

Executive Summary

Although the Center has been funded for less than 10 months, significant progress has been made in subject enrollment, optimization and validation of behavioral paradigms and imaging sequences for the human studies (Projects I and II); and validation and optimization of conditioning and behavioral stress paradigms, and molecular and histological measures for the mouse studies (Project III). A central focus of the Center cores has been in optimizing the data management and analysis as well as training infrastructure for the Center investigators and trainees and special attention has been given to training young investigators and trainees outside the Center as well as those within the Center. Although data acquisition has just begun, review papers highlighting the translational (across species) and transitional (across ages) approach of the Center to understand gene X environment X development interactions are in press and empirical publications emphasizing BDNF Val66Met molecular and histological effects, and optimization of behavioral and stress paradigms are under review and/or published.

We have developed an external advisory committee that includes Dr. Susan Bookheimer of UCLA for the genetic and pediatric imaging studies, Dr. Regina Sullivan of NYU for developmental and stress related rodent studies and Dr. Joe Ledoux of NYU for the translational rodent/human conditioning studies. These advisors are in addition to our external advisory board for DNA sharing (Drs. Ed Cook of UIC, Dan Geschwind of UCLA and Matt State of Yale) and our imaging consultants (Drs. Gary Glover, Bruce Fischl and Steve Smith).

We have held two center meetings. The first meeting was informal and occurred in conjunction with our annual summer institute on the biology of developmental disabilities in Ithaca, NY. Core PIs and investigators presented (Clark, Mezey, Glatt and Casey) as well as Project I and III investigators and fellows (Amso, Bath, Lee, Casey), and Center consultants (Glover, fellow of Fischl) and attendees included fellows of Gunnar and Thomas (Project II) during the dates of June 22-27, 2008. The second formal meeting is scheduled for Mar 9, 2009 with our internal consultants and advisory board members, and core and project investigators and trainees. This meeting will provide the opportunity for Center investigators to report on their progress, discuss plans for upcoming studies, and share ideas of general relevance to the Center as well as receive input from consultants and advisors on further optimization of Center studies and logistics.

The Center has been named the NIMH Center for Brain, Gene and Behavioral (CBGB) research across development to reflect the multidisciplinary and developmental focus of the center. A public website maintained at the WCMC site makes publications and tools and assays assessible to the public (see: <http://sacklerinstitute.org/cornell/CBGB/> and http://sacklerinstitute.org/cornell/assays_and_tools/, respectively).

Progress in each project has been considerable even in this short time as reviewed in the individual progress reports. Highlights include: Enrollment of nearly 400 participants in less than 1 year; 100% accuracy of 332 samples received by the ADM Core to date and seamless bio specimen processing data flow between the projects and ADM Core; new developments in an image analysis pipeline for the imaging projects within the Statistical Analysis Core; over a dozen publications; several training initiatives and center interactions illustrating cross fostering of trainees across projects and support for grant submissions by junior investigators.

Transitions and changes within the Center include the appointment of Dr. Thomas from Co-investigator to Co-PI on Project II, the addition of Dr. Doug Ballon, Director of the Imaging Facility and Core at WCMC as key personnel on Project I. There has been one design change in Project II. In order to match ages with Project I which serves as a comparison group, Project II has shifted the targeted age for neuroimaging from 13-15 years to 12-13 years. There has been an addition of a dependent measure for Project I. In addition to slope in response latency during conditioning, galvanic skin response is being recorded in parallel (see Project I report). There has been a sample broadening by Project I. To increase the sample of Center subjects, Project I has begun collecting DNA from a sample of previously institutionalized children in NYC identified as part of NIMH R01 MH73175 *Brain Development Following Institutionalization* who have been phenotyped using similar measures as those proposed in the Center and relevant to Project II aims (Casey, Glatt, et al. under review *Neuroscience*). Funds for genotyping this sample have been provided internally as part of the commitment of the college to the Center and detailed in the official response to program review.

Project I: Impact of BDNF Genotype on Brain Development and Learning

A. Specific Aims

The specific aims of this project have not changed from the original competing application. This project examines how the uniquely human BDNF Val66Met polymorphism mediates contextual, cued and reversal learning (extinction) across development and how everyday stress and cumulative stress may alter these effects.

A1. Specific Aim 1. To examine the influence of BDNF genotype on the development of behavioral and neuroanatomic measures of different forms of learning using both MRI and fMRI (Center Aim 1).

