Appendix B5c. Center for Brain Sciences and Health (CBSH).

Name of Center: Center for Brain Sciences and Health/Center for Behavioral Neuroscience

Center Director: H. Elliott Albers

A. General Information

1. When was the center created and to which department/college/office was it originally designated? The Center for Brain Science and Health (CBSH) was founded in 1992 by faculty in the Departments of Biology and Psychology in the College of Arts and Sciences. This Center became the administrative home of the Center for Behavioral Neuroscience (CBN) when it was funded in 1999. Its director, Elliott Albers, is a Regents professor of Biology and Psychology.

If the designation has changed, in which department/college/office does the center currently reside? The center resides under the Dean’s Office.

2. To whom does the center’s director report? The center’s director reports to the Dean of the College of Arts and Sciences.

3. If there is an advisory board to this center, describe its function and composition. The External Advisory Board of the Center for Behavioral Neuroscience is composed of a Chair and 12 additional members chosen by the director of the Center. The Board is composed of internationally recognized experts in behavioral neuroscience and science education from academia, government, and industry; selected to provide guidance and advice to the Center.

4. If the center is considered interdisciplinary, describe the interdisciplinary focus. The vision for the Center for Behavioral Neuroscience is that it become an internationally recognized center for research elucidating the brain mechanisms of social behavior, that it educate new generations of research scientists and students in innovative, interdisciplinary ways of investigating these mechanisms, and that it transmit the excitement of behavioral neuroscience to the general public. In doing so, the CBN will become a national resource for the field of behavioral neuroscience, contributing new knowledge, training a diverse student population, and bringing an appreciation of science to the public at large. The CBN involves collaborations across the disciplines of biology, psychology, anthropology, neurology, psychiatry, computational neuroscience, and biomedical engineering.

5. Describe in detail the amount of start-up support available. The CBN receives four million dollars per year (1999 – 2009) to run the center. In addition, the Georgia Research Alliance has provided 1.5 million plus per year for the first five years of the CBN and 1.7 millions for years 6-10 to be used for research infrastructure including start-up for new faculty. This money is used for new faculty.
recruits at all eight partner institutions (GSU, Emory, Ga. Tech, Clark-Atlanta University, Morehouse School of Medicine, Morehouse College, Spelman College, Morris Brown College). Start-up packages paid from the GRA money vary from institution to institution and from faculty to faculty depending upon their needs.

B. Goals and Objectives
1. Please enumerate the initial goals and objectives and describe the current goals and objectives if they have changed.

Our initial and continuing goals involve bringing together the unique resources from a consortium of Atlanta colleges and universities, backed by considerable state and federal support, to build a nationally recognized program that will (a) define the interaction of brain processes and complex behaviors, (b) create a cadre of interdisciplinary investigators focused on behavioral neuroscience, and (c) transfer relevant discoveries from the laboratory to the public. We have more recently developed more specific objectives towards these overarching goals.

The new objectives include:

Objective #1: Initiate a Center wide discussion of new research themes and approaches to using a combination of collaboratory meetings and meetings centered around new research themes.

Objective #2: Establish new approaches to facilitate collaborations between faculty who study non-traditional animal model systems and the technology cores.

Objective #3: Establish a forum for evaluation of the role of bioinformatics in the CBN; Explore partnerships with bioinformatics initiatives within the partner institutions and at NSF.

Objective #4: Have AUC faculty identify new ways to enhance their research programs by interacting with CBN.

Objective #5: Enhance the national exposure of the Center in behavioral neuroscience in scientific circles.

Objective #6: Increase overall student interest in studying behavioral neuroscience by providing experiential education opportunities for students at all levels, provide science curriculum enhancement at the pre-college and undergraduate levels, and conduct proactive recruitment activities at the high school, and undergraduate levels. We will continue with current educational initiatives and recruitment for these initiatives at the pre-college, undergraduate, graduate, and post-doctoral levels; continue teacher training and implementation of neuroscience in the pre-college curriculum.

Objective #7: Promote an awareness of alternative careers in or related to behavioral neuroscience beyond that of the professoriate by providing information to students at all levels about careers in or related to behavioral neuroscience such as science policy, journalism, biotechnology, pre-college teaching, and industry positions. We will provide career counseling and development as requested; aid students with guided career decision-making processes as requested; provide opportunities for extensive career exploration with an emphasis on experiential and action learning through seminars, workshops, information sessions and interviews, and panel discussions.

Objective #8: Promote behavioral neuroscience in K-12 curriculum in the local school systems by providing a number of programs and activities that target K-12 students and
teachers to educate them in behavioral neuroscience; develop and implement a plan for effective and efficient dissemination of curricular information. We will provide CBN sponsored K-12 teacher-training and workshops utilizing behavioral neuroscience material generated by CBN institutions and other sources; develop and provide behavioral neuroscience curricular materials that can be employed by Atlanta schools and other school systems nationwide; continue to work with the Georgia Internship for Teachers (GIFT); promote the Center’s Lending Library of Learning Resources among K-12 science teachers in the local school systems; continue to provide hands-on science activities to K-12 students through Center activities and programs such as the Neuroscience Exposition, Brain Camps and the ION program.

**Objective #9:** Broaden student exposure to and interest in behavioral neuroscience by providing exciting and informative experiences in behavioral neuroscience with information and activities in K-12 education for students and their families. We will continue development and facilitation of middle-school Brain Camps, BrainsRule and other Brain Awareness Month activities, the Atlanta Brain Bee, and the Institute of Neuroscience (ION) that targets high school students; continue development of exhibits and events at partner non-profit organizations, such as Zoo Atlanta and the Fernbank Museum of Natural History; continue professional development for K-12 teachers and utilization of behavioral neuroscience curriculum in the classroom.

**Objective #10:** Increase the percentage of female and minority faculty members, postdocs, graduate students and undergraduate students in the Center closer to the overall goals of 50% female and 20-30% minority.

**Objective #11:** Promote information about Center members and their research in public venues.

**Objective #12:** Increase the visibility of the CBN in the local community and nationally.

2. **What are the major institutional, administrative, and/or financial resources that facilitate achieving the center’s goals and objectives?**

The CBN has successfully leveraged institutional support from all eight of the partner institutions to promote the success of the center. Without support from the leadership, faculty and students at all of these institutions, the CBN would not have been able to accomplish many of the things it has over the six years of its existence. The funding for the center comes primarily from the National Science Foundation and from the Georgia Research Alliance. These funds pay for administrative staff and supplies, support for students and postdocs, support for the center’s many educational programs, start-up for faculty recruits, support for some of the center’s innovative research and most of the other needs of the CBN.

