive map of developmental trajectories. Nevertheless, the data and skills/expertise from this award will lead to a future, larger-scale longitudinal study tracking changes of fear generalization and its underlying corticolimbic circuit.

B. INNOVATION

This application is innovative in four reasons:

- 1) Novel examination of *fear generalization and the underlying neural substrates in youth*:** No prior studies have examined the neural circuit of fear generalization in youth. This approach will not only provide a cross-sectional perspective on its developmental course, but will also advance our understanding of the neurobehavioral mechanism of sensitivity to potential threat.
- 2) Novel application of *multiple units of analysis*** (behavior, task-fMRI, structural, diffusion, and resting-state fMRI, and self-reports of anxiety symptoms) in youth: No prior studies have combined fear generalization and multi-modal MRI in youth with pathological anxiety. This approach will allow linking dysfunction within specific neural regions to anomalies within a neural circuit and to psychology.
- 3) A *sampling strategy* that is both trans-diagnostic (i.e. inclusion of multiple anxiety disorders) and dimensional (i.e., inclusion of normal to abnormal anxiety):** No prior studies have used either approach in neuroimaging studies of pediatric anxiety. The trans-diagnostic approach will allow examining fear generalization as a *general mechanism* across different anxiety diagnoses; the dimensional approach combined with “metastructure” of DSM-5 anxiety disorders will allow investigating the spectrum of normal to abnormal fear generalization, and thus could link neuroscience to psychopathology of pediatric anxiety.
- 4) The proposed training plan is *highly interdisciplinary and novel*** in the field of child psychiatric research, because it combines expertise in (i) psychopathology of anxiety disorders, (ii) translational and developmental neuroscience to youth, and (iii) computational approach to multimodal MRI. This will be an excellent training vehicle towards translational neuroscience research studying pathological anxiety.

C. APPROACH

C1. Preliminary Studies. This section describes pilot data supporting the design and feasibility of the proposed project: The applicant's previous studies demonstrate that (1) in healthy adults the vmPFC activation tracks safety signals⁹, (2) in adults with GAD the vmPFC activates less to safety but more to threat signals¹⁰, (3) in both healthy adults and adults with GAD, the fidelity of the vmPFC threat-safety discrimination is closely linked to connectivity of the corticolimbic system¹¹. The applicant's pilot data also show (4) validation of the fear generalization fMRI task in youth with pilot data in five cases.

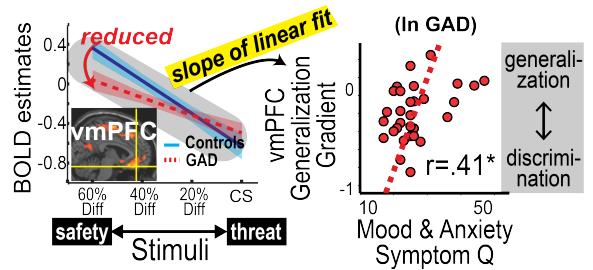


Figure 3. A, VMPFC activation plotted as a function of stimuli. Healthy adults showed greater vmPFC activation to safety than to threat (solid line). Steepness of this activation gradient was significantly reduced in GAD (dotted line). **B, A scatter plot showing association between VMPFC generalization gradient and anxiety symptom severity in GAD** (anxiety subscale in Mood & Anxiety Symptom Qn²). * $p < .05$

1. VMPFC activation tracks safety signals in healthy adults (n=25). In the applicant's PhD study, young healthy adults (ages 18-24 years) were tested on a fear generalization fMRI task. VmPFC activation tracked safety signals⁹ (Figure 3A; solid line). *This supports feasibility of using fear generalization fMRI to study vmPFC threat processing (Aim 1).*

2. Abnormal vmPFC function in adults with GAD (n=32). Adults with *GAD* group showed less discriminating vmPFC generalization gradient across threat vs safety cues, that is, reduced activation to safety cues, compared with HC¹⁰ (Figure 3A; dotted line). A less discriminating vmPFC generalization gradient, in adults with GAD, correlates with a greater anxiety symptom (Figure 3B). *These data demonstrate that abnormal vmPFC function is linked to GAD in adults. This K01 will investigate this link in youth with pathological anxiety for the first time.*

3. Abnormal vmPFC function is associated with abnormal corticolimbic circuit. Using multimodal MRI, the applicant showed during his PhD training that, in adults with GAD, specific abnormal corticolimbic circuit measures have significant impact not only on the abnormal vmPFC function during fear generalization (a direct effect; dotted line in Figure 4), but also on the diagnosis of GAD (an indirect effect; solid line in Figure 4)¹¹. *These data suggest that abnormal*

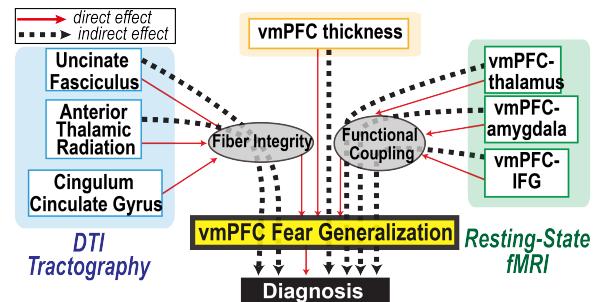


Figure 4. VMPFC threat processing is associated with multivariate patterns of the corticolimbic circuit. Only significant paths are shown ($p < .05$). Methods: *Structural Equation Modeling*. Parameters were estimated using bootstrapping simulation.

corticolimbic connectivity may be associated with psychopathology of adulthood anxiety disorders via its impact on the vmPFC function.

4. Validation of fear generalization fMRI in youth. In a pilot study of fear generalization fMRI, healthy youth (n=5; age 11-17 years; mean=15.2) showed significant generalization gradients in self-reported fear and activation of the corticolimbic system (**Figure 5**). VmPFC activation tracked safety signals showing greater activation towards the safety cues (i.e., cues 60% different (small or bigger) from the conditioned stimulus (CS)). These data support that youth may generalize fear.

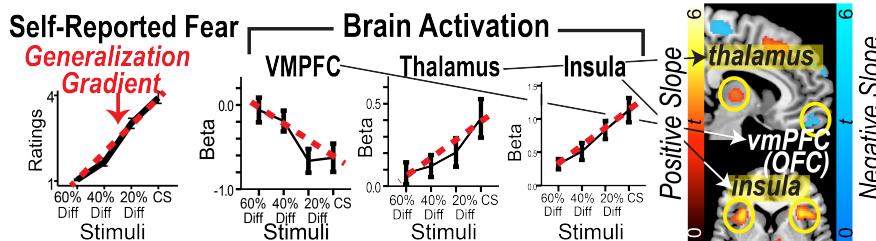


Figure 5. Fear generalization gradient in healthy youth (n=5). Healthy youth (ages 11-17 years) showed significant linear gradients in risk ratings ($p < 0.001$) and brain activation ($p's < 0.05$) within the corticolimbic system ($p's < 0.05$). Methods: risk ratings and brain activation are plotted as a function of stimuli. Repeated-measures of ANOVA were used using stimuli as the within-subject variable. Error bar is 1 se.

C2. Research Plan The overall objective of this proposal is to test the impact of pathological anxiety in youth on fear generalization behavior and the underlying corticolimbic function and connectivity. To this end, we propose a multimodal MRI study combined with a fear generalization fMRI task in youth (ages 8-17 years) across the spectrum of anxiety, from normal to abnormal. In Specific Aim #1, we will investigate fear generalization behavior (using self-reported fear and fear-potentiated SCR) and its relationship with corticolimbic function (vmPFC activation) and connectivity (diffusion tractography and rsFC) in healthy youth (n=25). In Specific Aim #2, we will investigate fear over-generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in youth with pathological anxiety (n=50).

Subjects. Children and adolescents (n=75) will be recruited across 3 anxiety symptom levels:

- The anxiety levels will be based on Pediatric Anxiety Rating Scale⁴⁷ (PARS; range 0-30): (i) low to mild anxiety ($\text{PARS} \leq 12$; n=25; Aim #1); (ii) mild to moderate ($13 \leq \text{PARS} \leq 19$; n=25; 13 is considered clinically meaningful anxiety; Aim #2); (iii) moderate to severe ($20 \leq \text{PARS}$; n=25; an inclusion criterion used in the Child/Adolescent Anxiety Multimodal Study¹⁵; Aim #2).
- Within each group, subjects will be matched on age, sex, handedness and socioeconomic status.

All subjects will meet the following inclusion criteria: 1) ages 8-17 years; 2) medication-free for a minimum of 2 months prior to participation in the study, and exclusion criteria: 1) Current DSM-5 disorder other than an anxiety disorder or mild-to-moderate Major Depressive Disorder (Children's Depression Inventory or CDI<20; a cut-off for moderate depression^{48, 49}); 2) MRI contraindication; 3) History of major neurological or medical illness or head trauma with>10 min loss of consciousness; 4) History of substance dependence; 5) IQ<80 (Wechsler Abbreviated Scale of Intelligence).

Key Design Decisions. The current proposal is designed to generate pilot data to inform a R01 longitudinal study tracking developmental changes of fear generalization from children to young adults using a dimensional and trans-diagnostic approach. **Selection of ages 8-17 years.** Fear generalization has been tested in adults in previous studies (young adults with ages 18-24 years in the applicant's PhD study), but no report exists in children and adolescents. **Trans-diagnostic sampling.** In previous studies fear generalization is shown in adults with various anxiety disorders^{8, 50, 51}. Based on this, this study will begin to study fear generalization in youth across anxiety disorders, starting with the most common triad of anxiety diagnoses in youth—GAD, Social Phobia, and Social Anxiety Disorder, which are highly comorbid³⁸. **Dimensional sampling.** We chose to focus on the dimension of anxiety severity (not diagnosis) in keeping with the NIMH Strategic Plan and the RDoC initiative.

Recruitment. **Aim 1.** We will recruit 25 healthy youth (low-to-mild anxiety) through community outreach and public advertisements. Dr. Posner's lab (mentor) recruits, on average, more than 30 healthy children and adolescents per year across developmental stages. We thus do not anticipate difficulty in reaching the recruitment goal of 25 youth in year 1 of the award. **Aim 2.** We will recruit 25 mild-to-moderate youth and 25 moderate-to-severe youth from patients and subthreshold anxiety individuals through 3 resources: (i) the Pediatric Anxiety and Depression Research Clinic (directed by Dr. Rynn, advisor), (ii) the Anxiety Disorders Clinic (directed by Dr. Simpson, co-mentor), and (iii) referrals from mental health clinicians in the Columbia Division of Child and Adolescent Psychiatry (directed by Dr. Rynn). These recruitment sites can provide referrals across the range of anxiety symptom severity. Over the past 2 years, patients in the Pediatric Anxiety and Depression Research Clinic have had PARS total scores from 10-27 (95% confidence interval; mean=19). A similar range of anxiety severity is present in the Columbia child and adolescent outpatient clinics. These recruitment sites

can also provide medication-free referrals. The Pediatric Anxiety and Depression Research Clinic recruits more than 25 drug-free patients per year to research protocols, and the Anxiety Disorder Clinic recruits on average 40 drug-free patients per year. Dr. Posner has also recruited more than 120 *medication-free* youth for the NIMH-funded neuroimaging studies over the last four years. We thus do not anticipate difficulty in reaching the recruitment goal of 40 anxious youth in Year 2-3 of the award period.

Clinical Assessments. *General assessment.* Children who meet study criteria will be assessed using Anxiety Disorders Interview Schedule-Revised (**ADIS-R**⁵²). Tanner Questionnaire⁵³ (to assess pubertal stages), and Female Menstrual Cycle (**FMC**⁵⁴). *Severity of anxiety symptoms:* We will use Pediatric Anxiety Rating Scale (**PARS**⁴⁷), State Trait Anxiety Inventory for children (**STAIC**⁵⁵), and Screen for Child Anxiety Related Emotional Disorders (**SCARED**⁵⁶). *Severity of other mood symptoms:* Children's Depression Inventory (**CDI**⁴⁸).

Participant Preparation. We will use a mock scanner session, frequent praise and reminders to remain awake and still. Using these, for the last 4 years Dr. Posner's laboratory has obtained over 120 high-quality scans in clinical youth populations difficult to scan (e.g., patients with ADHD).

Experimental Procedures. After screening, and providing consent in accordance with a protocol approved by the CUMC/NYSPI, participants will undergo multimodal MRI scanning. We will perform the following 38-min long MRI scanning (structural MRI (5 min), fear generalization fMRI (15 min), diffusion MRI (8 min), and resting-state fMRI (eyes-open) (10 min).