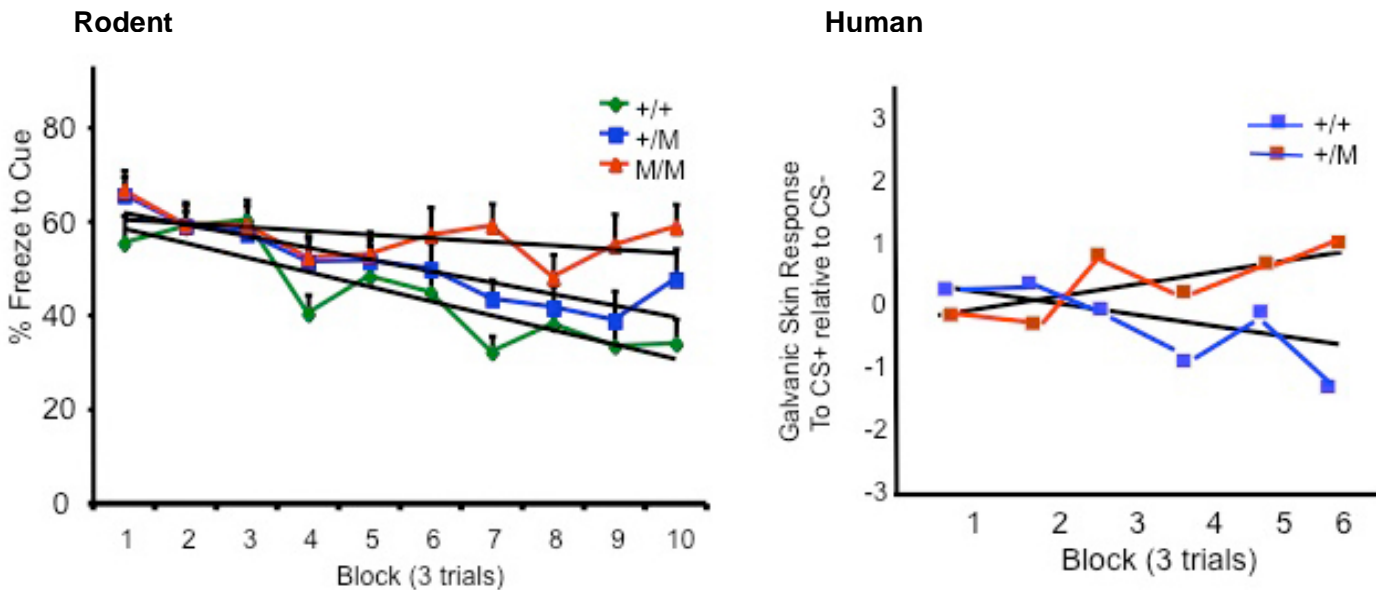
A2. Specific Aim 2. To examine the interaction of BDNF genotype and environment on behavioral and neuroanatomic measures of learning using both MRI and fMRI across development (Center Aim 2).

B. Studies and Results

The majority of year 01 has been in recruiting subjects for the proposed studies and developing a recruitment pipeline for recruitment, screening, collecting DNA and behavioral measures. We have already recruited 160 subjects, almost 100 more than projected. All subjects have been successfully genotyped (see ADM Core progress) and comprehensive behavioral phenotyping has begun on half that sample and matching of genotype groups by age, gender, race, SES and IQ is being overseen by the ADM Core.

B1. Specific Aim 1.

During this funding period we have been optimizing behavioral paradigms, imaging sequences, and analyses working with the Statistical Genetics Core and imaging consultants. As such, there has been a slight change in our behavioral measures, in addition to slope in response latency to conditioned stimuli during conditioning and extinction, galvanic skin response is being recorded to assess arousal during conditioning and extinction in humans to confirm validity of unconditioned stimulus (aversive sounds) and which may better parallel freezing measures in the mouse studies of Project III and show more reliable changes across subjects with unconditioned stimulus presentation than response latency. Figure 1 below shows freezing in the mouse (Project III) during extinction to the conditioned stimulus (left panel) and our preliminary results from 60 late adolescent to young adult humans (right panel) in galvanic skin response to the conditioned stimulus relative to the neutral stimulus. The Met allele carriers show slower extinction than the wildtype rodents or the humans with the Val/Val genotype. These findings have potential implications for exposure therapies, suggesting that Met allele carriers may be less responsive to such therapy.



B2. Specific Aim 2.

During this funding period we have been optimizing environmental exposure measures of cumulative risk and stress and characterizing their effects on prefrontal and limbic circuitry (e.g., amygdala and hippocampus). We now show decreased prefrontal connectivity and function (attentional shifts) following elevated everyday stress that returns once the stressor is removed (see Liston, McEwen and Casey, 2009 *PNAS*) using our prefrontal behavioral task. This work validates the importance of this originally proposed behavioral task as an outcome measure in all three projects.

In order to increase the sample of subjects in the Center relevant to Project II (subjects with varying degrees of early adversity due to prior institutionalization), Project I has begun collecting DNA from a sample of previously institutionalized children identified as part of an NIMH R01 MH73175 *Brain Development Following Institutionalization*. These participants have been phenotyped using similar measures as those proposed in the Center (Casey, Glatt et al., under review). Funds for genotyping this sample have been provided internally as part of the commitment of the college to the Center as detailed in the official response to program review.

C. Significance

A critical component of the proposed studies is to understand how genetic and environmental factors influence learning and brain development. This work is essential with direct implications for understanding basic mechanisms of atypical learning in disorders such as anxiety and depression and potentially broader implications for early intervention and personalized treatment.

D. Plans

We will continue to address our specific aims by recruiting, screening and imaging our proposed developmental samples during performance of the refined paradigms. These refined paradigms have been optimized based on preliminary data reported here and in our monthly interactions with other project PIs.