3. **What are the major institutional, administrative, and/or financial constraints that interfere with achieving the center's goals and objectives?**

The different missions of the partner institutions have occasionally produced challenges for the advancement of CBN objectives, but by making relationship building among the institutions a major focus of the CBN, these challenges have aided the development of stronger relationships among the CBN faculty and institutional leaders that will better serve the center and the participating institutions in the future.
4. What is your assessment of your achievement of your goals?
This assessment may be best adapted from the recent site evaluation report of the CBN written by a team of NSF and scientists from other institutions. The CBN is to be commended for many things. First and foremost, research, education and knowledge transfer have become extremely well-integrated. The CBN is an evolving model for real interactions among different labs across campuses that would not have collaborated in the past. The CBN has provided the structural network that typically does not exist and continues to focus on the needs of collaborations and to motivate the collaborations. Without the structural network that has been developed, it is expected that this community type of science would not have happened. The experience of the CBN can be used as a model/prototype of science that will hopefully extend to other institutions and to other levels of analysis, including biomedical and behavioral/ecological science. Finally, they have successfully extended science into other community institutions. The result of many of these positive features is that their national visibility continues to increase.

The CBN has continued to make progress in fulfilling their original mission. The members of CBN continue to become increasingly collaborative in their research. The many cross-laboratory and cross-institutional collaborations are an obvious strength of the Center. The CBN has extensive commitment to K-12 education through a wide and varied collection of programs. The CBN is committed to diversity as evident in its maintenance of the membership and communities served. The current population of graduate fellows (22% minorities and 74% females) is close to and above the national average and remains particularly impressive. The CBN has a very impressive level of commitment to, and implementation of, a variety of mechanisms for presenting concepts in behavioral neuroscience to the lay community. Partnering the basic science of the CBN with associations focused on applied aspects of neuroscience is good to help the public see the value of basic research.

C. Research of the Center
1. What research is currently being conducted in the center? Describe the major areas/topics of research. How has the center increased productivity of the faculty?
The CBN provides funding for a limited number of research projects through its venture grant program that is designed to promote innovative research in the field of behavioral neuroscience of social behaviors. In addition, the CBN promotes research related to certain social behaviors by sponsoring graduate scholars, postdoctoral fellows, undergraduate research opportunities, seminars, retreats, and workshops.

What follows is a brief discussion of select ed research projects from each of the four traditional collaboratories. It was our goal to give an overview of the variety of the research that has grown out of the CBN. Many, if not most, of these collaborations would not have happened had it not been for the CBN.

Affiliation Collaboratory
The **Affiliation Collaboratory** has made significant scientific progress in the past year on a wide variety of projects. There are a large number projects and collaborations
within the collaboratory involving faculty at Georgia State University, Emory and Spelman. The following highlight only some of the projects:

**Project 1: Social Recognition:** In 2004, Isadora Bielsky (CBN Scholar) and Larry Young demonstrated that vasopressin V1a receptor (V1aR) knockout mice displayed social amnesia, meaning that they had an inability to recognize conspecific mice to which they had previously been exposed. This paper, published in *Neuropsychopharmacology*, also demonstrated that this cognitive deficit was specific for social learning and memory since these same mice recognized nonsocial odors and performed normally in nonsocial memory tasks, such as the Morris Water Maze. In a follow-up study, Isadora Bielsky used a combination of pharmacology and viral vector gene replacement approaches to identify the lateral septum as the population of V1aR mediating social recognition. V1aR antagonist infused into the lateral septum of wildtype mice blocked social recognition, while re-expressing the V1aR in the lateral septum of knockout mice completely restored social recognition abilities. These data are published in the August issue of *Neuron*.

We had previously shown that oxytocin knockout mice also displayed social amnesia. In a study now in press at the *Proceedings of the National Academy of Sciences*, we confirm the role of the oxytocin system in social recognition by demonstrating that oxytocin receptor (OTR) knockout mice also display social amnesia. More interestingly, these mice also show defects in maternal nurturing. Both post-partum and virgin OTR knockout mice display severe deficits in maternal behavior. Additionally, male mutants display elevated levels of aggression. Finally, OTR knockout pups display fewer ultrasonic vocalizations in response to social isolation. Together, this study, which is an international collaboration between Katsuhiko Nishimori (Japan), Heather Ross (CBN Scholar), Isadora Bielsky (CBN Scholar) and Larry Young, demonstrates that disruption of the oxytocin receptor system results in pervasive social deficits.

**Project 2: Social Attachment:** In 2004, several papers published in *Journal of Comparative Neurology, Neuroscience*, and *Nature* as part of the thesis work of Miranda Lim (CBN Scholar, PhD 2004), greatly enhanced our understanding of the neurobiological mechanisms underlying pair bonding in male prairie voles. This year, Jose Morales (a CBN post-doc) completed a microdialysis study examining the release of vasopressin in the ventral pallidum of male prairie voles during pair bond formation. His results, which were presented at the 2004 Soc. for Neuroscience meeting, demonstrate that vasopressin is released in the male prairie vole ventral pallidum with ejaculation. More recently, Dr. Morales has demonstrated using microdialysis that extracellular glutamate increases concomitantly with vasopressin and that infusion of a vasopressin receptor antagonist, which blocks pair bonding, also blocks this increase in glutamate. This study suggests that an interaction of vasopressin and glutamate in the ventral pallidum may result in pair bond formation. This data is currently being written up and will be submitted J. Neuroscience. Based on this paradigm, Charlene Cole (minority CBN Scholar) has begun similar experiments examining the interactions of oxytocin and glutamate in the nucleus accumbens in the female prairie vole.

**Project 3: Individual Variation in Social Behavior.** An exciting new direction in the Affiliation Collaboratory is the investigation of the neurobiological mechanisms
underlying individual variation in social behavior. In a 2004 paper published in *Molecular Biol. Evolution*, Elizabeth Hammock (CBN Scholar, PhD 2005) demonstrated that a polymorphic microsatellite DNA sequence in the promoter of the vole vasopressin receptor (V1aR) regulates gene expression in a cell-type specific manner and suggested that species differences in this microsatellite might be responsible for species differences in social behavior between the monogamous prairie vole and the non-monogamous meadow vole. Dr. Hammock then hypothesized that natural variation in the length of this microsatellite might be responsible for individual variation in receptor expression in the brain and social behavior within the prairie vole species. To test this hypothesis, Dr. Hammock used a breeding paradigm to create lines of voles with either longer than average microsatellites, or shorter than average microsatellites. She found that male prairie voles with longer microsatellites display higher levels of paternal care, were more interested in social olfactory stimuli, and were more likely to form pair bonds than males with shorter microsatellites. The strains also displayed different levels of V1aR binding in the brain. This study, published in *Science*, demonstrates that instability of microsatellite sequences in the promoters of genes that regulate behavior create diversity in social behavioral phenotypes, which can be acted upon by natural selection. A collaborative venture grant between Larry Young and Zoo Atlanta was recently awarded to compare microsatellite structure in the V1aR of several primate species that differ in their social structure.