Fear Generalization fMRI Task. Inside a MRI scanner, participants will complete a 14.5-min long fear general-

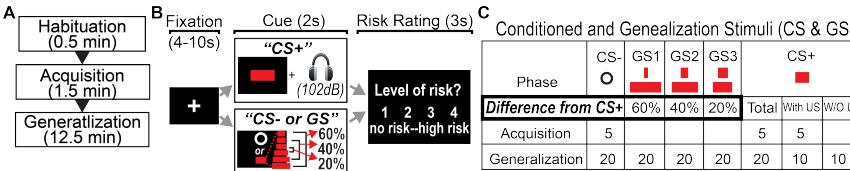


Figure 6. Fear generalization fMRI task.

A. Overall plan. **B.** Generalization phase.

C. Trial types and frequencies during the

study: For an acquisition phase, only CS+ or CS- will be presented. For a generalization phase, all the stimuli (CS+, CS-, and GS) will be presented.

ization task. Of note, we will use an instructed fear generalization paradigm (i.e., participants will be informed of CS-US (unconditioned stimulus) contingency before the task), because it allows minimizing the iteration of stimuli representation to produce a robust CS-US pair and robust generalization of conditioned fear^{10, 57}.

The task consists of habituation, acquisition, and generalization (Figure 6A). The entire task is conducted inside the MRI scanner but participants will be scanned only during the generalization phase, the phase of our interest. *For a habituation phase*, participants will hear an auditory startle probe (40msec, 102dB⁵⁸) six times without any visual cues to order to minimize startle-caused head motion. The startle probe will serve as the Unconditioned Stimulus (US). Next, *for an acquisition phase*, participants will be instructed on CS-US contingency: Presentation (2sec) of a mid-sized rectangle (56 pixels in height and 280 pixels in width) will be paired with the auditory startle probe (occurring 1.9s after the onset of the rectangle). This rectangle will thus serve as CS+. Participants will then view a white ring without a startle (CS-), and the mid-sized rectangle (CS+) presented in a pseudo-random order. During the presentation, CS+ will be always paired with the US to induce rapid fear acquisition. Lastly, *for a generalization phase*, the same CS+ (the mid-sized rectangle) and CS- (the white ring) will be used, and 6 other rectangles in of identical height (56 pixels) but with varying widths (112-448 pixels) will be also used. The 6 rectangles will differ by ±20%, ±40% or ±60% in width from the CS+. Note that, for GS, widths are counter-balanced. These rectangles will never be paired with the US; thus they will serve as the generalization stimuli (GS). *Altogether, these create a continuum of threat and safety.* During generalization, we will present pseudo-randomly a stimulus for 2s followed by a jittered fixation cross. This will be followed by a risk rating on a 4-point scale using a button box (Figure 6B).

Data Acquisition. FMRI. Participants will be scanned with a 3T GE Signa scanner with 32-channel head coil at CUMC/NYSPI. We will collect **rs-fMRI**, **SMRI** and **DMRI** as in the applicant's previous study¹⁴ (**Table 1. Self-Reported Fear.** Participants will rate their perceived fear or risk immediately after viewing each stimulus on a four-point Likert scale using a MR-compatible button box. **Fear-potentiated SCR.** SCRs will be collected from the second and third digits using a MR-compatible electrodermal activity recording system (BIOPAC Systems, Goleta, CA). An SCR will be scored if the trough-to-peak response begins 1-4s after stimulus onset, lasts .5-5.0s with amplitude greater than .02 microsiemens^{43, 59}. Scored SCRs will be normalized in each individual to compare across subjects⁶⁰.

Table 1. Pulse sequences of multimodal MRI

fMRI	T2*-weighted echo planar images, TR=2000ms, TE=23ms, FOV=224 mm, slices=37, voxel-size= 2.33×2.33×3.5 mm, volumes: 300 for fear generalization task ; 150 for rs-fMRI (2 sessions).
SMRI	fast-spoiled gradient-recall sequence, 256 × 256 matrix; FOV=250 mm; 1 mm isotropic
dMRI	TR=8500ms; FOV=240 mm; voxel size=1.7×1.7×2.5 mm; 60 slices; 25 non-collinear directions ($b = 1,000 \text{ s/m}^{-2}$)

Image Processing. We will use the same image processing procedures in the applicant's recent publications^{11, 14, 57, 61, 62}. Briefly describing, for **Task-fMRI**, we will perform slice timing correction, motion correction

(motion parameters will be used in GLMs as nuisance variables), spatial normalization, smoothing in SPM12, and physiological noise correction⁶³. For **rsFC**, after the same preprocessing, voxel-wise seed based correlation will be applied using the CONN toolbox⁶⁴. For **DMRI**, we will perform eddy current correction, rotation of gradient matrices, and diffusion tensor model fitting, and Bayesian estimation of diffusion parameters. **Probabilistic tractography** in FSL will be performed as it offers more reliable tract estimation than a deterministic method⁶⁵.

Data Analysis 1–Risk ratings and fMRI. Risk Ratings. We will build a group-level GLM (general linear model) containing subject, age group, anxiety level group (for Aim #2), and covariates (sex, BMI, ethnicity, socioeconomic status, handedness, and IQ). **fMRI.** Our fMRI analysis to test generalization effects has been previously used in multiple publications^{9–11, 57}. First, preprocessed images will be entered into a subject-level general linear model (GLM) in SPM12. Regressors of interest include onsets of GS 60%, GS 40%, GS 20%, CS+, CS-; and nuisance variables include in-scanner motion parameters. Planned contrasts are ‘each condition vs rest’. To test generalization effects, we will use the four contrast images, GS60%, GS40%, GS20%, and CS+, in a **group-level, mixed-effect GLM** with stimulus as the within-subject factor. Extended models will also test the influence of covariates (sex, BMI, ethnicity, socioeconomic status, handedness, and IQ). Significance of linear gradients of the fear generalization gradient will be tested using a contrast of [1.5 .5 -.5 -1.5] (analogous to a trend analysis). For this, regions of interest (ROI) will be based on an anatomical atlas of the vmPFC¹¹. **Fear generalization gradient.** We will extract parameter estimates for the subsequent correlation analyses with the corticolimbic connectivity measures using the coordinates of the peak voxel showing the significant linear gradient as in the applicant’s prior study¹¹ (see **Figure 5**).

Data Analysis 2–dMRI and rsFC. We will estimate structural and functional connectivity of the corticolimbic system using dMRI and rsFC. **ROI definition.** ROIs for the connectivity analysis will be defined using structural segmentation results in Freesurfer. These include vmPFC (the medial orbital frontal gyrus in Desikan atlas⁶⁶, previously used in fear generalization¹¹ and fear extinction studies³⁰), the PFC regulatory regions (e.g., the dorsolateral PFC and ACC) and, the ventral affective subcortical regions (e.g., the amygdala, hippocampus, caudate, striatum, and brain stem) based on a prior fear generalization study¹¹. **Seed-based connectivity mapping.** Using the vmPFC as a seed, we will perform diffusion probabilistic tractography and rsFC following the methods used by the applicant^{11, 14, 61, 62}. **GLMs.** We will examine whether the corticolimbic connectivity correlates with age or vmPFC fear generalization (i.e., slopes of vmPFC fear generalization gradient; **Figure 3**). For **dMRI**, a GLM will contain vmPFC fear generalization index as the outcome variable; probabilistic tractography measures or mean fractional anisotropy (FA) of the estimate pathway as the predictor; sex, IQ, and ethnicity as the covariates. For **rsFC**, a GLM will contain vmPFC-seeded connectivity maps (parameter estimates) to the ROIs as outcome variable; vmPFC fear generalization index as the predictor; sex, age, BMI, ethnicity, and IQ as the covariates. Alternatively, we will apply network-based statistics (NBS)⁶⁷ as in the applicant’s previous study¹⁴, which control family-wise error rates for inter-correlated connectivity matrices. Lastly, GLMs will be used to determine correlation between vmPFC fear generalization estimates and corticolimbic connectivity.

Bayesian Learning Modeling: In order to infer learning mechanisms (e.g., prediction error of threat) underlying fear over-generalization in youth, we will apply Hierarchical Gaussian Filter (HGF)^{68, 69} to fMRI and behavioral data (self-reported fear and SCR). An important advantage of HGF is that it allows estimating subject-specific learning parameters. From the behavioral data, we will first estimate ‘precision-weighted’ prediction error in each individual⁷⁰. Then, these estimates will be used as the parametric modulator in a GLM of fMRI activation to identify brain activation correlating with the learning estimates.

Hypothesis Testing and Power Analysis. Aim 1: Investigate fear generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in healthy youth (n=25). **Hypothesis 1:** Healthy youth will generalize conditioned fear: Self-reported fear and fear-potentiated SCR will increase, as cues resemble the ‘threat’ cue. Conversely, vmPFC activation will increase, as cues resemble the ‘safety’ cue. **H1-Methods:** We will use GLMs with stimulus as the within-subject factor and with self-reported fear, SCR, or vmPFC activation as the dependent variable in each model, respectively. $P < .05$ will be considered significant; in the voxel-wise vmPFC activation analysis, we will use the false discovery rate to correct p -values, and a corrected $p < .05$ will be considered significant. **H1-Power Analysis:** Based on the effect size f of .97 (partial eta square of .49) of linear effect of the linear gradient of the vmPFC activation in the applicant’s prior study in healthy adults⁹, when power $> .95$ and alpha $< .01$, required sample size is 15. Our planned sample of 25 may be sufficient. **Hypothesis 2:** Steepness of the vmPFC activation gradient across threat vs safety will correlate with vmPFC connectivity patterns within the corticolimbic circuit. **H2-Methods:** We will use GLMs with generalization index of vmPFC activation as the independent variable, and functional and structural connectivity of the corticolimbic system as the dependent variables, respectively in separate models. The false discovery rate will be used to correct p -values for multiple comparison and a corrected $p < .05$ will be considered significant. **H2-**

Power Analysis: In our prior study with young adults, a hierarchical regression analysis showed the corticolimbic circuit measures (e.g., diffusion tractography, and resting-state functional connectivity) significantly explained vmPFC fear generalization index—with an adjusted r^2 of 0.565, equivalent to Cohen's f^2 of 1.3. Based on this effect size, with power > 0.80 and alpha < 0.05 (one-sided), we require an sample size 18. This justifies our planned sample size of 25. **Possible Confounds:** Motion estimates for MRI, sex, age, BMI, handedness, socioeconomic status, minority status, and IQ will be used as nuisance variables in extended models. **Exploratory Analyses: Model-Based fMRI**—We will assess brain activation associated with the learning parameters (e.g., precision-weighted prediction error) from HGF. The parameters will be used as the parametric modulator in a GLM for fMRI. Effects of anxiety symptom severity on the brain activation will be then examined.

Aim 2: Investigate fear over-generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in youth with pathological anxiety (n=50). **Hypothesis 3:** As anxiety severity increases, generalization of conditioned fear will also increase (over-generalization of fear): Self-reported fear, fear-potentiated SCR, and vmPFC activation will show less discriminating responses to threat vs safety cues.

H3-Methods: To assess impact of anxiety on the generalization gradients, we will perform ANOVA (analysis of variance) or MANOVA (multivariate ANOVA). Fear generalization gradients of self-reported fear, SCR, and vmPFC activation will be used as the dependent variables, in a separate GLM, respectively; and anxiety group, as the independent variable. We will consider $p < .05$ as significant. **H3-Power analysis:** Based on the effect size $f = .24$ (partial eta square=.06) of the interaction between within-subject (stimulus) x between-subject (group) factors in repeated measures of ANOVA in the applicant's prior study in adults with GAD¹⁰, a sample size of n=75 will provide adequate power (> .95) to detect an alpha < .01. **Hypothesis 4:** Less discriminating vmPFC activation across threat vs safety cues (i.e., decreased steepness of the gradient) will correlate with decreased corticolimbic connectivity. **H4-Methods:** Same as in H2, we will use GLMs with generalization index of vmPFC activation as the independent variable, and functional and structural connectivity of the corticolimbic system as the dependent variables, respectively in separate models. **H4-Power Analysis:** As in H2, the sample size of n=75 is adequately powered (> .95) to detect an alpha < .01, based on the effect size in the applicant's prior study. **Possible Confounds:** In addition to the nuisance covariates as in Aim #1, we will also include depression scales as a covariate, given frequent comorbidity with anxiety^{71, 72}.

Exploratory Aim: Assess impact of age and the interaction of age-by-anxiety on fear generalization behavior, the corticolimbic function and connectivity. Given the age range (8-17 years) in this proposal, development of the brain may involve differences in fear generalization behavior and the underlying corticolimbic system. Furthermore, pathological anxiety may impact the normal development. We will thus explore impact of age and the age-by-anxiety interaction using univariate GLMs and growth curve modeling. **Method:** (1) **GLMs.** **Healthy youth (n=25)**—In the existing GLMs with self-reported fear, SCR, vmPFC activation, and corticolimbic connectivity, as the dependent variables in healthy youth for Aim #1, respectively, we will add age as an additional covariate. **Healthy & anxious youth (n=75)**—In the GLMs for Aim #2, we will add age and will examine age-by-anxiety group interaction (analysis of covariance).