E. Publications

- Casey, BJ, Hare, T & Galvan, A Decision Making In Adolescents. M Delgado, E. Phelps & T Robbins (Eds). Attention and Performance: Processes of Change in Brain and Cognitive Development. Cambridge, MA: MIT (under review)
- Casey, BJ, Glatt, CE, Tottenham, N, Soliman, F, Bath, K, Amso, D, Altemus, M, Levita, L Jones, R, Thomas, KM, Gunnar, M, Mezey, J, Clark, A, Leon, AC, Hempstead, B, and Lee, FS. BDNF as a Model System for examining Gene by Environment Interactions across development. Neuroscience (in press)
- Levita, L, Hare, T, Voss, H, Ballon, D & Casey, BJ The bivalent side of the nucleus accumbens. Neuroimage 1;44(3):1178-87 (2009)
- Liston, C, McEwen, B & Casey, BJ Psychosocial stress reversibly disrupts prefrontal processing and attentional control PNAS 106:912-917 (2009)
- Somerville, L, Jones, R & Casey, BJ A time of change: Behavioral and neural correlates of adolescent sensitivity to appetitive and aversive environmental cues Neural correlates of adolescent behavior. Cognition and Brain. (in press)
- Tottenham, N., Hare, TA, Quinn, BT, McCarry, TW, Nurse, M, Gilhooly, T, Milner, A, Galvan, A, Davidson, MC, Eigsti, IM ... Casey, BJ (in press) Prolonged institutional rearing is associated with atypically larger amygdala volume and difficulties in emotion regulation. Developmental Science (in press)
- Soliman, F., Levita, L., Millner, A., Amso, D., Voss, H., Glover, G., & Casey, B.J. (June, 2008). Recruitment of frontolimbic circuitry in reversal and extinction learning. Proceedings of the Organization for Human Brain Mapping, Melbourne, Australia (abstract).

F. Project Generated Resources

None.

Project II: Impact of BDNF Genotype and early life stress on learning in adolescents

A. Specific Aims

The Specific Aims of Project II have not changed. However, during the planning of this project, Drs Gunnar and Thomas conceived of their roles as shared PIs with efforts are shared commensurately. However, the paperwork was submitted listing Dr. Thomas as a Co-Investigator. During this year, it was requested that the subcontract be amended to reflect her role as a multiple PI; that is, this project will have two Principle Investigators: Megan R. Gunnar and Kathleen M. Thomas.

B. Studies and Results

Project II involves two phases. Phase I includes the recruitment and genotyping of a large sample of post-institutionalized, internationally-adopted children. Phase II involves behavioral assessments and neuroimaging of a subset recruited to balance genotype, sex, and birth region (reflecting race and population genetic background). A total of approximately 600 children are expected to be represented in Phase I and 250 children (over sampled to achieve 200 with usable neuroimaging data) in Phase II. Progress in phase I and II is described separately below.

There has been one design change. In order to match ages with Project I which serves as a comparison group, we have shifted the targeted age for neuroimaging from 13-15 years to 12-13 years.

B.1. Phase I Progress

1. Hiring and training of participant recruiter: Our prior work indicates that it is critical to employ in this position individuals with prior international adoption experience. Spring of 2008, we hired Nancy Ward, MS Social Work, who had 30 years experience in international adoption and served for many years as a case worker and supervisor at the major adoption agency in Minnesota. Ms Ward is responsible for recruitment and collection/return of phase I material and recruitment and scheduling for phase II.

2. Recruitment for Phase I began in June, 2008. Phase I participants complete Oragene saliva collection for gene determination, and parents complete a pre-adoption a number of questionnaires (pre-adoption conditions, adoptive family SES, diagnostic conditions and medications, educational services use, and the MacArthur Health and Behavior Questionnaire of emergent psychopathology. After 7 months of recruitment, we are ahead of target, with 172 participants who have returned Oragene samples and questionnaires, and 54 in process. Agreement and return rate is over 80%. Questionnaire data from Phase I are being entered and checked as they arrive in the laboratory.

3. A total of 172 Oragene samples have been collected and processed by the ADM Core of the Center, with 100% yielding data now coded by whether or not they possess one Met allele (see ADM Core progress).

B2. Phase II Progress

1. Hiring and training of Phase II personnel: In June 2008, Ms. Amanda Hodel was hired (30% time). As Phase II coordinator, she works with the recruiter (Ms. Ward) to schedule Phase II behavioral and neuroimaging testing sessions, trains experimenters for behavioral testing sessions, and manages purchasing and supplies. In addition, training was conducted for 3 GRAs and 4 clinical interviewers who are responsible for obtaining consent and conducting behavioral, neuropsychological, and/or interview portions of the behavioral testing session. The GRAs also conduct the MRI simulation, attend the MRI scan, obtain saliva samples for hormone measurement, and monitor behavioral performance during the neuroimaging session.

2. Scanner pulse sequence optimization (Dr. Mueller) and pilot testing of neuroimaging tasks (Dr. Thomas) began in June 2008. Testing of Phase II participants began in July 2008. Phase II requires 2 visits to the university, the first for behavioral and interview assessments (computerized cognitive tasks, IQ assessment, psychiatric interview) as well as acclimation in an MRI simulator; the second for structural and functional

neuroimaging. Based on data from Phase I, children targeted for Phase II were contacted and exclusion criteria were assessed during phone screening and recruitment. Of the children meeting criteria, 16 families declined to participate in Phase II, 28 have completed or begun Phase II assessments (behavioral session, or both sessions completed), and an additional 12 are scheduled for Phase II testing prior to the end of current grant year (April 30, 2009). Of the 28 children tested, 26 have completed all assessments and the remaining 2 have only partial MRI data.