A second project examines individual differences in the need for social buffering in rats. This project represents a collaboration between the Affiliation Collaboratory (Larry Young) and the Fear Collaboratory (Mike Davis) and was conducted by a CBN post-doctoral fellow (Hemanth Nair). In this study, recently accepted for publication in the *Journal of Neuroscience*, Dr. Nair used a “discovery-based” approach to identify neuropeptide receptor distribution patterns that predict individual differences in social isolation-induced potentiation of startle (IPS) in rats. This approach, based in multivariate statistics, provided predictions as to the brain regions where oxytocin (OTR), vasopressin (V1aR), or corticotropin releasing factor (CRF1 and CRF2) receptor binding contribute to IPS. Dr. Nair then directly tested one of the predictions – that CRF1 receptors in the Nucleus Accumbens are in part mediating IPS – by directly infusing CRF into the region. As predicted, CRF facilitated the IPS effect. Hence, the collaboratory successfully developed a novel, discovery-based approach to identify neuropeptide receptor binding patterns that predict a complex, affiliative behavior and then successfully tested the prediction using conventional pharmacology. This study provides both insight into the neuropeptide basis of socially-related psychiatric disorders and a novel approach to study their neurophysiological basis.

**Project 4: Imaging the Social Brain:**

Two brain-imaging studies were funded in the last year though the venture grant mechanism. Both projects are designed to develop technologies to better image human and primate brains. Dr. Lisa Parr’s project is designed to ultimately image brain activation in chimpanzees and monkeys while they perform face-processing tasks. Chimpanzee brain scanning using PET was started in earnest in 2004 using 18F-FDG. Dr. Parr has currently completed scanning with three chimps under 2 conditions: a face matching task and an object (clip art) matching task. These scans have now been
coregistered to an individual, anatomical MRI from each subject (performed at the new Yerkes Neuroscience Facility, opened spring 2005) for localization of brain regions. Analyses are currently underway. Monkey imaging has been awaiting training of subjects on face matching tasks. This past year, 5 monkeys have reached criterion on an object matching to sample task and additionally, one of these subjects has reached criterion on a face-matching task. Monkey scanning is pending the completion of the Yerkes Neuroimaging Facility. This venture grant helped to develop techniques for functional neuroimaging that led to Dr. Parr receiving an R01 entitled “Neuropsychology of primate social cognition” from NIMH in 2004. It also aided in the development of a large Program Project Grant with other investigators from Yerkes, Emory University, and Georgia Tech entitled “Aging and dementia in female primates.” This grant has been scored and is currently under its first review.

A second project led by Drs. James Rilling and Todd Preuss is designed to develop Diffusion Tensor Imaging (DTI) techniques that could be used to compare monkey, chimpanzee and human fiber tracts between brain regions involved in social processes. To date, the human scans are of high quality, however chimpanzee scans suffer from considerable distortion that may be due to subject movement under anesthesia. The CBN Imaging Core staff has developed a segmented DTI sequence designed to solve this problem, but the resulting images have been noisy. The Imaging personnel are currently trouble-shooting the sequence. Nevertheless, the images are of sufficient quality to conduct some preliminary analyses that were presented at both the Physical Anthropology meetings in April and the Organization for Human Brain Mapping meetings in June. At these meetings, Drs. Rilling and Preuss received several suggestions for data acquisition and analyses that they are currently pursuing.

Simultaneous with their in vivo scanning, Rilling and Preuss have resumed scanning of post-mortem brains with the new imaging center at Yerkes. The Yerkes imaging physicists have produced a protocol that generates high quality DTI images from post-mortem brains. Rilling and Preuss have been using tractography programs to dissect the fiber tract connecting Wernicke’s and Broca’s language areas (the arcuate fasciculus) and compare it in chimpanzees and humans. If there are specializations of the human brain beyond its overall size, they expect to find them in circuits like these that support human social cognitive specializations. The data resulting from this venture grant have been included in two grant applications to NIH, one of which is pending and a second that was declined on its first submission.

**Project 5: (Venture Grant) Measuring attachment security in rhesus monkeys**

The main goal of this project, which is a collaboration between Mar Sanchez (Affiliation) and Kim Wallen (Reproduction), is to develop a methodology that bridges the research on mother-infant relationships in humans and non-human primates. Specifically, they aim to adapt a widely used method of assessing attachment security in human children, the Attachment Q-Sort, for use with captive rhesus monkey mother-infant pairs. Attachment security measures the balance between exploration and comfort-seeking behavior in infants/children and is associated with developmental outcomes. A second aim is to determine whether measures of attachment security from the non-human primate version of the Attachment Q-Sort (AQS) are associated with behavioral, socio-emotional and neurobiological development. The human Attachment Q-sort (AQS) has
been previously adapted for use with Japanese macaques (Kondo-Ikemura and Waters 1995), and the authors reviewed the appropriateness of this instrument for measuring attachment security in infants of that species, supporting the instrument’s content validity. The main goal of this study was to assess its discriminant and predictive validity in rhesus macaque infants at the Yerkes National Primate Research Center, through funding received from a CBN venture grant.

Dr. Sanchez, working with Dr. Warfield, has generated a “Yerkes infant attachment Q-set”, consisting of 95 items, and its corresponding criterion sort. The primate coders (blind to attachment theory) have finished training with Dr. Warfield, reached reliability, and are currently coding the experimental tapes. Their current Q-security scores correlations with Dr. Warfield’s range from 0.7-0.88. Throughout the process of achieving reliability, they have already selected several “training tapes” that will be provided together with this instrument to the primatology community for use to standardize this type of training (one of the aims within this venture grant). The long-term aim is to analyze whether infant abuse or maternal separation (current models of early adverse care in rhesus monkeys) affect attachment scores in infant rhesus monkeys and whether attachment security early in life is associated with the infants’ socioemotional development and functioning of the stress-emotion system. Preliminary results from studies funded by this CBN venture grant and based on a random sample of tapes scored by Dr. Warfield in the 0-6 months age block show that abused infants had lower attachment security scores (0.19) than controls (0.39) towards their mothers. This work has led to a new collaboration between Dr. Sanchez and Dr. Kathy Stansbury (CBN faculty at Morehouse College) to compare the results found in monkeys with a human cohort.

**Aggression Collaboratory**

The research of the **Aggression Collaboratory** is focused on the neural and behavioral mechanisms that underlie the perception and formation of dominance hierarchies and the subsequent effects of social status on the structure and function of the central nervous system. The research is strongly comparative, using six disparate animal models: monkeys, Syrian hamsters, rats, cichlid fish, crayfish and termites. All six species use aggression to garner resources during initial interactions with conspecifics, and all form dominance hierarchies based on the outcome of initial agonistic interactions. These similar patterns of behavior occur despite extensive differences in the animals’ brains, behavior, and ecological niches. This led us to ask whether these behaviors are subserved by a common set of physiological mechanisms. We are using these models to address the following: (a) what are the key behaviors associated with dominance hierarchy formation and maintenance?; (b) what are the brain areas and circuits that are involved?; (c) what are the neurochemical signals that are released or that gate these behaviors?; (d) what triggers their release, what is the pattern of release, and how does that pattern change?; (e) what changes occur in the effect of these chemicals on their targets in response to a change in social status?; (f) how do changes in the receptors or their pattern of expression account for the change in a neurochemical effect or in social behavior?; and (g) how do social species learn about dominance hierarchies? It is important to note that we have focused on both the dominant and the submissive behaviors emitted by organisms in social conflict situations; thus, we are investigating a
wide range of agonistic behaviors. Significant progress has been made in these areas in the past year.