C3. Anticipated Problems and Alternative Strategies. (1) If univariate GLMs (e.g., ANCOVA) fail to show a significant interaction of age-by-anxiety on vmPFC fear generalization or the corticolimbic connectivity measures, we will then use combinations of categorical variables (anxiety group or age groups) and continuous variables (anxiety scales or age in years). For anxiety scales, we will first select scales significantly predicting the neural measures in a stepwise manner (e.g., stepwise regression), and then model the interaction with age. (2) Regarding the correlation between vmPFC fear generalization and corticolimbic connectivity, power may be lost because of multiple comparisons. If so, we will use multivariate partial least square regression as in the applicant's recent study¹⁴. This will allow identification of patterns across all connectivity measures instead of treating each measure as an independent variable. (3) It is possible that generalization effects in healthy youth or over-generalization in anxious youth may be masked by significant sex differences in threat processing and its developmental trajectories⁷³. To address this, we will examine each sex separately; or include sex, 'Tanner stage', sex-by-'Tanner stage' (given differential developmental stages across sex) into the GLMs testing generalization effects on the behavioral and MRI measures.

C4. Expected Outcomes and Future Directions. **Aim 1. Investigate fear generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in healthy youth.** We expect to demonstrate for the first time that healthy youth show normal fear generalization measured through behavior and MRI and to delineate the neural correlates of this behavior. **Aim 2: Investigate fear over-generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in youth with pathological anxiety.** We expect to demonstrate for the first time that anxiety symptom severity in youth, irrespective of diagnosis, correlates with fear over-generalization measured through behavior and MRI. These out-

comes are important because they will support the theory that fear over-generalization is one mechanism underlying pediatric anxiety. ***Future Directions.*** These outcomes will lead to a larger-scale R01-funded study to longitudinally track developmental changes of fear generalization using trans-diagnostic, dimensional sampling with the aim of identifying a ‘tipping point’ marking the transition from normal to pathological anxiety or disruptions in brain development. The long-term goal of this line of research is to promote early detection, prevention, and new interventions for youth with pathological anxiety.

5. TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

5.1 Rationale.

Ethical conduct is the crucial prerequisite for biomedical research, particularly when involving vulnerable populations such as anxious children and adolescents. In my former PhD training in the Department of Neurobiology and Behavior and the Department of Biomedical Engineering (School of Medicine), I have had extensive training in the responsible conduct of research. I will continue this training throughout the award period through didactic courses, seminars, and one-on-one meetings with mentors at Columbia University Medical Center and the New York State Psychiatric Institute (CUMC/NYSPI).

5.2 Previous Training.

1. PhD at Stony Brook University • **Ethical Research** at Stony Brook University (directed by Dr. David Talmage, Associate Professor in the Department of Pharmacological Sciences, 1hr/wk). This course covered data collection and management, attribution of credit, plagiarism, peer review, and conflicts of interest.
2. Postdoctoral Fellow at CUMC/NYSPI • Online courses required by CUMC through Research Compliance and Administration System. This training was specifically designed for researchers working with human subjects. Topics included: Information Security, Privacy & HIPAA (Health Insurance Portability and Accountability Act), Human Subjects Protection and Good Clinical Practices.

5.3 Plan for Training.

1. Course • **Responsible Conduct of Research and Related Policy Issues** at CUMC (directed by Dr. Richard Kessin, Professor in the Department of Pathology and Cell Biology; G4010, 1hr/wk). This course explores ethical and policy issues during the conduct of basic, translational, and clinical biomedical scientific research. Specific topics include: research misconduct, human research participants and scientific research, data sharing and data secrecy.
2. Seminar • **Seminar in Applied Psychiatric Ethics** at CUMC (*Columbia Psychiatry Residency Program*, directed by Drs. David Lowenthal, Clinical Director, and David Strauss, Director of Psychiatric Research and Vice Chairman for Research Administration, Ethics and Policy). This course covers concepts in psychiatric ethics, such as sexual misconduct with patients and colleagues, research ethics, and ethical problems in various clinical circumstances.
- **Annual Ethics Symposium** at NYSPI (directed by Dr. Ilene F. Wilets, the Chair of the NYSPI-IRB). This is a day-long workshop that addresses special issues concerning the capacity to consent to research and guardianship.
3. One-on-one meeting • **Weekly Meetings with Drs. Posner (mentor) and Simpson (co-mentor)**. We will discuss practical issues in the ethical treatment of youth, particularly those with clinical anxiety disorders, and research conduct in our weekly one-on-one meetings.
- **Monthly Meetings with Dr. Rynn** at NYSPI (**advisor**, Chair of the Division of Child and Adolescent Psychiatry and Director of Pediatric Anxiety and Depression Research Clinic). We will also discuss issues of informed consent, evaluation and responsible practices with regard to assessment and monitoring of children and adolescents with anxiety disorders.

Research Foundation for Mental Hygiene, Inc.

New York Psychiatric Institute Division
1051 Riverside Drive • New York, New York 10032
(646) 774-6500 • (646) 774-6540

June 2, 2015

Dear Colleagues:

I whole-heartedly and unequivocally support Dr. Jiook Cha's application for Mentored Research Scientist Career Development (K01) Award, entitled "Identification of Neural Correlates of Fear Over-generalization in Pathological Anxiety in Youth". I recruited Dr. Cha to come to Columbia after completing his PhD at Stony Brook University, and have had the distinct pleasure of working closely with him since then. As the associate training director for research in child psychiatry at Columbia University, I have worked with many post-doctoral research fellows and I can assure you that Dr. Cha is at the top of the pack.

Dr. Cha is an investigator of exceptional intelligence, determination, persistence, and intellectual curiosity, who has a steadfast commitment to advancing our understanding of the neural mechanisms of psychiatric disorders. He has demonstrated unprecedented facility with inter-disciplinary methodologies imperative to successfully meeting the challenges of a career path focused on neuroimaging of anxiety disorders in children and adolescents. Dr. Cha is both clear and creative in his thinking and ardent in the pursuit of his academic and career goals. During his doctoral and postdoctoral trainings, he has demonstrated the ability to lead independent projects and tackle crucial questions in affective neuroscience and psychiatry. I have no doubt that Dr. Cha will become a leading investigator in pediatric anxiety research with this K01 training, and I assure that I am fully committed to supporting his research career.

Dr. Cha joined my lab after completing his PhD training in the Department of Neurobiology and Behavior at Stony Brook University. As his postdoctoral advisor, I work closely with Dr. Cha and have firsthand experience of his innovative research, strong work ethic, and valuable contributions to the field. Dr. Cha's has a unique academic background. He obtained his Bachelors of Science in Environmental Engineering at Korea University in Korea and his Master's degree in Neurobiology studying basic neurobiology at Catholic Medical College in Korea. Dr. Cha then completed his PhD in Neuroscience at Stony Brook University, New York. For his thesis research, entitled, "*Neural underpinnings of anxiety: Multi-modal magnetic resonance imaging approach*," he brought his interests in integrative science to human cognitive and affective neuroimaging research. In his four first-authored articles published in the *Journal of Neuroscience*, *Cerebral Cortex*, and *Cortex*, he reported mesocorticolimbic substrates of anxious behaviors in clinical and non-clinical individuals using an elegant methodology combining structural, functional, and diffusion MRI and connectivity mapping, and various behavioral paradigms (e.g., fear generalization and negative attention bias). These are an exceptional series of studies bridging affective neuroscience, cutting-edge neuroimaging, and clinically oriented research.

I am delighted that Dr. Cha has decided to pursue patient-oriented, translational neuroscience research in child psychiatry. Child psychiatric research needs talented and dedicated young investigators like Dr. Cha. His innovative approach to research and his drive for studying the neural underpinnings of anxiety disorders in youth will have a significant impact on the field. During his first year of his postdoctoral fellowship, his aim was to apply his neuroimaging expertise to clinical research in childhood mental illnesses. Toward this end, he worked on an MRI study of aggression in children with ADHD. This led Dr. Cha to publish a first-authored article in *Neuropharmacology* reporting that structural connectivity (diffusion tractography) within the corticostriatal reward circuit predicts aggression in children with ADHD. Dr. Cha is also working on two additional manuscripts together with Dr. Simpson (co-mentor) and myself. In one, he describes a common neural substrate of pathological anxiety and depression by combining functional (task-fMRI) and structural (dMRI) analyses of the hippocampus in young adults. In the other, he describes abnormal connectivity (structural and functional) within reward circuitry in individuals with anorexia nervosa. His study on depression and anxiety led Dr. Cha to win a prestigious travel grant award and a Career Development Leadership Program at the Anxiety and Depression Association of America (ADAA) conference in 2015. Most recently, in March 2015, Dr. Cha successfully competed for the 2nd Human Brain Project (HBP) Education Program Workshop (Future Medicine-Medical Intelligence for Brain Diseases), supported by European Research Council. He was not able to attend the program because of his VISA status in the US; and thus, we included this workshop in his training plan. Dr. Cha's re-

markable achievements within a short period of time clearly indicate the quality of his research and its impact on the field, as well as the fruitful synergism of his mentoring program.

To become a successful, independent researcher at the interface of translational neuroscience and psychiatric research, Dr. Cha has so far proven to possess an extraordinary command of methodologies in neuroimaging and affective neuroscience. His ambition is to use a longitudinal approach to chart the development course of fear generalization and over-generalization in healthy and pathologically anxious youth. Ultimately, Dr. Cha aims to use this research to fuel the development of novel prevention strategies and treatments for pathological anxiety. This Career Development Award is a superb way to launch this important line of research by first examining fear generalization in youth using a cross-sectional design. His training plan will also solidify his knowledge of: (1) the phenomenology of childhood and adulthood anxiety disorders, (2) patient-oriented translational neuroscience in youth with pathological anxiety, (3) advanced computational methods in neuroimaging, and (4) responsible conduct of research. Under each goal, we have designed well-balanced programs consisting of didactic coursework, supervision and hands-on practicum, as well as seminars and conferences.

I will supervise and ensure the overall progress of Dr. Cha's Career Development Award. I will provide training in pediatric neuroimaging and developmental neuroscience in our weekly one-on-one meetings. I will oversee the coordination and completion of each specific goal of the training plan together with co-mentor, Dr. Simpson, in our monthly meeting. I am also committed to providing training in the Responsible Conduct of Research. Dr. Cha will attend a formal course provided by CUMC/NYSPI in Year 1 of the award, with additional training specific to his research project in our weekly and monthly (with Dr. Simpson) meetings.

We designed Dr Cha's training plan to provide an outstanding mentoring team with complementary expertise: **Dr. Blair Simpson** (co-mentor; Director of Anxiety Disorder Clinic at CUMC/NYSPI), **Dr. Moira Rynn** (Chief of the Division of Child and Adolescent Psychiatry at CUMC/NYSPI), **Dr. Daniel Pine** (Chief of the Section on Development and Affective Neuroscience at NIMH), and **Dr. Dubois Bowman** (Chair of the Biostatistics Department at CUMC/NYSPI). I have on-going collaborative relationships with all of these colleagues and I am fully confident that we will continue to work together productively. Two additional advisors include **Drs. Stephan Klass** (Director of Translational Neuromodeling Unit at ETH Zurich and University of Zurich) and **Shin-Jae Yoo** (Associate Computational Scientist at Brookhaven National Laboratory). Each member of the mentoring team will supervise complementary components of the training as described in Dr. Cha's training proposal. With this mentoring team and the excellent resources available at CUMC and NYSPI, Dr. Cha is ideally positioned for this Mentored Research Scientist Development (K01) award. Dr. Simpson (co-mentor) and I, together with the advisors, will convene quarterly meetings to review Dr. Cha's research and training progress. I will also provide Dr. Cha with annual written feedback that represents his progress towards his training, research and academic goals, which we will also discuss in person.

My qualifications and mentoring experience make me well suited for the role of primary mentor on Dr. Cha's application. I have extensive expertise in using multimodal MRI to study a range of disorders in youth. I have published more than 20 peer-reviewed scientific articles. I am the associate training director for research training in the Division of Child and Adolescent Psychiatry at CUMC and have significant experience as a supervisor and mentor. I have been a principal investigator on 3 NIH grants including NIMH-funded K23, R01, and R21 awards, and I am the project leader for an MRI Core within a Conte Center examining the effects of serotonergic modulation on brain development. I also have extensive experience working with Dr. Blair Simpson (co-mentor). We are currently collaborating on two NIH funded studies, and have published together previously.