3. Dr. Thomas continues to mentor graduate research assistants in the transfer, maintenance and processing of behavioral and neuroimaging data. Ms. Raquel Gabbitas, a first-year doctoral student in Child Psychology, has taken primary responsibility for initial MRI data processing. In addition to her coursework in child psychology, Ms. Gabbitas has been training with Drs. Thomas and Gunnar in the areas of developmental cognitive neuroscience and early adversity and stress. Beginning Spring semester, Ms. Gabbitas will also be attending the weekly project meetings held jointly by Drs. Gunnar and Thomas. Ms. Gabbitas will be traveling to and participating in the next Center meeting to be held in New York on March 9, 2009.

4. Phase II preliminary analyses from the first 25 participants are underway and will be presented at the Center meeting on March 9th.

C. Significance

Data from this project will be important in expanding our understanding the factors that contribute to risk and resilience in children experiencing early life stress. Using a multi-method approach including clinical, behavioral, neuroimaging, and genetic measures will allow us to examine critical interactions between genes and environment.

D. Plans

We plan to continue to enroll and test children in both Phases I and II at the current rate, with an expected increase in Phase II testing during the summer (non-school) months. We will continue to work with the genetics core of the Center to genotype our sample and to ensure appropriate recruitment for later statistical analysis. We will work with the neuroimaging core to analyze structural MRI data using FreeSurfer software and plan to send a graduate student to train in these analysis techniques.

E. Publications

Casey, BJ, Glatt, CE, Tottenham, N, Soliman, F, Bath, K, Amso, D, Altemus, M, Levita, L Jones, R, Thomas, KM, Gunnar, M, Mezey, J, Clark, A, Leon, AC, Hempstead, B, and Lee, FS. BDNF as a Model System for examining Gene by Environment Interactions across development. Neuroscience (in press)

F. Project Generated Resources

None.

Project III: Impact of stress and enrichment on a mouse model of the BDNF Val66Met SNP

A. Specific Aims

A.1. Specific Aim 1. Determine the biological consequences of genetic variant BDNF_{Met} on hippocampal, prefrontal cortex, and amygdala function across development.

A.2. Specific Aim 2. Determine the interaction between stress and genotype across development in BDNF_{Met} mice through the assessment of hippocampal, prefrontal cortex, and amygdala function.

A.3. Specific Aim 3. Determine the effect of BDNF gain of function on hippocampal, prefrontal cortex, and amygdala function of the BDNF_{Met} mouse across development.

B. Studies and Results

Specific Aim 1. Determine the biological consequences of genetic variant BDNF_{Met} on hippocampal, prefrontal cortex, and amygdala function across development.

B.1.a. Molecular, anatomy, and neuronal morphology analyses across development. In this past year, we have been focused on optimizing the molecular signaling and anatomical analyses in the the BDNF_{Met} mice. We have been able to detect activated BDNF receptor (phospho-TrkB) via immunohistochemistry in a variety of brain regions (Bath et al., 2008), and have begun analyses in the hippocampus, amygdala, and vmPFC at earlier postnatal times. In addition, we have recently optimized measurement of vmPFC volume by stereology, which will allow for comparison of brain volume in this region as compared to hippocampus and amygdala (BLA) across postnatal development. In parallel we have been continuing to measure neuronal morphology in these same regions at P15, P30 and P60. Consistent with our preliminary studies, we find significant decreases in dendritic complexity in hippocampal and BLA neurons even as early as P15. Interestingly, the onset of deficits in neuronal morphology in prefrontal cortical (vmPFC) neurons occurs at later time points (between P30-P60) suggesting that the variant BDNF_{Met} may have differential effect on the vmPFC, as compared to hippocampus and amygdala.

B.1.b. Behavioral Analyses. In this past year, we have begun behavioral analyses of BDNF_{Met} mice in the behavioral battery we proposed across postnatal development. While the results are preliminary, we have found that in tests of anxiety-related behaviors (open field, elevated plus maze) adolescent P30 BDNF^{Met/Met} mice do not appear to exhibit any alteration in anxiety-related behaviors as compared to their age matched, wild-type littermates. Extinction in these mice appears slow relative to wild type mice, paralleling initial findings in human Met allele carriers, shown in Figure 1 of Project I. These studies suggest that the form of anxiety resulting from the variant BDNF_{Met} emerges at a time after P30, during a peri-adolescent time period.

B.2. Specific Aim 2. Determine the interaction between stress and genotype across development in BDNF_{Met} mice through the assessment of hippocampal, prefrontal cortex, and amygdala function.

B.2.a. Behavioral Analyses following Stress. In this past year, we have begun validating the stress paradigms that will be used across development. We have determined in adult mice that a 7 day restraint stress regimen will increase anxiety-like behavior (open field, elevated plus maze) in the BDNF^{Met/Met} and BDNF^{+Met} mice. This is of significance as at baseline the heterozygous BDNF^{+Met} mice do not display altered anxiety-like behavior, and these results suggest that this environmental intervention can elicit a behavioral phenotype in these mice that is not present in the wildtype littermates, who underwent similar restraint stress.

B.3. Specific Aim 3. Determine the effect of BDNF gain of function on hippocampal, prefrontal cortex, and amygdala function of the BDNF_{Met} mouse across development.