**Project 1. Monkeys:** These projects are new initiatives that are part of our cross-collaboratory emphasis. Two venture grants have been funded (PIs: Bachevalier; Hampton) that involve both the Aggression and Reproduction Collaboratories.

The Bachevalier project will examine, among other things, agonistic behavior of monkeys receiving early amygdala lesions. Early medial temporal dysfunction is associated with many developmental mental disorders (schizophrenia, autism, Williams syndrome) that have significant impact on the normal cognitive development of a young individual that spans the entire life. Because little is known about the long-term behavioral and cognitive consequences of early damage to specific structures within this brain region, specifically when the animals are raised in a naturalistic environment, the specific aims of this venture grant are to prepare 10-12 day old male monkeys with bilateral neurotoxic amygdala lesions, place them together with their mothers in a large semi-naturalistic social group at the Yerkes field station, and follow in great detail the emergence of species-specific behaviors during the first year of life relating to affiliation, social status and fearfulness, the appearance of sexually dimorphic behaviors, and the maturation of cognitive function.

The Hampton project will develop a novel, ethologically-grounded test of nonhuman primate social cognition. Monkeys will be trained to select the dominant individual from a pair of monkeys displayed in video clips on a touch screen. Monkeys will then be tested for the ability to deduce a linear dominance hierarchy based on several such pairs. Mark Wilson (Reproduction Collaboratory) will collaborate by arranging social interactions for filming. Andrew Fischer (Behavioral Technology Core) will provide programming and engineering expertise for production of automated computerized testing equipment. This project is designed to generate pilot data to support a grant proposal to study the neural basis of social cognition using imaging, lesion, and single unit recording techniques.

**Project 2. Hamsters:**

Dominance relationships among hamsters are formed as the result of social conflict and are maintained, at least in part, by communication. This project will continue to define the circuitry for aggression and flank marking. Collaborative experiments between Dr. Albers, Dr. Moore and Dr. Huhman will determine the precise roles of glutamate, AVP, OT and 5-HT and their receptors as they affect these behaviors. Several publications examining AVP and OT receptors in dominant and subordinate hamsters have been submitted this year. These projects have involved undergraduate students, CBN scholars and CBN postdoctoral fellows. These projects will also offer an opportunity to increase student involvement in behavioral neuroscience at CAU.

The Huhman lab, in collaboration with several other CBN labs (Davis, Young, Albers), continues to explore the neurobiology of submission in hamsters, an aspect of agonistic behavior that has been largely overlooked. We have demonstrated that the long term increase in submissive behavior that is observed in hamsters following social defeat (i.e., conditioned defeat) is mediated by glutamatergic neurotransmission, and inhibited by GABA neurotransmission, in the amygdala. New data indicate that the plasticity underlying this change in behavior is mediated by NMDA receptors in the basolateral
amygdala that contain the NR2B subunit. We have also shown that overexpression of CREB or blockade of neurotrophin receptors in the basolateral amygdala augments or reduces conditioned defeat, respectively. We have recently obtained data that suggest that conditioned defeat is not dependent on the hippocampus. Finally, we have continued to examine sex differences in behavioral responses to social defeat. Females are less likely than are males to respond to social defeat with a long-lasting change in aggression. We have demonstrated that the behavioral response to defeat differs in females across the estrous cycle and we are currently examining the role of gonadal hormones in the formation of conditioned defeat in male and female hamsters.

**Project 2. Rats:**

The project on rat dominance and aggression arose from discussions within the collaboratory as to whether the neural mechanisms of aggression and dominance signaling in rats are similar to those in hamsters. Rats are highly social and respond aggressively to conspecifics much less reliably than do hamsters. The initial challenge was to identify conditions in which rats reliably establish persistent dominance relationships. The laboratory at Morris Brown College has worked to establish a variation of the Visible Burrow System wherein they can observe the formation of dominant:subordinate relationships in rats competing for food. They have been able to establish this model, but have found that it was extremely labor-intensive to review videotapes and score which animals were accessing the food port. With the help of the Behavioral Core technician and Rob Poh, the CBN information technology director, Morris Brown has been able to automate their system with a MiniRraKer microchip ID system from AVID Inc. in order to determine dominance. This group also has one paper in press examining V1a receptor binding in rats following food competition, as well as other papers submitted or in preparation. A number of undergraduate students from Spelman College, Morehouse College and Clark Atlanta University have been involved in this project, as was one CBN postdoctoral fellow, Dr. Alicia Askew (MB, PD) who this year moved on to a tenure-track faculty position at Presbyterian College (S.C.).

**Project 3. Fish:**

This research theme focuses on changes in behavior, physiology, and neuroendocrine systems in response to social stressors, particularly dominance and subordination, in fishes (convict cichlids: *Archocentrus nigrofasciatus*; swordtails: *Xiphophorus helleri*; gobies: *Lythrypnus dalli*). A CBN Venture Grant awarded to Drs. Lawrence Blumer (Morehouse College) and Matthew Grober (Georgia State University) in 2002 facilitated the first aim of this project – to identify robust physiological markers of social stress. In last year’s annual report, we described gallbladder hypertrophy in response to chronic subordination in convict cichlids, the first study to identify social stress as a diagnostic criterion for gallbladder dysfunction in any vertebrate. We have been pleased to see that this work on fishes has been recognized, and has been the subject of editorials, in international medical journals (e.g., *Medicina*). In addition to the gallbladder work, Dr. Grober and Dr. Ryan Earley (CBN postdoctoral fellow) have explored differences between dominant and subordinate animals at multiple levels within the hypothalamic-pituitary-interrenal (adrenal) axis. After a considerable period of cohabitation, dominant and subordinate animals showed no difference in cortisol
concentrations or magnocellular/parvocellular corticotropin-releasing-factor (CRF)-ir cell number or density. In collaboration with Dr. Kim Huhman and Alicia Faruzzi (CBN scholar), however, we have identified qualitative status differences in CRF receptor 1 and receptor 2 densities in the forebrain preoptic area of these fishes; quantification of autoradiographic films and emulsion-dipped slides is now under way. We also have examined the acute cortisol response following fighting experience in winners and losers of two species of fish (*A. nigrofasciatum* and *X. helleri*). Results indicate that variation in the cortisol response of cichlids immediately following an aggressive encounter was predicted more by contest intensity than by outcome, but the opposite held true for swordtails. We are now pursuing potential causes for interspecific differences in the peripheral neuroendocrine response to fighting experience (e.g., social environment, interaction frequency). This research on the convict cichlids was the foundation for Dr. Earley’s NIH National Research Service Award application, which was funded in June 2004.

Dr. Earley’s research under the NRSA has focused primarily on the role of the neuroendocrine stress axis in mediating reproductive allocation in a bi-directional sex changing goby. The first wave of experiments has unveiled status and sex differences in basal cortisol levels, with males showing low cortisol concentrations relative to dominant and subordinate females. We are now in the process of analyzing brain tissue to ascertain differences in CRF immunoreactivity, CRF receptor 1 and 2 binding, and CRF mRNA expression patterns between the sexes, and plan to conduct manipulative studies (using metyrapone and cortisol) to investigate whether the HPA axis affects the “decision” to change sex. In the spirit of the cichlid studies, we also have extracted a myriad of tissues that might respond to social stress, including the gallbladder, spleen, gills, liver, muscle, and gonad in attempts to understand the manifold physiological effects (e.g., osmotic competence, liver glycogen content, muscle lactate) of occupying dominant or subordinate social statuses.