Over the period of this K01 award, several publications are expected. First, towards the end of Year 1, Dr. Cha will analyze data from Aim #1: *Investigate fear generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in healthy youth*. We expect to write at least one peer-reviewed article from this analysis (publication #1). Considering the paucity of literature on the neural underpinnings of the fear generalization in youth, the expected paper will be a significant contribution to the field. Second, towards the end of the Year 3, Dr. Cha will have analyzed data collected under Aim #2: *Investigate fear over-generalization behavior and its relationship with vmPFC function and corticolimbic connectivity in youth with pathological anxiety*. We expect to write a second paper using these data (publication #2). Third, towards Year 4, after Dr. Cha completes his training in advanced computational methods, he will apply this knowledge to data collected under both Aims. We expect at least two papers from this (publication #3 and 4). Given Dr. Cha's track record of publications (5 first-authored publications in the PhD and postdoctoral training over the last 4 years), this is a

very reasonable expectation. All aspects of the proposed research project will constitute Dr. Cha's own research program. The publication plan that we've put together will provide an opportunity for me to continue to work with Dr. Cha on writing manuscripts and giving oral presentation. Although Dr. Cha has a successful publication history, English is his second language. Continuing to polish his writing skills is essential to research independence.

Another essential component of this training plan is to provide effective training for Dr. Cha to successfully compete for research grants. Completing for research grants is crucial to transitioning to an independent, research career. I will mentor Dr. Cha on applying and successfully competing for various research grants at CUMC and independent foundations. Dr. Cha and I have identified several pilot research grants, which fit well with the specific research aims and training goals of his proposal:

- (1) International Short Visit Fellowship Program (Swiss National Science Foundation; Aim #1 and 2; Training Goal #3-Advanced Computational Neuroimaging)
- (2) MRI Unit Pilot Grant (NYSPI; Aim #1 and 2; Goal #2-Translational Neuroscience)
- (3) Clinical and Translational Research Grant (CUMC; Aim #2; Goal #2-Translational Neuroscience)
- (4) Research Opportunities and Approaches to Data Science (Data Science Institute, CU; Aim #2; Goal #3-Advanced Computational Neuroimaging).

Drs. Cha, Simpson and I designed this training plan to maximize Dr. Cha's research related career development. During the award phase, therefore, Dr. Cha will not be required to teach, serve on committees, or hold any administrative assignments. Dr. Cha will commit 100% of full professional effort to the career development program and related career development activities.

Lastly, we have designed a plan to ensure successful transition from a mentored to an independent investigator. I will enthusiastically assist Dr. Cha in his search for a University-based tenure-track position both by providing suggestions for jobs openings and reaching out through my network of colleagues. I will review Dr. Cha's drafts of research statements, and cover letters when he applies for independent positions towards the later phase of the award period. I will also assist Dr. Cha in preparing his job talks, interviews, and job negotiation.

In conclusion, Dr. Cha is an exceptionally talented young scientist and I am fully committed to advancing his career. He is an ideal candidate for a Career Development Award – talented, dedicated, ambitious, intelligent, and creative – and I have no doubt that he will make important scientific contributions. NIH cannot go wrong in investing in such an outstanding candidate.

Sincerely,



Jonathan Posner, MD
Assistant Professor of Clinical Psychiatry
Columbia University Medical Center

Research Foundation for Mental Hygiene, Inc.

New York Psychiatric Institute Division
1051 Riverside Drive • New York, New York 10032
(646) 774-6500 • (646) 774-6540

June 4, 2015

Dear Colleagues:

I am writing this letter with great enthusiasm for Dr. Jiook Cha as he applies for a Mentored Research Scientist Career Development award (K01) entitled "Identification of Neural Correlates of Fear Over-generalization in Pathological Anxiety in Youth". Born in Korea with a PhD from Stony Brook University in Neuroscience and Biological Engineering, Dr. Cha is a brilliant young scientist who seeks to understand the brain mechanisms underlying pathological anxiety in youth. Ambitious, creative, and very hard working, Dr. Cha has the right stuff to become a future leader in our field. This K01 application will give him the necessary skills and preliminary data to launch an independent research career.

Jiook's proposal aims to investigate fear generalization and the underlying corticolimbic circuit in youths with anxiety disorder and in healthy controls. Normative fear generalization is an important adaptive learning mechanism, and excessive fear generalization is one potential process that leads to pathological anxiety. This will be the first fear generalization neuroimaging study in youths. By combining fMRI task, behavioral measures, and multimodal neuroimaging, Jiook's proposal embodies the RDoc's approach aiming at delineating neurobehavioral mechanisms of clinical anxiety with multiple units of analysis. His proposal employs a sophisticated approach, which he has previously, successfully applied to young adults with GAD in his PhD training.

Closely linked to this research plan, Jiook's training plan is designed to enable him to successfully transition to research independence. He has three main training goals: 1) to learn about the clinical phenomenology of pathological anxiety in both youth and adults; 2) to learn how to conduct patient-oriented translational research, bridging the gap between neuroscience and psychiatry; and 3) to learn advanced computational methods in neuroimaging. This training will equip Jiook with knowledge and expertise critical not only for successful completion of the research plan, but also for his ambition towards developing neuroimaging-based precision medicine in psychiatry.

I am excited to serve as his co-mentor along with my collaborator Dr. Jonathan Posner. As co-mentor, I will help Dr. Posner supervise Jiook's overall progress of research and training, with Dr. Posner supervising the neuroimaging progress and my supervising the clinical research piece. I will also specifically help Jiook with two of his training goals as outlined in his training plan. Specifically, I will help to train Jiook in **the phenomenology of anxiety disorders** through our weekly meetings and didactic course that I teach in the Psychiatry Residency program, Anxiety Disorders, at New York Presbyterian Hospital-CUMC/NYSPI. I will also help to train Jiook in how to conduct **patient-oriented translational research** through our individual meetings and his joining the weekly scientific meetings of the research clinic that I direct, where we discuss patient-oriented translational studies in anxiety disorders. Finally, I will organize a quarterly meeting, together with Dr. Posner (primary mentor) and other members of the mentoring team to review his scientific and career progress; we will ensure that he completes his training goals, develops data that can lead to a successful R01 application, and develops superior skills in oral and written presentations that will be critical for his success.

I am well-suited to serve as Jiook's co-mentor. I have been continuously funded by NIMH since 1999 to pursue patient-oriented translational research in patients with anxiety disorders. Throughout my career, I have mentored numerous junior faculty and students in career development awards at the post-doctoral, and doctoral levels. My post-doctoral and junior faculty mentees have gone on to assume productive faculty positions at major research institutes. As a result, I know the skills that young scientists need to develop to become independent researchers. In recognition of my mentoring abilities, I was awarded a NIMH midcareer investigator award in patient-oriented research (K24). I also have been contributing to the RDoc project at the National Institutes

of Mental Health, first as a member of the Negative Valence Systems Workshop, and then as the Principal Investigator of an NIMH-grant (R01) consistent with the RDoC approach. Finally, I have worked closely with other members of Jiook's mentoring team, including Drs. Posner, Rynn, and Pine.

Jiook is one of the more promising young scientists that I have worked with to date. With a background in engineering and neuroanatomy from Korea, and his PhD and post-doctoral training in neuroimaging in America, he has a unique combination of skills. He has also chosen a very important area of study: the neural mechanisms underlying pathological anxiety. With the K01 training, I am extremely confident that Jiook will become a future leader in our field. .

Truly yours,



Helen Blair Simpson, MD PhD

Professor of Psychiatry at Columbia University Medical Center

Director of the Anxiety Disorders Clinic and the Center for OCD and Related Disorders at the New York State Psychiatric Institute

Research Foundation for Mental Hygiene, Inc.

New York Psychiatric Institute Division
1051 Riverside Drive • New York, New York 10032
(646) 774-6500 • (646) 774-6540

June 5, 2015

Dear Jiook,

I am pleased to fully support your K-01 Mentored Research Scientists Award proposal titled, "Identification of neural correlates of fear over-generalization in pathological anxiety in youth". I will happily serve as an advisor as well as a collaborator on your study. I believe that this is an extremely important study aimed at delineating the developmental roots of clinical anxiety. The outcomes of your proposed research will not only impact childhood anxiety disorders, but also adulthood anxiety disorders. I am particularly excited for your inter-disciplinary and trans-diagnostic approach in this proposal to study neurobehavioral mechanisms of pediatric anxiety disorders. I strongly believe that your prior training at Stony Brook University and Columbia University has prepared you as an ideal candidate for this award.

You have expertise in multimodal neuroimaging and cognitive neuroscience, but I believe additional training in the phenomenology of anxiety disorders across the lifespan (from childhood to adulthood) is essential for successful transition to research independence considering your long-term goal of to translate neuroscience to anxiety disorder. As Chief of the Division of Child and Adolescent Psychiatry and Director of Pediatric Anxiety and Mood Research Clinic, I believe I can provide necessary training in childhood anxiety disorders as follows: (1) I will meet you in our weekly Research Meetings in the Child Pediatric Anxiety and Mood Research Clinic at New York State Psychiatric Institute. In the meetings, you will learn about various clinical cases, research, and therapeutics. I will also arrange your observership for clinical assessments or relevant research activities. (2) I will discuss and review the progress of your training in our monthly one-on-one meeting. Lastly, as an advisor, I will also coordinate this career development training plan with your mentors, Drs. Jonathan Posner and Helen B. Simpson. We will have quarterly meetings together in person or via conference call to discuss the progress of your training, research, and career development.

Your application brings together an exceptional candidate and an exciting study. I strongly believe that reception of the K-01 Award will allow you to become a future leader in child psychiatry, bridging cognitive, developmental, translational neuroscience, and cutting-edge neuroimaging and data analysis in your research. Moving this field forward requires such innovative, multidisciplinary approaches, and I am delighted to be a part of your training towards achieving this goal.

Sincerely,



Moira Rynn, MD
Director, Division of Child and Adolescent Psychiatry
Ruane Professor for the Implementation of Science for
Child and Adolescent Mental Health (in Psychiatry)
at the Columbia University Medical Center
Columbia University, College of Physicians and Surgeons
New York State Psychiatric Institute



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

Dear Jiook,

May 28, 2015

This letter expresses my commitment to working with you as part of your K01 Award, entitled "Identification of neural correlates of fear over-generalization in pathological anxiety in youth". In accordance with NIH policies, the content of letters of collaboration from intramural scientists is restricted to a description of collaborative work proposed under the grant.

I understand that your career development goals focus on an application of translational neuroscience to youth with pathological anxiety to delineate the neural basis of fear over-generalization. I am eager to mentor you on application of fear conditioning (e.g., generalization) combined with fMRI to youth with pathological anxiety and concurrent fMRI and psychobiological experimentations. I have already sent you our fear conditioning paradigms that we have previously used in youth with anxiety disorders. I will continue to provide such consultation and advice during the award period. I will help you examine both the behavioral and fMRI data collected from anxious youth and troubleshoot issues that arise. I will help you interpret your findings in the broader contexts of current neurobehavioral models of anxiety disorders in youth. This line of research aligns tightly with the focus in my own group and is consistent with other themes emphasized in the larger NIMH research portfolio.

I will happily take an active part in your training in translational, developmental neuroscience research in youth with pathological anxiety as an advisor and a collaborator through:

- 1) Hands-on training in psychobiological/fMRI experiments in youth with pathological anxiety in my lab at NIMH (1-2 wks/yr);
- 2) Monthly meetings via conference calls to discuss the progress of your training and research;
- 3) Quarterly meetings via conference calls to discuss the progress of the overall research and career development with your mentors;
- 4) Face-to-face meetings at conferences (e.g., Biological Psychiatry and ADAA).

Since I am currently employed at the Intramural Research Program of NIMH and would participate as part of my Official Duties, I will not require any compensation for my efforts to assist you. Moreover, no funds from this grant can be used to support any of the work that I conduct as part of my Official Duties. Finally, should you be successful in your application, I do plan to visit you in New York using funds available in my group, and I would be able to support you for a visit to the NIMH to so that I might better assist you in your training.

Best regards,

Daniel S. Pine, M.D.
Chief, Section on Development and Affective Neuroscience
National Institute of Mental Health (NIMH)
Bethesda, MD 20892-1381

DuBois Bowman, PhD
Professor and Chairman

May 27, 2015

Dear Dr. Cha:

I am writing to fully support your proposed research and training outlined in your NIMH K01 proposal, entitled "Identification of neural correlates of fear over-generalization in pathological anxiety in youth". It is with great pleasure that I support this extremely interesting project, and I will be happy to serve as an advisor and a collaborator on this application. Specifically, I will provide supervision for your training in advanced computational methods in neuroimaging.