B.3.a. Generation and analyses of overexpression BDNF^{overexp/Met} mice. This past year, the breeding and expansion of the BDNF^{overexp/Met} mouse colony has proceeded in anticipation of analyses to begin in Year 2.

C. Significance

There are two major areas of significance in Project. First, the BDNF^{Met/Met} mouse serves a model system for studying the contribution of this variant BDNF to core symptoms of neuropsychiatric disorders (i.e. difficulty in adjusting to new environments or recognizing safe ones as measured by contextual, cued and reversal learning) across development and as a function of stress. There is a prevailing dilemma from the human genetic association studies as to whether this variant BDNF is a risk factor gene for these neuropsychiatric disorders. The findings from the BDNF_{Met} mouse provides an in vivo model system to study in a controlled

manner the molecular, anatomical, and behavioral consequences of this genetic variant that the parallel human studies in this Project that are assessing the developmental trajectory of the hippocampus, amygdala and ventromedial prefrontal cortex, function involved in different forms of learning. Second, the impact of environmental inputs (stress, enrichment) on this genetic background can be rapidly tested in this variant BDNF mouse model, not only to delineate “sensitive periods” of vulnerability, but also optimal timing for intervention to rescue impairments in these forms of learning.

D. Plans

D.1. Specific Aim 1. Determine the biological consequences of genetic variant BDNF_{Met} on hippocampal, prefrontal cortex, and amygdala function across development.

D.1.a. Molecular, anatomy, and neuronal morphology analyses We plan to assess further signaling, brain region volume, and neuronal morphological alterations in different brain regions (hippocampus, prefrontal cortex, amygdala,) in BDNF_{Met} mice across development. Initial experiments will focus on early postnatal time points (P7, P15, P30), in an attempt to map the onset of the neuronal morphological deficits we have observed in the adult mice. In the case of the vmPFC, an additional P45 time point will be added to monitor to the emergence of deficits in volume and neuronal morphology.

D.1.b. Behavioral Analyses. In this upcoming year, we plan to continue the proposed behavioral studies across development. Of particular interest will be mapping the emergence of the anxiety phenotype after P30, by assessing these behaviors at P40 and P50, as well as mapping the emergence of the fear extinction deficit.

D.2. Specific Aim 2. Determine the interaction between stress and genotype across development in BDNF_{Met} mice through the assessment of hippocampal, prefrontal cortex, and amygdala function.

D.2.a. Behavioral Analyses following Stress. In this upcoming year we will be completing the 7 day restraint stress studies in P30 and P60 BDNF_{Met} mice, as well as assessing BDNF-dependent signaling in the hippocampus, BLA, and vmPFC after the mice receive stress. This 7 day stress restraint protocol appears to be optimal for inducing increased anxiety-like behavior in the BDNF_{Met} mice without affecting the basal anxiety-like behavior in the wild-type littermate controls.

D.3. Specific Aim 3. Determine the effect of BDNF gain of function on hippocampal, prefrontal cortex, and amygdala function of the BDNF_{Met} mouse across development.

D.3.a. Generation and analyses of overexpression BDNF^{overexp/Met} mice. In this upcoming year, we will begin our baseline analyses of the BDNF^{overexp/Met} mice. Initially only the adult mice will be analyzed, to assess the validity of this new transgenic mouse line. Baseline BDNF levels in the the hippocampus, BLA, and vmPFC will be measured in these mice will be assessed in the adult mice, as well as molecular signaling as in **Aim 1**. In addition, baseline hippocampal memory, anxiety-related behaviors, and fear extinction will be assessed in the adult BDNF^{overexp/Met} mice.

E. Publications

Bath KG, Mandairon N, Jing DQ, Rajagopal R, Kapoor R, Chen ZY, Khan T, Proenca CC, Kraemer R, Cleland T, Hempstead BL, Chao MV, Lee FS. Variant brain-derived neurotrophic factor (Val66Met) alters adult olfactory bulb neurogenesis and spontaneous olfactory discrimination. *J. Neurosci.* 28:2383-2393, 2008. NIHMSID: NIHMS43608.

Bath, K.G., Voss, H.O., Jing, D., Anderson, S.A., Hempstead, B.L., Lee, F.S., Dyke, J., and Ballon, D. (*in press*). Rapid quantitative phenotyping of genetically engineered mice via magnetic resonance microscopy. *Magnetic Resonance Imaging*.

Carim-Todd, L.*, Bath, K.G.*, Fulgenzi, G., Sudhirkumar, Y., Barrick, C.A., Becker, J., Buckley, H., Dorsey, S., Jing, D.Q., Lee, F.S., and L. Tessarollo (2009). Endogenous truncated TrkB.T1 receptor regulates neuronal complexity and TrkB kinase receptor function in vivo. *Journal of Neuroscience*, 29(3):678-85.

Chen Z.Y., Bath K.G., McEwen B.S., Hempstead B.L., and F.S. Lee (2008). Functional analyses of variant BDNF (Val66Met) polymorphism. *Novartis Foundation Symposia*, 289:180-187, *Review*.

Hui Y., Wang Y., Pattwell S., Jing D.Q., Liu T., Zhang Y., Bath K.G., Lee F.S., and Chen, Z.Y. (*in press*). Variant BDNF Val66Met polymorphism affects extinction of conditioned aversive memory. *Journal of Neuroscience*.