A second research theme is to examine whether central components of the stress axis (i.e. CRF cell size/density and mRNA expression) respond in the same way to domination by conspecifics versus heterospecifics. This project involved a CBN venture grant collaboration between Dr. Grober and Drs. Gary Grossmann (University of Georgia) and Michael Wagner (Michigan State University) who have studied two species of fish (yellowfin shiner: *Notropis lutipinnis*; rosyside dace: *Clinostomus funduloides*) that cohabitate and exhibit overlapping microhabitat preferences in the Little Tennessee River of western North Carolina. Yellowfin shiners are an introduced species and are significantly more aggressive than the native (and threatened) rosyside dace. Thus, this project also has implications for conservation biology: assessing the physiological effects of social stressors imposed by aggressive yellowfin shiners on the rosyside dace and subsequent consequences for the livelihood of the dace. In the 2004 annual report, this project was still in its infancy. The investigators developed a successful protocol for immunohistochemical localization of CRF-ir cells in the forebrain preoptic area of both species, and are in the process of obtaining all tissues from the experimental fish from Dr. Wagner. In the past year, an undergraduate student has sectioned approximately 120 brains (of the 218 total). In addition, an oligoprobe was developed to detect species differences in CRF mRNA expression patterns. The laboratory was prepared for *in situ* hybridization protocols. Given the large volume of brain tissue that still needs to be sectioned, we are
unable to report on the results at this time. Once the sectioning is completed (within six months), the project will proceed with immunohistochemical and in situ hybridization for quantification of differences in forebrain preoptic CRF-ir cell size/density and CRF mRNA expression. We anticipate that the results of this study will underscore the importance of integrating behavioral ecology, conservation biology, and neuroendocrinology.

**Project 4. Arthropods - Crayfish:**

Although the brain structures of crayfish differ considerably from those of rodents, the behaviors observed during the formation of dominance hierarchies, as well as some of the neurochemicals involved, are similar. Moreover, the prominence of identified neurons and well-studied circuits in producing relevant behavior patterns facilitates analysis of the neural mechanisms that mediate changes in aggression and dominance. One aim of this project is to identify crustacean 5-HT receptors and to determine how patterns of receptor expression change with social status. Drs. Don Edwards and Charles Derby and postdocs and students from their laboratories have identified two putative crustacean 5-HT receptors, characterized them pharmacologically in heterologous expression systems, and measured changes in the levels of expression of one of them from the whole crayfish CNS following changes in social status. Levels of expression in dominant animals were greater than and significantly more variable than levels in subordinates (Spitzer et al., 2005). In addition, as in rodents, the effects of dominance status on neurogenesis and olfactory learning are being studied in crustaceans. Social interactions reduced proliferation of olfactory projection interneurons in crayfish, and increased the survival of olfactory local interneurons in social dominants and decreased it in social subordinates (Song et al., 2005).

Social status affects a variety of behavior patterns in crayfish, including burrowing (Herberholz et al., 2003), predator/prey interactions (Herberholz and Edwards, unpublished), and sexual behavior (Issa and Edwards). Social subordinates avoid burrowing near dominants and avoid approaching a crayfish prey taken by a dragonfly nymph predator. In contrast, dominant crayfish display no reluctance to burrow and will actively attack and try to eat a crayfish prey seized by a dragonfly nymph. Some subordinate male crayfish also display female sexual behavior in interactions with dominant males, which display male copulatory behaviour. Seen in about half of male dominant/subordinate pairs, this behavior correlates with significantly reduced levels of aggressive interaction compared to pairs that do not display pseudo copulation. Moreover, subordinate members of pairs that display pseudocopulation survive indefinitely as members of the pair, whereas subordinates that do not display the behavior are killed within 24 hr by their dominant partners.

Octopamine has opposing effects on the aggressive behaviors of insects and crustaceans. Furthermore, the effects of octopamine vary with social experience. Edwards and colleagues have recently hypothesized that state- and species-specific effects may be due to differences in the distribution of highly conserved octopamine receptors. To begin to test this hypothesis they are generating antibodies that recognize a known arthropod octopamine receptor in all arthropod species currently used by the members of the aggression collaboratory (termite, crayfish, lobster, prawn). The antibodies will be used to define receptor distribution in dominants and subordinates across species. To date, Dr. Deb Baro has isolated RNA and produced cDNA from
crustaceans (lobster and crayfish) and is currently using this cDNA in degenerate PCR experiments to clone a fragment of the octopamine gene.

One venture grant was funded to use manganese-enhanced magnetic resonance imaging (MEMRI) to identify patterns of status-related neural activity in the brains of crayfish. This project was very successful in adapting MEMRI, a technique previously used only in vertebrates, for imaging of an aquatic invertebrate. Results from this project were published our results in the December 2004 issue of the Journal of Experimental Biology. Efforts to image neural activity in the brain of live animals were hampered by the maximum spatial resolution obtainable with the equipment available. The relative low field strength provided by the scanner proved to be insufficient for fast acquisition of high-resolution images. Thus, the Biology Department at GSU decided to upgrade the current NMR with a micro-imaging supplement that allows imaging at much higher temporal and spatial resolution. Initial results are promising in the tests with the new equipment. To date, the project has produced one peer-reviewed publication and several conference contributions. A collaborative grant proposal to the NSF or NIH by Herberholz (University of Maryland) and Edwards (GSU) is planned for the near future.

**Project 5. Termites:**

Termites are eusocial insects and one of the few species that engage in organized warfare. Despite this, aggressive behavior is poorly understood in termites and nothing is known of its neural bases. The Aggression collaboratory has provided a unique opportunity to correlate behavioral changes with neurochemical and neuroanatomical changes produced by the social interaction of competing termite populations, work that would never have been pursued without the influence of the CBN (Jackson- Morehouse College; Edwards – GSU).

The work this year has focused on the importance of group size vs. fighting abilities as they effect dominance between groups of termites. This work has indicated that group size is the best predictor of dominance when groups of termites are paired. Dr. Jackson, with the help of several undergraduate students from Morehouse College, has also observed that groups of termites that live close to one another are less likely to exhibit high levels of aggression towards one another than are groups that live further apart geographically. They hypothesized that these termites might be more closely related to one another genetically and that a form of kin recognition might play a role. Further tests of this hypothesis have failed to support this hypothesis.

Numerous students at Morehouse College have been trained by GSU faculty and postdoctoral associates to remove termite brains and prepare them for serotonin immunohistochemistry. In the future, the plan is to pair small groups of termites drawn from different colonies in three-chambered arenas where the middle arena contains food and the other two chambers house the two groups. Fighting should lead to one of the two groups claiming and controlling the central chamber. HPLC and immunohistochemistry would then be used to measure levels of CNS monoamines and their distribution in the winning and losing animals immediately after the deciding contest and at intervals thereafter over weeks. Controls would consist of paired groups of animals drawn from the same population.