My research is committed to develop biostatistical methods to integrate multimodal brain imaging data (including structural, functional, and diffusion MRI) with an aim to determine biomarkers for various psychiatric disorders, such as major depression, schizophrenia, drug addiction, and Parkinson's disease. Your research focused on multimodal MRI investigating the corticolimbic circuit and function to study pathological anxiety and your training objective (training goal #3) resonates well with my core research interests. Therefore, my expertise will complement both your research and your training in this proposal.

I will meet with you in person on a monthly basis to supervise that component of your training plan (review the training progress and discuss challenges) and to provide hands-on training in the **application of advanced statistical and computational approaches** that I have been developing (e.g., anatomically weighted functional connectivity integrating diffusion and resting-state functional MRI) **to the multimodal MRI data** that you are planning to collect in your research proposal. In addition to our monthly meetings, I will collaborate with your mentors (Drs. Posner and Simpson) to ensure the success of your training and career development during the award period. **In quarterly meetings**, I will review the progress of your research and training with your mentors and other advisors, to ensure that all the goals of this proposal are adequately met.

I believe that your training will prepare you to play a key role in advancing neuroimaging data analysis to develop a biomarker of clinical anxiety in children and adolescents and thus make a significant contribution to the progress of precision medicine in psychiatry. I have no doubt that you will become a future leader in this important field. I look forward to working with you soon.

Sincerely,



DuBios Bowman, Ph.D. Chairman and
Professor Department of Biostatistics
The Mailman School of Public Health
Columbia University



Jiook Cha, PhD
Postdoctoral Research Fellow Columbia University
Medical Center Department of Psychiatry
New York State Psychiatric Institute

Prof. Dr. med. Klaas Enno Stephan, PhD
Telefon +41 44 634 91 25
Telefax +41 44 634 91 31
Stephan@biomed.ee.ethz.ch

Zurich, 21st May 2015

Letter of support for Jiook Cha, PhD

Dear Jiook,

I am delighted to express my support of your application for a NIMH K01 Award, titled “Identification of neural correlates of fear over-generalization in pathological anxiety in youth”. In my capacity of the Director of Translational Neuromodeling Unit at University of Zurich and ETH Zurich, your proposal to combine an fMRI paradigm of fear generalization, multimodal MRI, and computational modeling is of tremendous interest and promise.

My research group is committed to developing computational models of brain dysfunction as tools for better diagnostics or treatment prediction of psychiatric disorders. You are proposing to use Bayesian learning models, particularly *Hierarchical Gaussian Filtering*, a modeling framework recently developed by our group, to investigate maladaptive associative learning during fear generalization in anxious youth. I am excited by this proposal, which could provide much-needed knowledge about the cognitive mechanisms of pediatric anxiety disorders. I believe that your training plan for the computational modeling of fear generalization will lead you to become a leading investigator in translational neuroscience and psychiatry.

As we have already discussed since 2014, I will happily advise and collaborate with you on this exciting project. I will **provide hands-on training on Hierarchical Gaussian Filtering** at ETH Zurich in Switzerland. Specifically, I will host your **two-week visit to my laboratory, the Translational Neuromodeling Unit, in Zurich**, to learn about implementation of Hierarchical Gaussian Filtering and to apply it to your fMRI and behavioral data. In addition to direct training with one of my postdocs who has long-term experience in computational modeling, you will also **participate in my weekly supervision meetings, including the Computational Neuroimaging Clinic (CNC)**, which deals with practical neuroimaging data analysis problems and our Translational Neuromodeling Colloquium. These seminars will provide additional excellent training opportunities for you. Beyond your visit in Zürich, **my group will provide additional input and help via email and Skype as needed to support your project**. With your mentors and other advisors, I will review and discuss your training and research to ensure that the goals of this proposal are met.

Lastly, to supplement the travel costs to visit Zurich, as we have discussed, I will sponsor your application for the International Short Visit Fellowship Program of the Swiss National Science Foundation. I understand that you will submit this application in June 2016.

You are undoubtedly an exceptional candidate for this award. I wish you the best of luck and I look forward to working with you soon.

Yours sincerely,

Prof. Dr. med. Klaas Enno Stephan, PhD
Director, Translational Neuromodeling Unit



Brookhaven National Laboratory
Computational Science Center
Bldg. 463B - P.O. Box 5000
Upton, NY 11973-5000

phone: (631) 344-3751
email: sjyoo@bnl.gov

June 2, 2015

Jiook Cha, PhD
New York State Psychiatric Institute
Columbia University
1051 Riverside Drive, Unit 74
New York, NY 10032

Dear Jiook,

I am very happy to serve as an advisor and collaborator on your K01 Mentored Research Scientist Award. Your study aims to investigate the neural correlates of fear over-generalization in pathological anxiety in youth. I think this is a terrific project and I am delighted to be a part of it.

In my role as an advisor and collaborator, I will **meet you monthly** at Stony Brook University in Long Island, NY as we have already done several times or via Google chat throughout the award period. The goal of our work together will be to **help you learn and apply cutting-edge big data science approach** that has yet to be widely applied to MRI and relevant clinical data in psychiatric research. This includes state-of-the-art **predictive modeling using machine-learning algorithms** (e.g. Manifold Learning, Bayesian Graphical Modeling, Semi-supervised Network Analysis, etc.) that I have developed and applied to other data-intensive fields, such as bioinformatics, physics, climate science, and social network analysis. I will give you an access to all of these methods. To add to this, I have already given you an **access to our core computing system**.

Your career development and training thus far have well prepared you for this award. You are an exceptional young scientist and I have no doubt that this award will launch your career as an independent investigator bringing the inter-disciplinary expertise in your field.

Sincerely,

A handwritten signature in black ink, appearing to read "Shinjae Yoo". To the right of the signature is the date "06/02/13" in a standard digital font.

06/02/13

Shinjae Yoo, PhD
Associate Computational Scientist
Computational Science Center
Brookhaven National Laboratory

Adjunct Assistant Professor
Institute of Advanced Computational Science
Stony Brook University

9. DESCRIPTION OF INSTITUTIONAL ENVIRONMENT

Columbia University Medical Center and the New York State Psychiatric Institute offer numerous training opportunities, resources for the candidate's career development and research proposed in this K01 application.

ENVIRONMENT – Contribution to success

Dr. Cha and his research team are supported by significant clinical research and imaging resources in the Department of Psychiatry at Columbia University Medical Center and the New York State Psychiatric Institute (CUMC/NYSPI). Its resources, research faculty and staff, combined with those of the Research Foundation for Mental Hygiene, have made the Department of Psychiatry at CUMC/NYSPI one of the nation's most respected psychiatric research centers with long-standing experience in conducting clinical research studies. The facilities and other resources available to Dr. Cha's research team include everything needed to commence and complete the proposed project successfully. They have research space that is contiguous and extends from the ground level (the MRI suite) to two floors above within the same building where the PI (Dr. Cha) and his mentoring team have offices down the hall from the pediatric neuroimaging laboratory (Dr. Posner), Anxiety Disorder Clinic (led by Dr. Simpson), Pediatric Anxiety and Mood Research Clinic Children's Research Day Unit (led by Dr. Rynn). This ensures easy access to resources, communication, and provides the foundation for this research team to grow and expand their collaborative work. The support from the Department of Psychiatry, the Division of Child and Adolescent (Dr. Rynn) will ensure the successful recruitment of children and adolescents with anxiety disorders and subthreshold anxiety in this study. Furthermore, weekly science meetings at the Anxiety Disorder Clinic and Pediatric Anxiety and Mood Research Clinic Children's Day Unit Children's Research Day Unit will also provide excellent opportunities for Dr. Cha to learn and collaborate with junior and senior investigators with diverse expertise. The intellectual environment in the Department of Psychiatry is rich with other extramurally funded investigators who are doing work that is complementary to the proposed project. These facilities provide a scientific environment that is supportive of the proposed research and success of the project.

CUMC/NYSPI maintains an extensive array of departmental and divisional seminars, symposia, and lectures as well as a highly regarded weekly Grand Rounds program providing a forum for renowned researchers to interact with faculty. The MRI Unit, in particular, has a number of resources especially pertinent to the new investigator. Each semester, MRI Unit faculty teaches weekly advanced-level courses in computational modeling of learning and multimodal MRI data analysis. The MRI Unit Meeting occurs once every 1-2 week. This meeting offers a forum for new investigators to interact with MRU personnel and senior investigators, to become apprised of activities, updates, and MRU-related research projects, and as a forum for intellectual exchange through research presentations, and discussion of journal articles.

The research program at CUMC/NYSPI is exceptionally strong and diverse. This includes work in psychiatry (Drs. David Shaffer, Laurence Greenhill, Hector Bird), eating disorders (Drs. Tim Walsh, Michael Devlin), as well as basic work in neurophysiology (Dr. Eric Kandel), developmental psychobiology (Drs. Myron Hofer, William Fifer), affective disorders (Drs. John Mann, Myrna Weissman), anxiety disorders (Drs. Donald Klein, Michael Liebowitz), schizophrenia (Drs. Jeffrey Lieberman, Claudia Schmauss, Anissa Abi-Dargham), substance abuse (Drs. Herbert Kleber, Edward Nunes), medical genetics (Drs. L. Erlenmeyer-Kimling, James Knowles), and epidemiology (Drs. Myrna Weissman, Ezra Susser, Madeline Gould).

The facilities and resources available to this research team therefore include everything needed for the proposed project and career development.

INSTITUTIONAL COMMITMENT TO EARLY STAGE INVESTIGATORS

The PI qualifies as an Early Stage Investigator who has extensive evidence of institutional commitment to his development as an academic researcher. His 12-month academic appointment includes a total of 12.0 person months (100% effort) dedicated to research. He already has sufficient laboratory space and access to equipment for this project, as evidenced by his ability to yield the preliminary data for this first K01 application. His access to the clinical research resources described below is evidenced by his recruitment of 5 youth in his fMRI study on fear generalization (see Preliminary Data in Research Strategy-Approach). His access to the imaging resources in the Department of Psychiatry is also evidenced by these studies, as well as by his recent neuroimaging (structural and diffusion MRI) study with children with ADHD (see Biosketch-Cha). These imaging data were acquired, processed and analyzed (cortical surface analysis and probabilistic tractography) using the MRI scanner in the Department of Psychiatry and the High-Performance Computing (HPC) system (see Resources) at CUMC.

Research Foundation for Mental Hygiene, Inc.

New York Psychiatric Institute Division
1051 Riverside Drive • New York, New York 10032
(646) 774-6500 • (646) 774-6540

Institutional Commitment to Candidate's Research Career Development

June 1, 2015

We are delighted to provide this letter for Dr. Jiook Cha for the submission of his NIMH Mentored Research Scientist Career Development Award (K01). Dr. Cha is an innovative and accomplished young investigator with a passionate commitment to become an independent investigator. On behalf of the New York State Psychiatric Institute and the Department of Psychiatry at Columbia University, we offer our wholehearted commitment to his development as a post-doctoral research fellow in Child and Adolescent Psychiatry.

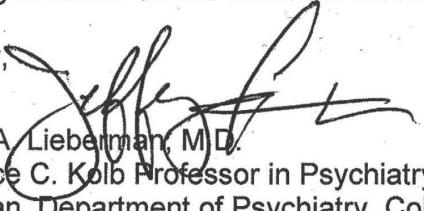
We are extremely excited about this K01 application, entitled "Neurodevelopmental Trajectories of Fear Generalization Across Anxiety Spectrum". In this proposal, Dr. Cha and his mentoring team have prepared a comprehensive training plan through which he will develop expertise and gain experience in psychopathology of anxiety disorders; in developmental and translational neuroscience to youth with pathological anxiety; in computational approaches to multimodal MRI. His training program includes structured didactics, private tutorial, and hands-on training that will primarily take place in our institution. To support the execution of his research and training plans, Dr. Cha has assembled an impressive team of mentors and advisory committee at our institution—who are highly accomplished investigators in their fields and have extensive experience in the successful mentoring of young investigators. Drs. Posner, Simpson (Director, Anxiety Disorders Clinics), Rynn (Chair, Child and Adolescent Psychiatry), and Bowman (Chair, Department of Biostatistics) at our institution will supervise Dr. Cha's research and training proposal.

Our Department of Psychiatry has a longstanding track record of nurturing young faculty along the path to intellectual independence. We are fully committed to the retention, development, and successful advancement of Dr. Cha along this path. Dr. Cha will have access to all the resources within the Division of Child and Adolescent Psychiatry, such as office and laboratory space, and related resources. Dr. Cha will also have access to the facilities of the New York State Psychiatric Institute and Columbia University as outlined in his proposal, including the MRI Research Unit, the Pediatric Anxiety and Mood Research Clinic. We commit with full assurance that Dr. Cha will have necessary support of his mentors, advisory committee, NYSPI, and Columbia University to collaborate with and recruit community members in his research endeavors. Since Dr. Cha's successful transition to a NIH-funded, independent investigator will be a crucial integral part of our research program, the division and department will provide him with sufficient mentoring and administrative support his successful preparation of R01 in this K01 award period.