Program Director/Principal Investigator (Last, First, Middle): Casey, Betty J

- Lee FS, Chao MV. Neurotrophic factors. In Kaplan and Sadock's, Comprehensive Textbook of Psychiatry, Ninth Edition. (eds. Sadock BJ, Sadock VA). (In press), Chapter.
- Magarinos, A.M., Li, C.J., Bath, K.G., Jing, D.Q., Lee, F.S., and B.S. McEwen (*under review*). Stress-induced hippocampal dendritic remodeling in mice: effect of a partial deficiency in brain-derived neurotrophic factor. *Journal of Neuroscience*.
- Yang J, Siao CJ, Nagappan G, Marinic T, Jing DQ, McGrath K, Chen ZY, Mark W, Tessarollo L, Lee FS, Lu B, Hempstead BL. Neuronal release of proBDNF. Nature Neurosci. 12:113-115, 2009.

F. Project Generated Resources

None.

Administrative and Data Management (ADM) Core

A. Specific Aims

The Administrative and Data Management Core (BJ Casey, Director) is the central node in the operations of The Center. In addition to centralizing critical administrative support functions for The Center, the ADM Core facilitates the scientific goals of The Center by managing the flow of data and processed results throughout The Center. This function of data management is particularly important in the proposed center because it involves a research model in which information from each project informs the scientific hypotheses and methodologies of all other projects. Specifically, the ADM Core is responsible for three major functions:

- 1) Administrative functions such as logistical activities, overseeing fiscal and reporting functions, planning regular meetings of The Center, developing and implementing The Center's educational initiative, and developing and hosting The Center's website;
- 2) Management of data from all projects to all cores; and
- 3) Management of biospecimens and processing of DNA for Projects I and II which involve human subjects to maximize the efficiency and integration of the operations of The Center, facilitate HIPAA compliance for human subjects, and ensure a double blind mechanism in the human behavioral measures.

B. Progress

B1. Administrative Functions.

Meetings/Committees. We have held two center meetings. The first meeting was informal and occurred in conjunction with annual summer institute on the biology of developmental disabilities that Casey (center director) has directed for nine years. Core PIs and investigators (Clark, Mezey, Glatt and Casey) and Project investigators and/or fellows (Amso, Bath, Lee, Casey, fellows of Gunnar and Thomas) and Center consultants (Glover, fellow of Fischl) presented and/or attended the meeting during the dates of June 22-27, 2008. The second formal meeting is scheduled for March 9, 2009 with our internal consultants and advisory board members. This meeting will provide the opportunity for the project and core investigators to report on their progress, discuss plans for upcoming studies, and share ideas of general relevance to the Center as well as get advice from our external advisory board and consultants to further strengthen the logistics, experimental design and analytic approaches of the Center.

We have developed an external advisory committee that includes Dr. Susan Bookheimer of UCLA for the genetic and pediatric imaging studies, Regina Sullivan of NYU for developmental and stress related rodent studies and Joe Ledoux of NYU for the translational rodent/human conditioning studies. These advisors are in addition to our external advisory board for DNA sharing that consists of Drs. Ed Cook of UIC, Dan Geschwind of UCLA and Matt State of Yale and our external consultants for the imaging component of The Center that include Drs. Bruce Fischl of Harvard, Gary Glover of Stanford and Steve Smith of Oxford.

Financial Functions. The ADM Core provides assistance with budgeting and monitoring expenditures, overseeing and authorizing purchases, distributing monthly expense and projection reports, rebudgeting, preparing invoices for subawards, assisting with financial reports, and assisting with the initiation of subcontracts to other institutions;

Administrative and Logistic Support to Projects and Cores. The ADM Core provides support for Center investigators including purchasing DNA and other lab supplies, arranging travel, assisting with publication needs, maintaining animal and human subjects protocol lists, handling subject payments, assisting with annual progress report preparation, oversight of research facilities including maintenance, supplies, repairs and safety, and regulatory compliance, and assisting with any other routine business as required.

Training Initiatives. The Center has been centrally involved in training initiatives via the ADM Core. Casey (PD and PI of ADM Core) is currently the Director of the Neuroscience Graduate Program and Co-Director of the Ithaca-Manhattan Graduate Initiative in Translational Developmental Neuroscience -IMAGINE) and part of the Tri-Institutional MD PhD Program as well as the founder and director of the annual Summer Institute on the Biology of Disabilities. The Center has taken a high profile role in each of these programs in addition to increasing roles in the training of medical students and residents through curriculum development and lectures and cross fostering of trainees across basic and human laboratories (e.g., Conor Liston, MD, PhD resident working with consultant, Bruce McEwen and Casey; Fatima Soliman MD, PhD candidate working with Francis Lee, coPI: Project III and Casey). All projects and cores were represented at the past summer institute and will be represented again this summer (see http://sacklerinstitute.org/cornell/summer_institute/).