**Fear Collaboratory**
A large number of projects have been completed or are ongoing in the Fear Collaboratory. This collaboratory continues to be supported by a large number of venture grants that support collaborations among CBN faculty and labs.

**Project 1:** Neural Mechanisms of Extinction of Conditioned fear. PIs: Michael Davis, Kerry Ressler, David Walker

**a. Rescue of inhibitory deficits in GAD 65 knockout mice.** Recent postmortem studies in humans suggest that defects in GABAergic neurotransmission might contribute to the neuropathology associated with schizophrenia. We recently reported that mice that lack the GABA synthesizing enzyme glutamic acid decarboxylase 65 (GAD 65) knockout (KO) mice showed robust deficits in prepulse inhibition (PPI) which were reversed by the atypical antipsychotic agent clozapine. Furthermore, these mutant mice show deficits in the expression of conditioned fear. These results lend support to the view that abnormalities in GABAergic systems might contribute to the basic pathophysiological mechanisms in schizophrenia and disorders related to fear and anxiety. Currently, we are examining whether PPI deficits displayed by GAD65 KO can be alleviated by microinjection of a GAD65-expressing lentivirus into various brain regions that we have identified as lacking normal levels of GAD65. In vitro anti-GAD65 antibody analyses indicate that our lentivirus vector express the desired GAD65 protein. We will soon be examining whether our lentivirus successfully expresses GAD65 in vivo and examine the relationship among GAD65 rescue, PPI, and fear expression in mutant mice. In addition we are currently examining the development patterns of PPI and fear deficits in GAD65 KO mice. Initial results indicate that in addition to KO mice, heterozygous mice also show a deficit in PPI during the first few weeks of birth. However by 2 months of age, heterozygous animals no longer show abnormal levels of PPI. Along with recent evidence indicating younger, but not older, heterozygous mice express low levels of GAD65, these data further support a role of GAD65 in the PPI deficits seen in null mutants.

**b. Expression mRNAs encoding various GABA-related genes.** There are converging lines of evidence suggesting that changes in GABAergic transmission may also be involved in the control of aversive memories as highlighted by the fact that patients suffering from anxiety disorders are commonly treated by the administration of benzodiazepines which mediate their actions via GABA(A) receptors. We hypothesize that the acquisition, retrieval, and extinction of fear are associated with dynamic changes in GABAergic function that are required for the normal molecular processes involved in the production and reduction of fear. To test this hypothesis we are currently involved in a number of studies. First, we are identifying and quantifying the expression pattern of mRNAs encoding various GABA-related genes within limbic structures that have been previously established to be involved in acquisition and production of fear and anxiety. Currently, ten in situ hybridization probes have been cloned and successfully used to label and map the distribution of various GABA receptor subtypes and associated proteins. Also, these probes are currently being used to examine training-induced changes in the expression of GABA-related genes and examining changes in GABA(A) binding patterns in the amygdala and hippocampus after the acquisition, retrieval, and extinction of Pavlovian fear. As of this date, we have found significant changes in a1-GABA(A) receptors, and gephyrin, GAD65, GABA transporter, and GABA RAP expression levels associated with the acquisition or extinction of fear. We are also examining the function...
of amygdala a1-GABA(A) receptors in the acquisition, retrieval, and extinction of conditioned fear in a1-GABA(A) inducible knockout mice using the Cre-loxP system. In these mice we have successfully demonstrated a knockdown of a1-GABA(A) receptor expression after microinjections of a CRE lentivirus. Initial behavioral studies indicate that after knockdown of a1-GABA(A) receptors, mice show a significant increase in anxiety as measured in the elevated plus maze and an increase in motor activity as measured in the open field exploratory test.

c. Differential mechanisms of extinction of conditioned fear dependent on length of time since acquisition. Fear extinction is defined as a decline in conditioned fear responses (CRs) following nonreinforced exposure to the feared conditioned stimulus (CS). Behavioral evidence indicates that extinction is an inhibitory learning process: extinguished fear CRs reappear with the passage of time (spontaneous recovery), a shift of context (renewal), and unsignaled presentations of the unconditioned stimulus (reinstatement). However, there also is evidence to suggest that extinction is "unlearning." Certain studies have documented a recruitment of phosphatases and suppression of fear-related gene expression in animals exposed to nonreinforced CS presentations when extinction is carried out soon after fear conditioning. Perhaps the mechanism of extinction varies with the interval at which extinction training is initiated, such that extinction initiated relatively soon after acquisition disrupts consolidation of the fear memory trace whereas extinction initiated at longer intervals engages secondary plasticity. We examined this issue by comparing groups of rats extinguished 10 min, 1 hr, 24 hrs, or 72 hrs following acquisition on their susceptibility to reinstatement, renewal, and spontaneous recovery of conditioned fear. Additional experiments examined the involvement of the protein phosphatase calcineurin in short and long interval extinction. Rats extinguished 72 hrs after acquisition exhibited reinstatement, renewal, and spontaneous recovery reliably while rats extinguished 10 min after acquisition exhibited none of them. Rats extinguished at 1 hr and 24 hr were intermediate. These findings suggest that extinction initiated shortly following acquisition may result from an erasure of fear whereas extinction initiated at longer intervals may be mediated by a parallel inhibitory memory trace. Calcineurin protein (measured via Western blot) increased in the BLA following short interval but not long interval extinction. Calcineurin mRNA (measured via in situ hybridization) in the BLA did not differ among short and long interval extinction groups and comparison acquisition-only groups. This suggests a specific upregulation of calcineurin activity in the amygdala following short interval extinction, mediated through a translational but not a transcriptional mechanism, and may be consistent with the hypothesis that short interval extinction occurs via an erasure mechanism. Several small pilot experiments examined the effect of administering calcineurin inhibitors (cypermethrin, FK-506, and cyclosporin A) prior to short interval extinction. Thus far problems with toxicity (targeted administration) and dosing (systemic administration, i.p. and s.c.) have prevented any conclusions from being drawn. Currently in progress is a pilot experiment examining the feasibility of i.v. administration of cyclosporin A prior to short interval extinction. The prediction is that short interval extinction but not long interval extinction will be impaired by pre-extinction training inhibition of calcineurin.

d. Role of neuropeptide Y (NPY) in extinction and expression of conditioned fear. Previous studies have shown that NPY inhibits both baseline startle and the expression of
fear-potentiated startle (FPS). More recent work has evaluated the role of NPY in the extinction of conditioned fear. We see an increased rate of within-session extinction of FPS in animals that have received i.c.v. NPY one hour prior to the test session. This effect was corroborated by another experiment in which animals were given extinction training (30 light-alone presentations followed by a test including 15 light-tone and 15 tone-alone trials). During the test immediately following extinction with drug onboard, a similar pattern of enhanced within-session extinction was observed for the NPY group as in the previous experiment. In addition to this within-session effect, we have observed an enhanced retention of extinction of contextual fear conditioning with administration of NPY. This effect was observed as a reduction in startle amplitude during tone-alone trials 48 hours after administration of either NPY or vehicle during an extinction training session. Further studies will seek to disentangle the contextual and cued components of fear conditioning to better understand how NPY is involved in extinction.