In March 2016, it is the Division's intention that Dr. Cha be appointed as an Assistant Professor of Psychiatry in the Division of Child and Adolescent Psychiatry in the Department of Psychiatry at Columbia University and as a Research Scientist at Research Foundation for Mental Hygiene, Inc. The faculty appointment is not contingent upon receipt of this award. We will provide Dr. Cha 100% of protected time to devote to the research and training activities outlined in his proposal upon receipt of his K01.

Dr. Cha has tremendous capability, interest, and drive to become a leader in the field of translational neuroscience and psychiatric research. The Division of Child and Adolescent Psychiatry, the Department of Psychiatry, and the New York State Psychiatric Institute are in an ideal position to provide the vital resources to ensure the successful completion of Dr. Cha's proposed research and training plans. We are fully committed to making available all the essential resources and mentoring to ensure his success.

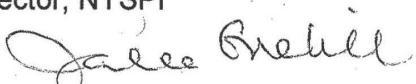
Sincerely,



Jeffrey A. Lieberman, M.D.
Lawrence C. Kolb Professor in Psychiatry
Chairman, Department of Psychiatry, Columbia
University
Director, NYSPI



Moira Rynn, M.D.
Ruane Professor for the Implementation of Science
for Child and Adolescent Mental Health
Director, Division of Child and Adolescent
Psychiatry,
Columbia University/NYSPINYSPI



Janelle Greenhill, M.P.H.
Director of Administration, Research Foundation for Mental Hygiene, Inc. @ NYSPI

14. PROTECTION OF HUMAN SUBJECTS

1. Involvement of Human Subjects. We will recruit three groups of youth (n=75)—healthy youth (n=25), those with subthreshold anxiety (n=25), and those with DSM-5 anxiety disorders (n=25). We will not target a specific N for each subcategory of the DSM-5 anxiety spectrum disorder because of the limited research budget in K01 award. The entire sample will be sub-grouped by anxiety levels. For this we will use the clinician-rated Pediatric Anxiety Rating Scale. Both females and males will be recruited and we will group match the subjects for important biological or other variables, such as age, sex, socio-economic status, and handedness.

Children and adolescents (n=75) will be recruited across 3 **anxiety symptom levels**:

- The anxiety symptom levels will be based on clinician-rated Pediatric Anxiety Rating Scale (PARS)⁴⁷ and will consist of: (i) low to mild anxiety (PARS ≤ 9; n=30); (ii) mild to moderate (10 ≤ PARS ≤ 15; n=30); and (iii) moderate to severe (16 ≤ PARS; n=30).
- Each cell of the 3 X 2 factorial design will contain n=15 subjects (in each cell of the 3 x 2 matrix, subjects will be matched on socio-demographic characteristics (age, sex, handedness and socioeconomic status).

All subjects will meet the following *inclusion criteria*:

- 1) Ages of 8-17 years
- 2) Medication-free for a minimum of 2 months prior to participation in the study.

All subjects will meet the following *exclusion criteria*:

- 1) Current DSM-5 other than anxiety disorders or mild-to-moderate Major Depressive Disorder (Children's Depression Inventory or CDI<20; a cut-off for moderate depression^{48, 49}), such as PTSD, OCD, Bipolar Disorder, Psychotic Disorder, Substance Use Disorders, ADHD
- 2) Contraindication to an MRI scan
- 3) Positive pregnancy test
- 4) History of major neurological or medical illness or head trauma with>10-minute loss of consciousness
- 5) History of substance dependence
- 6) IQ<80 (Wechsler Abbreviated Scale of Intelligence).

2. Sources of Research Material. Research material will include: (1) MRI scans; (2) neuropsychological testing; and (3) questionnaires measuring demographic characteristics such as age, sex, medical history, medications, psychiatric history, substance abuse history, and parental occupation, as well as clinician ratings and diagnostic interviews.

3. Recruitment Procedures. Patients and subthreshold anxiety individuals will be recruited through referrals from (1) the Pediatric Anxiety and Depression Clinic (directed by Dr. Rynn, advisor), (2) Anxiety Disorders Clinic (directed by Dr. Simpson, co-mentor), (3) mental health clinicians in collaboration with the Division of Child and Adolescent Psychiatry (directed by Dr. Rynn) at CUMC/NYSPI. The Pediatric Anxiety and Depression Clinic recruits more than 25 drug-free patients per year to research protocols, and the Anxiety Disorder Clinic recruits more than 40 drug-free patients per year. Considering this, we do not anticipate difficulties in recruit-

ing 50 anxious children and adolescents during Year 2-3 of the award period (25 youth per year or 1 patient in every 2 weeks).

Healthy control youth will be recruited from community-based telemarketing lists of households characterized by zip code, age, gender, ethnicity, and income level (Donnelley Marketing, ph: 1-800-846-7338). Introductory letters will be sent to households within the same zip code as the ADHD subjects to recruit a control sample with a demographic profile that matches the clinical sample. These mailings will be followed up by phone calls that describe the study and address questions in detail. Using these same recruitment methods in the past, approximately 10% of eligible control families contacted have ultimately participated in studies conducted in Dr. Posner's laboratory. Considering we have recruited 6 control subjects for the pilot experiment in last two months and Dr. Posner's lab (mentor) has recruited more than 30 healthy participants across developmental stages (ages 8-17 years) in 2014 alone, we do not anticipate any difficulties in reaching the recruitment goal of 25 control subjects in Year 1 (1 health control youth in every 2 weeks).

4. Consent Procedures Consent and assent for the study procedures will be obtained from both the parent and youth. Parents will be informed that all data collected about the child is confidential and that no information will be shared with the parent. For subjects who meet preliminary eligibility criteria, structured diagnostic interviews will be collected from both child and parent by Dr. Posner. General interviews of the child and parent will provide social, academic, and medical functioning. Neuropsychological assessment and MRI studies will also be collected from the youth. No personally identifying information will be collected in the questionnaires, interviews, or other research materials thus assuring confidentiality. Moreover, subject identification numbers will be assigned to each participant and will be used to label research materials. All data will be stored in locked file cabinets or password-protected/encrypted computers in a locked office at the New York State Psychiatric Institute (NYSPI).

5. Potential Risks The risks associated with MRI scanning can be classified into one of four categories: a) Acoustic Noise Levels, b) Gradient or Time-Varying Magnetic Fields, c) Radiofrequency (RF) Magnetic Fields, and d) Static Magnetic Fields.

a) Acoustic Noise The acoustic noise associated with MR imaging is related to the mechanical movement of the gradient coils during the scanning process.

FDA Guidelines: "The acoustic noise levels associated with the device must be shown to be below the level of concern established by pertinent Federal Regulatory or other recognized standards setting organizations. If the acoustic noise is not below the level of concern, the sponsor must recommend steps to reduce or alleviate the noise perceived by the patient." Current FDA guidelines follow the regulations of the International Electrotechnical Commission (IEC) Standard 601-2-33, which stipulate that for MR equipment used in medicine, hearing protection is required when the system can produce acoustic sound levels above 99 dBA (maximum A weighted r.m.s.) and that the protection should be able to reduce noise levels to below 99 dBA.

The FDA has approved systems for which noise levels have been quantified, ranging up to 105 dB RMS for scanners operating at field strengths of 1.5 Tesla. It is important to note that the static magnetic field strength is only one factor, and not necessarily the most important one, in determining acoustic noise. Among the factors listed above, the design and construction of the gradient coils plays a major role in the noise level that MRI scanning produces. Therefore, noise levels are not necessarily greater when scanning at 3.0 T compared with 1.5 T field strengths. It

is nevertheless possible that, in some circumstances, our system could produce noise levels higher than 99 dB, as do many clinical systems operating at lower field strengths.

Summary: The acoustic noise levels perceived by human subjects when undergoing MRI examination in our 3.0 Tesla magnet constitutes a non-significant risk; specifically, our system will not be operated in a way that will present more noise to human subjects than is recommended by the FDA.

b) Time-Varying Magnetic Fields The concern about the time-varying magnetic fields used in MRI is that these can, in some instances, induce stimulation of peripheral nerves, thereby producing sensations such as 'twitching' or 'tingling'. In very rare instances, this nerve stimulation can be painful. Nerve stimulation is particularly likely when subjects are physically positioned in a way that increases the likelihood of inducing stimulation, such as with hands clasped or arms folded. It should be noted that the parameter of interest here, dB/dt (the rate of change in the magnetic field per unit time), is not a function of the strength of the static magnetic field, so evaluating risk in a 3T MRI scanner involves the same considerations as evaluating other MRI systems operating at lower magnetic field strengths (i.e., the same issues apply to all the commercially available, FDA approved scanning systems). Thus, it is the *gradient system only* that needs to be evaluated to determine the risk of producing nerve stimulation.

FDA Guidelines: The FDA Guidance of 1995 was developed specifically to consider the fact that many clinical systems were capable of exceeding levels of dB/dt that could produce nerve stimulation. It was originally considered that a warning level should be implemented to guard against peripheral nerve stimulation, but the FDA finally concluded that: '*... this warning level is not considered critical since there are no harmful effects associated with mild peripheral nerve stimulation*'. The current guidelines therefore include monitoring procedures to help avoid painful peripheral nerve stimulation, and without specific dB/dt limitations.

Summary: The gradients used in our 3.0 Tesla MRI system will typically be operated at levels below those considered to be negligible according to FDA guidelines. Our system, like most commercially available, FDA-approved systems, does have the capacity to exceed this level, but it will include the same safeguards that are included in other FDA-approved clinical systems. Furthermore, policies and procedures will be implemented according to FDA guidelines to avoid the possibility of painful peripheral nerve stimulation. Therefore, in all circumstances the system will be operated in a way that poses nonsignificant risk to the participant.

c) Specific Absorption Rate (SAR) MRI scanning induces some heating of body tissues. This specific absorption rate (SAR) that determines heating is the amount of radiofrequency (RF) energy deposited (typically by a coil or "helmet"-like apparatus placed over the subject's head) per unit volume of tissue per unit time. The SAR for RF radiation is primarily related to the amplitude of RF power, the duration of the RF pulse, the type of RF coil used, the frequency of RF radiation, the resistivity of the tissue, the configuration of the anatomical region being examined, and several other parameters.

FDA Guidelines: "The following are levels of concern at which the reviewer shall exercise appropriate actions to ensure that the safety of the device is substantially equivalent to a predicate device: A) If SAR # 0.4 W/kg whole body; and if SAR# 8.0 W/kg spatial peak in any 1 gram of tissue; and if SAR # 3.2 W/kg averaged over the head: **below level of concern**. Or B) If exposure to radiofrequency magnetic fields is insufficient to produce a core temperature increase in excess of 1°C and localized heating to greater than 38°C in the head, 39°C in the trunk and

40°C in the extremities: **below level of concern**. The parameter SAR cited above must be shown to fall below either of the two levels of concern by presentation of valid scientific measurement or calculation evidence sufficient to demonstrate that SAR is of no concern."

It should be noted that this guideline is based on the calculation of a system that has no thermoregulatory response, and thus it is a very conservative estimate compared with the temperature change that would be experienced in any living subject. Normal diurnal temperature variations in humans, for example, are about +/-1°C from the normal set point 37°C, and healthy people with normal thermoregulatory responses can easily dissipate any excess (or, in this instance, deposited) heat by increasing their peripheral blood flow or sweat rate. Thus, the heating effect of MRI with the SARs used in accord with these guidelines is extraordinarily unlikely to cause any acute effects in healthy human subjects. Furthermore, our scanner console calculates SAR based on the subject's body weight before running any pulse sequence and prohibits running of the sequence if it exceeds the FDA-approved limit.

Summary: Because all experiments performed on the 3.0 Tesla system will comply with FDA guidelines with regard to SAR, and because appropriate RF power safety checks are in place, this criterion for classification of NSR is satisfied.

d) Static Magnetic Fields The possible risks of static magnetic fields have received much attention in the lay press, but scientific consensus on these risks has yet to be fully reached. The FDA has deemed that systems operating at 8.0 Tesla or less do not pose a significant risk. Moreover, experience with thousands of clinical studies over the past decade, and with multiple human investigations carried out at higher field strengths over this period, have not revealed risks of exposure to higher static magnetic fields. The most significant risk associated with static magnetic fields is that ferromagnetic objects, such as aneurysm clips or heart valves, can interact with the magnetic field of an MRI scanner, causing the device to malfunction or to move, and injuring the subject.

FDA Guidelines: "Studies conducted at 8T or less are not considered significant risk" (FDA Center for Devices and Radiological Health, memorandum 7-14-03).