Research Initiatives/ New Grants. At least six new grants have been submitted or awarded that have been linked to the Center involving junior investigators. An NINDS presidential award was awarded to Francis Lee (CoPI Project III) in December 2008, an NIMH R01 was submitted by co-investigator, Charles Glatt in March 2009, two K99/R00 applications by Drs. Jeremy Skipper and Leah Somerville of the department of psychiatry at WCMC were submitted November 2008 and March 2009, an NIH R01 by Joann Difede of the department of psychiatry at WCMC together with center co-investigators, Charles Glatt (Project I) and Francis Lee (Project III) was submitted in February and a Howard Hughes training grant application will be submitted in April 2009 that includes investigators and trainees at two center sites (Ithaca and Manhattan Cornell sites). All six grants/applications take a neurodevelopmental approach to psychiatric and developmental disabilities and rely on genetic and/or imaging infrastructure provided by The Center. In addition, a NIMH center grant was awarded to Megan Gunnar (PI Project II) this year that is directly related to project II of our center.

B.2. Data Management and Data Sharing

Data Management. A new server, Intel-based Apple Xserve, along with an Apple sold (Promise VTRAK) redundant enterprise grade mass storage system was purchased with grant funds to house the mass of grant data in a safe and secure manner. A license and renewal for the backup software NETVAULT by bakbone software helps with safe and reliable back-up of data. Finally accessories from APC for power surge/regulation units were purchased to avoid data loss.

Data Sharing. We have used the server to both push and pull subject data and project related programs and software. Specifically, distribution of behavioral tasks for the scanner and for the behavioral battery (programmed by investigators of Project I) were pushed to the Project II site using this IT infrastructure.

The genotyping facility in the ADM Core has implemented a pipeline for receipt, data basing, processing and analyzing DNA samples from projects I and II using this infrastructure as well. We have received 160 and 172 samples from the two projects, respectively, all of which have been successfully processed.

The ADM Core has developed an external Center website: <http://sacklerinstitute.org/cornell/CBGB/> which it hosts at WCMC. All publications resulting from the Center projects and cores are posted on this website as well as computer programs and relevant tools and assays (see http://sacklerinstitute.org/cornell/assays_and_tools/). In addition, an external advisory board for DNA sharing that consists of Drs. Ed Cook, Dan Geshwind and Matt State is formally in place. Once sufficient DNA samples have been accumulated a call for proposals will be announced on the website. Since DNA is a finite resource, the external advisory board for DNA sharing will determine the recipients based on evaluation of the significance and rigor of written proposal requests.

B. 3 Management of Biospecimens

DNA samples have been processed to extract genomic DNA from these human samples. All samples have provided high quality DNA for genotyping analysis on first or second extraction (~95% of samples provided high quality genomic DNA on first extraction).

All processed samples have been genotyped at BDNF Val66Met (rs6265) and data has been returned to investigators in projects I and II. We have achieved 97% success rate with our genotyping assay. All samples that fail or provide equivocal genotypes on first run are rerun. Samples that fail on the second run are re-extracted and genotyped again. Using this intensive genotyping strategy we have made confident genotype calls on all samples received.

The genotype results are presented in table 1. Genotypes for projects I and II are in Hardy-Weinberg equilibrium and allele frequencies in line with the expected distribution for the ethnic composition of the populations in projects I and II. To date, Met allele carriers (met allele heterozygotes and homozygotes) are 43 (27%) and 86 (50%) of project I and II enrollments, respectively.

Table 1.

Project I

	val/val	val/met	met/met	total
Percent	0.73	0.22	0.05	1
Number	117	35	8	160

Project II

	val/val	val/met	met/met	total
Percent	0.50	0.44	0.06	1
Number	86	75	11	172

C. Significance

The activities of the ADM Core serve the needs of all the projects and cores within the Center, implementing and supporting administrative, data management, data sharing, training, fiscal reporting functions central to the success of the Center.

D. Plans

The ADM Core will continue its current functions and optimize pipeline for imaging data from projects to be shared with Statistical Genetics Core. Bi-annual meetings will be formalized in the summer and spring of each year to provide the opportunity for the project and core investigators to report on their progress, train new fellows and investigators, discuss plans for upcoming studies, and share ideas of general relevance to the Center as well as get advice from the external advisory board.

E. Publications

Casey, BJ, Glatt, CE, Tottenham, N, Soliman, F, Bath, K, Amso, D, Altemus, M, Levita, L Jones, R, Thomas, KM, Gunnar, M, Mezey, J, Clark, A, Leon, AC, Hempstead, B, and Lee, FS. BDNF as a Model System for examining Gene by Environment Interactions across development. Neuroscience (in press)

F. Project Generated Resources

None.

Statistical Genetics Core

A. Specific Aims

The statistical core is responsible for genetic and imaging data analysis and is preparing to handle the data that will be collected in the coming year by establishing a data analysis pipeline and by developing graphical modeling techniques for extracting features that reflect interactions between BDNF and environment and/or developmental covariates. The first of these is critical for data handling and the second will be a crucial tool set for identifying the relationships between BDNF, neural pathways, and downstream phenotypes, and for determining how these relationships are affected by environmental factors such as stress and developmental stage. This work has involved two postdoctoral associates Arindam RoyChoudhury and Larsson Omberg, a statistical technician Yuxin Shi, as well as the PIs.