c. The role of brain derived neurotrophic factor in the extinction of conditioned fear. Brain-derived neurotrophic factor (BDNF) and its receptor, TrkB, have been implicated as molecular mediators of synaptic plasticity underlying learning and memory. Previously, we reported that BDNF plays a role in amygdala dependent learning and memory, specifically in the acquisition of conditioned fear. Here we sought to address whether BDNF was involved in another form of amygdala dependent plasticity known as extinction. Although much is known about the neural basis of excitatory fear conditioning, we are just beginning to explore the molecular mechanisms underlying extinction, and it is not yet known whether BDNF is involved in this form of plasticity. In this study we examined the expression of BDNF mRNA in the basolateral amygdala (BLA) at various times following extinction using in situ hybridization. We found BDNF mRNA levels rise in the BLA 2 hrs following extinction training (90 lights presented in the absence of shock). In order to examine the specific role of BDNF signaling during extinction, we used a lentiviral vector that expressed TrkB.T1, a dominant negative TrkB receptor, to impair BDNF signaling in the amygdala. Rats were fear conditioned using light-shock pairings to establish an aversive memory to the light. 72 hrs later rats received bilateral intra-amygdala infusions of the TrkB.T1 or the GFP lenti-virus. Animals are currently being given 7 days to recover and allow for optimal infection of the virus. We will then extinction train the animals and test for the presence of fear-potentiated startle. If BDNF signaling is indeed required for the plasticity underlying extinction than rats receiving the TrkB.T1 virus should show impaired extinction and retain high levels of fear potentiated startle in response to the light. This experiment will allow us to definitively establish a role for BDNF in extinction of conditioned fear, and may have implications for the treatment of psychiatric disorders, such as PTSD, which involve an inability to extinguish fear memories.

Project 2: Measurement and Evaluation Of Pharmacological Agents To Reduce Fear.
PIs: Michael Davis, Erica Duncan, David Walker
a. Determine the contribution of NR2A- versus NR2B-subunit containing NMDA receptors in the amygdala to fear conditioning and fear expression. Initial experiments conducted with our standard fear-conditioning procedures (2 days of 10 light-shock pairings each) indicated that the blockade of NR2A-containing receptors (using NVP-AAM077) disrupts fear conditioning (pre-training infusion) and also fear
expression (infusion prior to fear-potentiated startle testing) in a dose-dependent manner, and that conditioning and potentiated startle are equally sensitive to this treatment. Blockade of NR2B-subunit containing NMDA receptors (using CP101,606 or ifenprodil) has had no effect on expression at the doses tested and has had inconsistent effects on fear conditioning. More recent experiments suggest that the NR2B antagonist may disrupt acquisition when weaker training procedures are used (1 day of 10 light-shock pairings). Using these procedures, we are currently testing the effects on expression.

b. **Evaluate the effects of compounds that act on the strychnine-insensitive glycine binding site (i.e., on the NMDA receptor) on fear-conditioning.** We previously found that the partial agonist D-cycloserine facilitates fear extinction. In recent experiments, we have evaluated the effect of D-cycloserine (DCS), D-serine (DS; which may be the endogenous and a possibly more effective ligand). At the doses and times tested, neither DCS nor DS have reliably influenced fear conditioning when injected systemically prior to light-shock pairings with weak training.

c. **Evaluation of a non-peptide CRF1 antagonist on fear and anxiety.** Systemic administration of this compound disrupted increases in startle produced by i.c.v.-infusions of CRH in a dose-dependent manner. At similar doses, the compound also appears to disrupt light-enhanced startle, but not fear-potentiated startle.

d. **Pharmacological characterization of m-opioid receptor agonist effect on the expression of fear potentiated startle.** There is a dearth of research geared toward the understanding of the role of opiates in anxiety-related behaviors. However, an understanding of the mechanisms of action of opiate effect on anxiety is important, in light of the vital need for novel and alternative treatments for anxiety disorders. Buprenorphine, a safe and efficacious partial m-opioid receptor agonist known to be 25-40 times more potent than morphine as an analgesic, has therapeutic potential for the treatment of anxiety-related disorders. In order to compare the potency of morphine to buprenorphine on the expression of fear-potentiated startle and the ability of the competitive, nonselective opioid antagonist, naloxone to block these effects, we established dose-response curves for the anxiolytic effects of buprenorphine and morphine in the presence of saline or a fixed dose of naloxone (2 mg/kg). We found that pretest administration of morphine and buprenorphine blocked fear-potentiated startle in a parallel, dose-dependent manner, without affecting baseline startle, suggesting a selective anxiolytic profile. Buprenorphine was 40 times more potent than morphine. Surprisingly, naloxone only partially blocked the anxiolytic effects of both morphine and buprenorphine at high doses, and had no effect at low doses. These results suggest that at least some of the anxiolytic effects of morphine and buprenorphine may involve non-opioid (naloxone insensitive) mechanisms. We are currently investigating possible non-opioid actions of opiates. Together, these findings may shed light on novel targets for the development of treatments for anxiety disorders.

e. **Studies of fear inhibition in humans.** We used an acoustic startle procedure in a modified conditioned inhibition experiment (AX+/BX-) to test the hypothesis that PTSD patients have decreased inhibition of fear-potentiated startle. We tested 41 healthy male and female volunteers in the AX+/BX- paradigm using airblast as the unconditioned stimulus. Participants were presented with one set of colored lights paired with aversive air blasts to the throat (AX+ trials), and a different series of lights presented without air blasts (BX- trials). We then presented A and B together (AB trials) to see whether B
would inhibit fear potentiation to A. We found significant potentiation to the danger cue, AX and significant inhibition to AB. More importantly startle amplitude was lower on the AB test trials compared to the AX test trials, indicating that the inhibition that had developed to B transferred to a novel test compound. The paradigm was also tested in 28 PTSD patients (14 with low current symptoms, 14 with high current symptoms). We found that PTSD patients had baseline startle amplitudes comparable to the control subjects. Both PTSD groups showed significant fear potentiation and discrimination between AX+ and BX-. However, the high symptom PTSD group did not show inhibition of fear on the AB trials, whereas both the controls and the low symptom PTSD patients potentiated more to AX+ than AB trials. These results suggest that PTSD patients with both low and high current symptoms show increased fear potentiation, but only patients with high symptom severity have impaired fear inhibition.

f. Examining the interaction of stress, environment, and genetics on the development of PTSD within the inner city in downtown Atlanta population. A number of ongoing studies are examining the prevalence and comorbidity of PTSD within the inner city in downtown Atlanta. These studies will provide a stepping off point for larger research programs. We also hope that as research in this clinic matures, we will be able examine some of the translational approaches, e.g. D-cycloserine, to study enhancement of extinction for treatment of PTSD using exposure therapy.