Summary: This category of risk applies to work conducted around superconducting magnets of any kind (including standard clinical diagnostic MRI units). It is not unique to our 3.0 Tesla facility, which will maintain a safety policy to safeguard subjects and staff members from these incidental risks. Systems with static magnetic field less than 8 Tesla have been considered to represent a nonsignificant risk by the FDA. The static magnetic field of our system (3.0 Tesla) is therefore to be classified as posing NSR to human subjects.

e) Possible Incidental Risks The physical confinement and isolation produced by the scanner could cause mild to moderate emotional distress, although in our past experience, subjects generally tolerated the procedures remarkably well. In addition, there is a risk of distress stemming from the detailed psychiatric history obtained during screening and diagnostic interviews. In our experience, however, subjects tolerate this well.

6. Protecting Against Potential Risks Our 3.0 Tesla scanner satisfies FDA criteria for non-significant risk (NSR) in all risk categories:

Acoustic Noise: As suggested by the FDA, I will take steps to reduce or alleviate the noise levels experienced by subjects in this protocol. The easiest and most reliable means of preventing

hearing loss is to use disposable earplugs, which I intend to do for all scans. I will also be using acoustically shielded headsets, which further attenuate noise.

Nerve Stimulation: All consent forms for studies that might induce peripheral nerve stimulation also will provide this information. A record of dB/dt value will also be included with the imaging data to help in analysis of levels of peripheral nerve stimulation possibly perceived by subjects. In addition, I will conduct detailed calculations of the changes in magnetic field over time that our gradient system is capable of, and conservative values will be selected as limits that will be used to determine when special additional monitoring is indicated. In these cases, I will use the monitoring procedures recommended by the FDA. The gradient switching times and strengths will also be monitored together with the routine assessment of all electrical components of the system, as described previously.

MR technologists will also receive special training to prevent peripheral nerve stimulation and before any scanning procedure that might stimulate peripheral nerves, a technologist will:

- a) Inform the subject that peripheral nerve stimulation may occur
- b) Describe the nature of the sensation to the subject
- c) Instruct subjects not to clasp their hands, since this may create a conductive loop which will increase the possibility of stimulation
- d) Maintain constant verbal contact with the subject
- e) Instruct subjects to inform the MR technologist if they experience discomfort or pain
- f) Terminate the scan if the subject complains of discomfort or pain
- g) Complete a report of any incidents involving severe discomfort or pain, including a description of the associated circumstances (imaging parameters, dB/dt value, level of pain, etc.), and submit this report immediately to the IRB.

SAR Absorption The magnitude of temperature increase during MRI scanning is minimal. Increases are always within FDA guidelines, which include core temperature increases less than 1°C , as well as localized heating to less than 38°C in the head, 39°C in the trunk, and 40 °C in the extremities. Our 3.0 Tesla system has in place a means to monitor RF power levels and ensure that energy deposition is sufficiently low to stay well within these guidelines for temperature increases. First, a "system security" unit is employed to integrate the output of the RF amplifiers. This integration takes into account the amplitudes and duty cycle of the transmitter. If system security detects an output that might exceed the guidelines noted above, it automatically shuts down the entire RF power system. Secondly, all pulse sequences are evaluated, based on calculations and sound scientific measurements, to ensure that SAR remains within FDA-approved guidelines, prior to their use in humans. Any experiment performed on our 3.0 Tesla system will comply with all FDA guidelines with regard to RF power deposition. Proper and routine monitoring of all RF electronics (e.g., coils, transmitters, system security, etc.) will be performed on a regular basis. All pulse sequences will be evaluated (by calculation and by valid scientific measurement) prior to use in humans.

Static Magnetic Fields The minimization of risks associated with the static magnetic field of 3.0 Tesla is mainly related to incidental risks (see below). These risks are the same as in other commercially available clinical systems, and like other clinical MRI centers, our facility will incorporate a complete range of procedures to assure security of the restricted access area, careful screening of potential subjects before they enter the restricted access area, and a metal detector positioned at the doorway leading into the magnet room within the MRI suite. Access to the MRI suite will be tightly controlled, allowing access only to personnel and research subjects who have legitimate reason to be there. Doors to the unit will be securely locked, with only MR technologists, physicists, or physicians controlling entry of ferromagnetic and other materials that could possibly cause injury to patients, research subjects, personnel, or equipment. In addition, entry-ways to the unit will be labeled with clear visible signs warning of the presence of the magnetic field and the exclusion from entry by individuals with implanted metal objects such as prostheses, pins, clips, IUD's, etc.

Incidental MRI Risks All subjects will be able to communicate directly with technologists and study staff to inform them of any emotional or physical distress during the scanning procedure. If they wish, the scan will be terminated immediately and the subject will be removed from the scanner.

Confidentiality All the information obtained from the subjects will be coded by number and kept in locked, confidential files. This information will not be accessible to anyone but the study investigators. Furthermore, all of the data (including MRI scans) will be stored behind firewalls at the New York State Psychiatric Institute. Finally, to ensure protection of confidentiality of the sensitive information that will be obtained in this project, I will apply for a Certificate of Confidentiality from NIH.

Clinical Issues I will make necessary referrals during the course of the study if the need arises and I will notify the treating clinician if abnormalities are found on the MRI.

Risk/Benefit Ratio Potential benefits to society are large in regard to improved understanding neural underpinnings of pathological anxiety specifically linked to heightened sensitivity to (potential) threat and its developmental changes. This knowledge in turn could lead to better interventions and prevention of the severe long-term consequences facing not only many youth but also adults with pathological anxiety. Children and adolescents participants and their parents will be informed of any clinically significant findings from the MRI. Each subject will receive payment for participating in the study. The risks of participating in this study are minimal, especially in relation to the potential benefits and public health importance of improved understanding of pathological anxiety.

15. INCLUSION OF WOMEN AND MINORITIES

We will attempt to recruit equal numbers of females and males, consistent with the epidemiology of child and adolescent anxiety disorders and that of the New York metropolitan community, which is 52% female and 48% male. Efforts will be made to have the study population reflect the ethnic distribution of the area covered by the Columbia University Medical Campus and the New York State Psychiatric Institute. The recruitment of Hispanic participants will be consistent with the Hispanic population for the NY-013 congressional district (about 50% Hispanic). Our figures will be about 50% White and 50% Black or African Americans. Overall, NIH guidelines for the inclusion of females and minorities as subjects in clinical research will be followed. We will attempt to recruit equal numbers of each gender from each of the above racial groups. Previous neuroimaging studies in Dr. Posner laboratory achieved these gender, racial, and ethnic representations, and we foresee the same for this study. We will analyze demographic data every three months and if we are failing to achieve these marks, we will increase our recruitment of the under-represented group by extending recruitment to other area clinics that include the under-represented group. There will be no exclusion of subjects based on gender, race, or ethnicity.

16. INCLUSION OF CHILDREN

The basic study design is to assess fear generalization behavior and underlying neural circuitry in youth (ages 8-17 year) across the spectrum of anxiety including both clinically anxious groups and subthreshold anxiety individuals. We will recruit three groups of youth (n=75): 25 healthy youth, 25 youth with subthreshold anxiety, and 25 youth with DSM-5 anxiety disorders.

The PI, Dr. Jiook Cha, the sponsor, Dr. Posner (who is a child psychiatrist and will provide clinical assessments), and the research assistants who will work directly with the children during this K award project have extensive experience conducting MRI experiments with children and adolescents. Dr. Posner's research team has tested more than 30 healthy young children and youth across developmental stages (ages 8-17years) in 2014 alone for a MRI study.

Over the past 12 years, the Division of Child and Adolescent Psychiatry and the MRI Unit at the New York State Psychiatric Institute has acquired more than 1,000 MRI scans in children and adolescents, many of whom have had severe anxiety disorders, ADHD, movement disorders, and cognitive impairments. Drs. Cha and Posner's research team has extensive experience in the use of relaxation procedures and behavioral techniques to help children enjoy their experience in the scanner and to lay sufficiently still to provide motion-free images. Parents are allowed to accompany their child into the scanning suite. As already described in the research strategy (please see Participant Preparation), subjects will be offered the opportunity to undergo a desensitization procedure and relaxation training prior to having their MRI study. These procedures minimize potential anxiety the subjects may have concerning the MRI scan, and they help to acquire motion-free imaging. Drs. Cha and Posner will also train the research assistants in how to help children feel calm and prepared for the MRI, and how to help the children if they become uncomfortable or frustrated at any point during the visit. Both Drs. Cha and Posner will be available to the research assistants at all times that a study visit is taking place in case questions arise during a visit. The MRI Suite and the assessment offices in the Child Psychiatry Division are all extremely child friendly. The offices have age appropriate art on the walls, and the furniture is comfortable for child seating. The spaces are specifically designed to help children feel at ease and happy to participate.

Including 75 youth, this research proposal may provide crucial pilot data for a future larger-scale R01 application aimed at charting the developmental trajectories of the threat processing corticolimbic system across the developmental stages from children to adults and across the spectrum of anxiety from normal to abnormal. Therefore including children in this proposal is essential to this purpose.

FACILITIES AND OTHER RESOURCES

Overview

The combined imaging study to investigate over-generalization of fear in pediatric anxiety will be conducted within the Division of Child & Adolescent Psychiatry at Columbia University (CU) and New York State Psychiatric Institute (NYSPI), both on the campus of the Columbia University Medical Center (CUMC). The facilities and other resources available to this investigative team include everything needed to undertake and complete the proposed project successfully. The collaboration between Drs Cha, Posner, and Simpson, who are experts in systems neuroscience, pediatric neuroimaging, and translational neuroscience, respectively, represents a realization of the emphasis on collaborative, translational neuroscience research that is central to the Division of Child & Adolescent Psychiatry at Columbia University.

Institutional Commitment to the Career Development Award Trainee

Dr Jiook Cha, the project's PI has extensive evidence of institutional commitment to his development as an academic researcher. Dr Cha already has sufficient laboratory space in Dr. Posner's laboratory and access to equipment for this project, as evidenced by his ability to yield the preliminary data for this K01 application. Dr Cha's access to the imaging resources in the Department of Psychiatry is also evidenced by his recent diffusion MRI study of children with ADHD (see Biosketch, Cha). These imaging data were acquired on the 3 Tesla MRI scanner that will be used for the proposed study.

New York State Psychiatric Institute

New York State Psychiatric Institute (NYSPI), a facility within the New York Office of Mental Health system, has been one of the world's leading institutions for psychiatric research for more than 100 years. NYSPI is renowned for its psychiatric research, its training and educational resources, and its clinical care, housing inpatient services and research units as well as 23 outpatient research units. Its resources, research faculty, and staff, combined with those of the Research Foundation for Mental Hygiene's Division at NYSPI and the Columbia University Department of Psychiatry, have made the NYSPI one of the nation's most respected psychiatric research centers. It comprises two entire buildings as well as several floors of a third building.

New York Presbyterian Hospital at Columbia University Medical Center

Columbia University Medical Center's (CUMC) affiliated hospital, New York-Presbyterian Hospital (NYPH) was ranked first among New York hospitals by *U.S. News and World Report*; it placed on the *U.S. News and World Report* Honor Roll as one of the 17 best hospitals in the United States. Research institutes and centers based at the CUMC campus include the Center for Community Health, the Howard Hughes Medical Institute Program, the Center for the Study of Society and Medicine, the Center for Medical Informatics, and the Columbia Genome Center.

Pediatric Anxiety and Mood Research Clinic (PAMRC, directed by Dr. Moira Rynn, advisor and collaborator): PAMRC is a specialty research clinic dedicated to improving the lives of children and adolescents suffering from anxiety and mood disorders. Directed by Dr. Moira Rynn, PAMRC is part of the Department of Psychiatry at Columbia University and is located in Washington Heights, New York City. PAMRC provides comprehensive evaluations, treatment with evidence-based therapy and medications, and a day treatment program with academic services for youth requiring intensive care. All assessment and treatment services at PAMRC are free of charge.

Children's Day Unit (directed by Dr. Moira Rynn, advisor and collaborator): The Children's Day Unit (CDU) is an outpatient day hospital program, directed by Dr. Moira Rynn, located at the New York State Psychiatric Institute/Columbia University Medical Center. The CDU is comprised of research participants and adolescents ages 8 to 17 years from the community who require intensive outpatient care. The CDU specializes in the treatment of Generalized Anxiety Disorder, Social Phobia, Post-Traumatic Stress Disorder, Obsessive-Compulsive Disorder, Depression, and other psychological disorders. All children and adolescents attending the CDU receive a comprehensive evaluation and then enter a structured therapeutic program. During the academic year, the CDU partners with the New York City Board of Education to provide education for students through the public school PS 186X located on the unit. Students attend class daily and are often eligible to earn school credits while attending the CDU. In addition, during the 6-week summer program in July and August, the CDU provides educational support and tutoring.