B. Studies and Results

fMRI Analysis Pipeline

Dr. Arindam RoyChoudhury has been spearheading the establishment of a pipeline for analysis of fMRI data in the context of the BDNF polymorphism. We have a design for the structure of the fMRI database, and a solid grasp of the normalization and registration issues for fMRI data. Among the many methods for analysis of fMRI data, many are unsuitable to our goal of contrasting BDNF (Sarty 2007). For example, univariate methods, which treat each voxel as an independent component and analyze them separately, fail to accommodate the spatial information from the position of the voxels. Consequently, we decided to look into other methods despite the simplicity of the univariate methods.

The multivariate methods of fMRI data analysis take into account the spatial information from the voxels. The multivariate method described by Bowman (2005) is particularly attractive and it is also well suited to the problem of contrasting fMRI patterns across genotypes. Bowman treats the intensities from the voxels with a smoothing process using a spatial autoregressive model. In this way the spatial information around a given voxel is also integrated into the model. After the initial data smoothing, we can treat each voxel as an independent regression. This method, inspired by Bowman can serve our purpose well. Because the method includes a regression step, we can jointly make inference of the population structure parameters as well as testing of homogeneity across BDNF genotypes.

We plan to treat the ethnic identities of the subjects as categorical independent variables. Any other known genetic and environmental factors relating to our phenotype will also be treated as independent variables. The phenotype at different point of development will be treated as longitudinal data. The model is flexible and will allow the incorporation of additional categorical variables as the group at the Sackler Institute proceed with the studies. We have started preparing a computational pipeline that is centered on the R package AnalyzeFMRI. We have created a R-based script that does the smoothing of the fMRI data for our purpose. The regression model has been designed and we have begun the generation of code for the regression analysis.

Graphical Modeling Approaches

Dr. Larsson Omberg has been spearheading the development of graphical modeling approaches for the identification of relationships between BDNF polymorphisms, neural activity, and downstream phenotypes that have conditional dependencies on environment and development covariates. The goal of developing this toolset is to model the conditional relationships among these levels and covariates and to discover potentially informative conditional relationships among spatial information extracted from fMRI. Intuitively, graphical models makes explicit the assumption that aspects such as neuronal activity in a region of the brain such as the hippocampus or amygdala is conditionally dependent on the allelic state at the BDNF locus. Similarly, downstream phenotypes such as contextual learning or learning/extinction may in turn be conditionally dependent on the brain activity states. The graphical modeling approaches being developed will allow us to both directly infer and model the importance of these various relationships. Once relevant relationships are identified, the graphical modeling approaches have a clear formalism for incorporating and testing the importance and interaction of these relationships with aspects of environment such as childhood stress and

stage of development. These techniques are an extension of linear modeling approaches that are likely to yield additional power for detecting such interactions, a central goal of the statistical genetics core.

Briefly, a graphical model has the following equation form (Mckeown et al 1998):

$$\mathbf{x} = \mathbf{Ax} + \mathbf{e} \quad (1)$$

where \mathbf{x} is a vector of variables which can represent the state of BDNF, neuronal activity, downstream phenotypes, the matrix \mathbf{A} reflects the structure of the conditional relationships among these variables and the \mathbf{e} is a vector of disturbances. With this structure, each of the \mathbf{x} can be represented as nodes of a graph where entries in the matrix \mathbf{A} determine the structure of directed edges that connect them. Environmental and developmental covariates and the effects of interactions are incorporated in this framework as variables in the vector \mathbf{x} , where there are directed edges from these covariates to other variables but not vice versa. Such graphs therefore model interpretable relationships among variables and can be used to discover relationships directly from data using inference approaches.

We are exploring the properties of two inference and discovery approaches that are designed for Gaussian (normal) disturbances and for non-normal disturbances. The first are methods associated with Structural Equation Models, the second uses the signal separation properties of Independent Component Analysis (ICA). We are exploring and developing extensions of both of these techniques. To date we have performed a simulation analysis for graphs of up to five components and have been adjusting aspects of our inference algorithms to improve performance based on these simulations. We plan to extend our simulation study to incorporate larger graphs and explore the properties directly for spatial fMRI data during this coming year. In particular, we plan to incorporate interaction effects in our simulations to assess the ability of our discovery algorithms to identify such effects at different levels of intensity. ICA approaches have been applied previously to fMRI data but our simulation work will be the first rigorous assessment of performance of this technique for relationship discovery for this data type. Conditional relationships among different spatial regions of the brain could also be discovered with our approaches and would be a natural application that we plan to explore during the coming year.

C. Significance

The activities of the Statistical Genetics Core serve the needs of the projects within the Center, implementing and supporting processing and analysis of neuroscientific datasets, and developing new methods in targeted areas that are central to the mission of the Center and essential for genetic studies.

D. Plans

We plan to perform simulations to quantify the power of these analytic approaches. These approaches will be examined and tested in consultation with Center imaging consultants (Drs. Gary Glover, Bruce Fischl and Steve Smith). Support and development activities will continue as outlined in the original proposal and above. One important focus will be the further development and dissemination of the analytic approaches that can be used widely both within the Center and the broader imaging genetic community.

E. Publications

Casey, BJ, Glatt, CE, Tottenham, N, Soliman, F, Bath, K, Amso, D, Altemus, M, Levita, L Jones, R, Thomas, KM, Gunnar, M, Mezey, J, Clark, A, Leon, AC, Hempstead, B, and Lee, FS. BDNF as a Model System for examining Gene by Environment Interactions across development. Neuroscience (in press)

F. Project Generated Resources

None.