Laboratory and Scanning Facilities

NYSPI and CU Department of Psychiatry 3.0 Tesla MRI Laboratory: The NYPSI MRI unit is the location of a General Electric (GE) 3.0 Tesla (T) high-performance MRI scanner. The site is state-of-the-art for image acquisition and analysis:

MRI Scanning Suite: NYSPI's GE 3.0 T whole-body scanner resides within a 3,200 square-foot MRI suite that includes the scanning room, a console area, a room dedicated for animal preparation, an electronics workshop, a laboratory for the design and construction of radio frequency coils, a subject "on-deck" waiting area (where scanning-related tasks can be practiced and the procedures for the scan can be reviewed), a family waiting room, a wheelchair-accessible changing room and lavatory, a break room for the technologist and other staff, an office for the MRI physicist, and a viewing room. The suite is equipped with 3 Hewlett Packard (HP) xw8000 workstations with full-image processing capabilities for all MRI modalities, one HP LaserJet 1320n color printer, two Sun Ultra 60 workstations, and six Pentium 4 class PC's, each with a 3GHz CPU, 2GB RAM, and 200 GB or more of hard disk space. The MRI scanner and other hardware are described below. The unit is staffed with a full-time MRI technologist, a physicist, an electrical engineer who builds and maintains equipment and software used for physiological monitoring, and a full-time, dedicated pulse programmer. The scanning suite is directly below the image-processing laboratory, which has an Ethernet fiber optic connection directly to the scanning console. Transfer of large-scale imaging data in variously formatted image files (DICOM, LX, GINX) from the MRI Unit to various computer platforms across laboratories within NSYPI occurs over a dedicated fiber optic network (with data transfer rate of 1 Gb/second) through a Storage Area Network (SAN). The SAN is a high-speed, special-purpose network that interconnects data storage devices with associated data servers of users on the network. SAN supports disk mirroring, backup and restore, archiving, and retrieval of archived data, data migration from one storage device to another, and the sharing of data among different servers. The SAN does not carry general-purpose traffic (e.g., e-mail or other end-user applications) and will avoid burdening a Local Area Network (LAN) with data storage, retrieval, and archival activities.

Whole Body 3.0 Tesla MRI Scanner: The Signa 3.0 Tesla magnet, with a 55 cm diameter patient bore, is a high-homogeneity, actively shielded, wide-open superconducting system, utilizing single cryogen unit technology, which provides a very low boil-off rate. The magnet delivers high, uniform homogeneity (< 0.05 ppm on water spectral FWHM for 20 cm DSV), which is essential for good image quality in demanding techniques such as spectroscopy and ultrafast echoplanar imaging approaches. The high-performance cardiac resonator module (CRM) gradient coil with enhanced gradient amplifiers can be operated at gradient amplitudes up to 4.0 mTesla/meter with slew rates as high as 150 mTesla/meter/msec, which enables ultrahigh spatial resolution (0.1 mm slice thickness in 3D) and ultrafast imaging (34.0 ms TR for 64 x 64 matrix EPI). Furthermore, 14 passive superconducting shim coils improve the main field homogeneity up to < 0.1 ppm on the spectral width of an 8cc brain volume by high-order (up to 2nd order) automated resistive shimming. Four-channel fast receiver modules, along with an ultralow-noise digital RF subsystem and frequency synthesizer, and a quadrature-drive transmitter/receiver head coil, provide high SNR and stability (< 0.05 ppm frequency variation, < 5% amplitude variation, and < 0.5 degree phase variation) to different neuroimaging studies. The camera room is actively shielded.

The scanner's hardware and software are upgraded to GE's 32-channel HDx hardware platform, the EXCITE 3.0T head coils, and ASSET (Array Spatial Sensitivity Encoding Technique) software. The 32 quadrature expandable channels and 16 high-bandwidth receivers, along with ASSET, deliver cutting-edge parallel imaging by making possible dramatically shortened TRs, TEs, and ESPs. Signal-to-noise is enhanced by approximately 100% over the prior LX platform, susceptibility artifacts are fewer, and resolution has been enhanced by these modifications. Furthermore, dual Intel Xeon 2.66 GHz processors with 2GB host memory linked to an EXCITE vector array processor make simultaneous computing technology possible (400 images/sec for 256 x 256 FFT), thereby benefiting real-time interactive imaging.

RF Coil Laboratory: A design laboratory in the NSYPI MRI Unit houses electronic device building and repair. It consists of 400 square feet with bench space and tool storage, equipped with electronics (eg, voltmeters, oscilloscopes, circuit design and construction). It has the capability to design, construct and test electronic circuits. The laboratory has developed specialized coils, such as surface coils, dual tuned coils, and coils for fetal baboon imaging and GABA spectroscopy, which reduce RF inhomogeneities and susceptibility artifacts.

Comprehensive Image Acquisition Capabilities: Multi-shot EPI sequences provide high-resolution functional applications (matrices up to 512 x 512) with FuncTool as a dynamic fMRI processing package. The Probe 2001 package enables proton spectroscopic applications on single voxel and multivoxel (3D chemical shift imaging; i.e., CSI) basis. A multinuclear spectroscopy package and broadband RF amplifier for phosphorous, lithium, and carbon is available. High B-value diffusion-weighted EPI technique with FLAIR preparation capabilities is installed, as is the latest diffusion tensor imaging (DTI) acquisition package, spiral sequences, and perfusion imag-

ing. Advanced vascular imaging includes time-of-flight (TOF) angiography and magnetization transfer contrast (MTC) methods. The GE MRI system also includes advanced image processing software (e.g., PROBE2000, PROBE 3D Brain, FuncTool, spiral, diffusion, fast spin echo, and Brain Wave packages). These permit easy visualization of single-voxel and multivoxel spectra MRS data, 2D and 3D chemical shift imaging, parametric metabolite mapping, diffusion tensor post processing, and functional brain mapping (BOLD), as well as fMRI stimulus sequencing and presentation.

MR Image Processing Laboratory: This is directly above the MRI scanning suite at NYSPI, where it occupies more than 2000 square feet. The imaging laboratory currently has one Sun Ultra 1, seven Sun Ultra 10, and two Sun Ultra 60 workstations, each with 512 MB RAM, dual 300-450 MHz SPARC-V9 floating point processors, 19-inch LCD color monitors, eight 75GB external hard drives for data storage, and an external optical drive. We also have two Sun Fire V880 servers with dual 900 and 1200 MHz CPUs, 4GB RAM, and 850 GB RAID 5 hard disk storage, along with an integrated fiber channel subsystem that provides an I/O performance of 1.2 GB/sec. Additionally, two Dell PowerEdge 6600 servers with four CPUs, 6 GB RAM, and 600GB disk space, running Microsoft Windows Server 2003, provides computing power and memory to even the most demanding image processing methods. From SAN to the servers, 4.5 TB high-speed disk array are connected via 2 Gbps fiber channels. A Network-Attached Storage (NAS) with 3.75 TB high-speed SCSI disk array is used for daily backup. A 1.9 TB tape library is also attached to the Dell server to perform monthly backup.

The lab also has two state-of-the-art Silicon Graphics workstations, 10 dual-processor Pentium 4 PCs with 3.4GHz CPUs, 3GB RAM, and 500GB hard disks, 12 Pentium PC's, three Dell Inspiron Pentium laptops, two Macintosh laptops, five external optical drives, an HP LaserJet 4700dn color printer, an HP LaserJet 4250dn laser printer, an HP LaserJet 4100dn laser printer, a Lexmark C910 color printer, and a Seiko Colorpoint 860 color printer.

These workstations are seamlessly interconnected on the LAN to provide a distributed computing environment for the most efficient use of computing power and storage space by research assistants. Processing can be run in "batch" jobs in the background during the day and in the foreground throughout the night, when staff members are not using the workstations.

Although the Image Processing Lab at NYSPI has extensive experience conducting structural MRI research, the laboratory uses manual techniques and analytic software that are not available to investigators outside of that laboratory. Dr Cha has gained experience analyzing structural MRI data using FreeSurfer (<http://surfer.nmr.mgh.harvard.edu/>; see the Key Preliminary Studies section in Research Strategy). FreeSurfer uses an automated algorithm for subcortical brain segmentation, is widely accepted in the imaging community, and is freely available to all investigators. Developing expertise in this approach to structural MRI research rather than the manual approach of the NYSPI Image Processing Lab will allow Dr Cha to become a fully independent investigator. Yaakov Stern, PhD, Columbia University Professor of Clinical Neuropsychology, will provide expert input into structural MRI data analysis (see Letter of Support) and the Taub Institute and Sergievsky Center at Columbia University, where Dr. Stern is the director, has extensive experience using FreeSurfer for structural MRI research.

High-Performance Computing (HPC) Systems: Columbia University's Advanced Research Computing Services (ARCS) maintains several high-performance computing systems, including multiple high-performance computing clusters as well as high-memory systems. In 2013 a new cluster with 6,336 CPU-cores and 73,728 CUDA-cores (GPU) replaced the previous Titan system. The new system has a maximum performance of 212 TFlops, almost nine times the performance of its predecessor. The system is on the Top500 list of supercomputers worldwide.

The new system has a number of improvements over the current system, including 10 Gbps Ethernet fabric throughout, 40 Gbps QDR InfiniBand for a portion of the system, GPU-enhanced computing, and a new, lower power hardware architecture. All of the clusters run current variants of the Linux operating systems, and are managed by Univa Grid Engine. Additionally, ARCS has two high-memory systems with 1 TB of system memory each, and a pool of computational servers for compilation, debugging, and job control.

The HPC system has most of the widely used neuroimaging data analysis pipelines (e.g., FSL, Freesurfer, SPM, AFNI) and other data analysis software (e.g., MATLAB). Using these, Dr. Cha has previously run diffusion probabilistic tractography analyses in a recent childhood ADHD study (see Biosketch, Cha) and currently runs the majority of neuroimaging data analysis.

Biostatistical Resources

The Department of Biostatistics at Columbia University's (CU) Mailman School of Public Health has a large faculty with expertise in diverse areas of statistics: clinical trials, analysis of microarray data, statistical genetics, neuroimaging data analysis, methods for handling missing data in medical research, clustered and longitudinal data

analysis, categorical data analysis, multivariate analysis, meta-analysis, and many others. These methods and developed programs are available to New York State Psychiatric Institute (NYSPI) researchers to help them answer their research questions. Chair of the Department of Biostatistics, **Dr. Bowman (consultant, advisor)** will provide consultation on biostatistical application in the research and training plans.

NYSPI's Biostatistics Division also provides researchers access to and training in state-of-the-art statistical techniques as needed for optimal use of their research data. The division's activities include developing and applying new methodologies for the design and analysis of psychiatric studies, participating in major funded research projects, teaching statistics to researchers and fellows of training grants, mentoring PhD candidates from the Department of Biostatistics at CU in methodological topics related to neuropsychiatric research, and providing consultations on data-analytic and design issues for grant submission and manuscript preparation.

Animal Facilities

Not applicable.

Computer Facilities

All personal computers are linked to a printer and to the NYSPI and CU computer networks. The study has access to all network support made available by NYSPI and by its Division of Child Psychiatry. The network consists of three Novell servers and four Windows servers. These servers are used to share files, host Web sites, and run the e-mail system. The data and e-mail databases are backed up to tapes regularly. Tapes are rotated on a two-week cycle and the month-end tape is saved. Data up to two years old can be restored from tape.

Library Facilities

The libraries of both NYSPI and CU are available to the study, including all of their online databases (e.g., MEDLINE, PsycINFO, PubMed, and Health and Psychosocial Instruments).

Office Facilities

All faculty members have private offices at the NYSPI. Each office comprises roughly 200 square feet and is provided with a phone, a desktop computer, filing cabinets, and Internet connectivity. Three research assistants will share an office provided with phones, desktop computers, filing cabinets, and Internet connectivity.

Clinical Facilities

Child Psychiatry Outpatient Service at Children's Hospital of New York at Presbyterian Medical Center: Under the direction of Lou Baptista, MD, the Child Psychiatry Outpatient Service at the Children's Hospital of New York (CHONY) is a group of specialized subclinics that handles approximately 34,000 patients annually. Clinicians from the clinic regularly consult with child psychiatry research faculty to ensure that patients are provided the most-up-to-date psychopharmacological and psychotherapeutic treatment.

Other Facilities

Interview rooms can also be scheduled for assessment and treatment visits. A comfortable waiting room stacked with toys and magazines is available for families as they wait for their appointments. Rooms with one-way mirrors and a room equipped for videotaping are available to be scheduled on the Children's Day Unit, which is also on the second floor of the NYSPI-Herbert Pardes building.

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