g. Hormonal influences on fear and anxiety models. The disparity in the occurrence of psychopathologies between males and females occurs in women after puberty. Thus far, we have found no effect of hormones on light-enhanced startle, a putative measure of anxiety in rats in female rats. Current literature postulates that it is in times of hormonal flux that females are particularly vulnerable to stress. The following series of studies looked at estrogen administered at various time points before testing to see if the rise and decrease of estrogen levels affected the response of ovariectomized rats to light-enhanced startle. In each experiment rats were pre-tested for light-enhanced startle and matched according to their response and then retested after treatment. 250 µg E2 s.c given 72, 48, 24, and 1 hr before retesting did not significantly change light-enhanced startle in females. We postulated that the lack of effect of E2 flux on light-enhanced startle may be due to the fact that it activates both estrogen alpha and beta receptor subtypes and therefore the activity of one may be off-setting the activity of another. This may be of particular importance in light of recent studies showing that the anti-anxiety effects of estrogen are due to the beta receptor activation, whereas anxiogenic effects may be mediated by alpha receptor activation. Following the same paradigm we tested the effects of 10 µg doses of a highly specific alpha and beta receptor agonist 48 hrs before testing on whereas in ovariectomized rats. Again we found no effect of treatment with either agonist on light-enhanced startle.

Project 3: Structural Changes In The Brain That May Mediate Long Term Memory Storage. PIs: Michael Davis, Kerry Ressler

a. Role of β-catenin in fear conditioning. A large body of evidence suggests that structural changes account for long-term memory storage. Among the most studied structural changes has been the elaboration of new synaptic architecture following a learning event. This process, known as dendritic morphogenesis, has been postulated by many investigators to be a physiologically relevant means of synaptic potentiation. The
processes governing dendritic morphogenesis are many and varied, but recent work has focused on the role of b-catenin in the remodeling of synapses in an activity-dependent way. Therefore, examining b-catenin function may provide an important link between neural activity and long-lasting synaptic change. Knockouts of b-catenin are embryonic lethal; thus, there have been no studies of the role of this protein in standard learning and memory behavioral tasks. By combining region specific viral infection with Cre-mediated recombination in animals carrying a floxed version of the b-catenin allele, we have devised a method for deleting the b-catenin gene in mature animals. Deletion of this gene either before the acquisition or the expression of conditioned fear, will allow us to determine when and if b-catenin function is required to elicit the fear response. Additionally, the ability of this method to be region specific will determine whether the presence of b-catenin in the amygdala is required to instantiate the fear response and will provide important information as to the site of the plastic changes underlying fear learning. We have found that b-catenin is highly expressed in the adult mouse brain, especially within the hippocampus and amygdala. There is a significant increase in b-catenin activation (decrease in phosphorylation) following the acquisition of fear. These results are consistent with an enhancement in activated, stable b-catenin, that would in turn enhance stabilization of cadherin junctions. We have successfully shown that injection with the LV-Cre virus results in a region-specific deletion of the b-catenin allele. Amygdala-specific b-catenin deletions do not affect baseline anxiety, activity measures, or novel object recognition. Amygdala-specific b-catenin deletions do not affect acquisition of conditioned fear because freezing is normal in these mice shortly after fear conditioning. Amygdala-specific b-catenin deletions appear to prevent consolidation of conditioned fear because there is little freezing either 1 or 24 hrs after conditioning under these conditions.

b. Fear potentiated startle in transgenic mice using olfactory cues. Because many complex social behaviors in animals use odor cues for communication and detection of prey, we wanted to use the advantages of the fear potentiated startle test to develop a way to measure fear-potentiated startle using odor cues. In addition, because of the unique sensory arrangement of the olfactory system we wanted to be able to use olfactory stimuli to understand gene expression and morphological changes following fear conditioning. In past years, we created a transgenic mouse line (M71-WGA:RFP-eGFP) that expresses fluorescent reporters within the correct zone of the epithelium and targets axons to the M71-specific glomerulus of the olfactory bulb. In our initial litters, a small number of juvenile animals had periglomerular cells apparently labeled with DsRed. In adult mice, no neurons past the glomerulus were visible with either DsRed or WGA antibodies. Endogenously expressed WGA may be toxic to these neurons or the DsRed conjugation may impair WGA transport. In addition, recent research by Thomas Bozza implies that M71 may not be activated by acetophenone in vivo. However, because the GFP-labeling of the glomerulus is reliably seen in adult olfactory bulbs, these animals may still be useful for the targeted injections of tracers (see below). To investigate possible morphological changes in axon ramifications with olfactory fear learning, we injected the anterograde tracer BDA-3K into olfactory bulb of trained and untrained mice. Axons were visible in piriform cortex and there were some labeled cells in olfactory tubercle. Pilot data indicates a difference in labeled olfactory tubercle cells between trained and untrained mice. We may be able to combine this technique with iontophoretic injections
targeted to the GFP-labeled cells of our putative labeled-line mice to overcome the limitations of the WGA-DsRed. In conjunction with the potential learning-induced morphological changes in the olfactory system, we are also investigating molecular changes with olfactory fear learning. Some preliminary evidence from rats suggests that BDNF mRNA increases in the piriform cortex following olfactory fear conditioning, but not with a presentation of odor alone or shock alone. We are presently engaged in investigating changes in BDNF mRNA and other molecular changes in the olfactory system with fear learning.

c. Development of a stress procedure that results in increased startle and evaluation of the involvement of the BNST in these effects. Rats that receive 14 shocks on each of 3 days in context A show an elevation in ‘baseline’ startle even when tested in a different context. These increases persist for many days but do decay eventually. In a recent experiment, we found that the increases were not influenced by context extinction procedures. Thus, they appear reflect a non-associative effect of stress. Intra-BNST infusions of the AMPA/kainate receptor antagonist NBQX prior to testing eliminated these increases whereas infusions into the amygdala attenuate but do not block them. Results from a single experiment suggest that pre-shock infusions into the BNST are also effective. The results point to an important role for the BNST in stress-induced anxiety.

Project 4: Analysis Of Limbic Circuitry In The Rhesus Monkey. PIs: Jocelyne Bachevalier, Kim Wallen, Stuart Zola, Mar Sanchez

a. Behavioral effects of neonatal amygdala lesions in monkeys living in a semi-naturalistic environment. Previous research has demonstrated the involvement of the amygdala in social cognition. However, little is known about its role in the early development and the long-term maintenance of these cognitive processes. This study proposes to investigate the effects of neonatal damage to the amygdala in the emergence and maturation of behavioral and cognitive processes in non-human primates living in a stable social group (Yerkes Field Station). Specifically, to assess the role of the amygdala in the development of 1) status achievement (independence from mother and acquisition of social rank in the group), 2) fearfulness (toward novel objects and peers) and affiliation (mother-infant attachment and establishment and maintenance of relationships with peers) and 3) maturation of cognitive processes related to social skills (perception and use of social signals) and control of goal-directed behaviors. Our hypothesis is that bilateral neonatal lesion of the amygdala will affect the emergence and/or maturation of species-typical behavior and that this may be due to more basic deficits in the development of cognitive functions. This pilot study proposes to use males, but a long-term goal will be to add females to assess sex differences in the effect of neonatal lesion of the amygdala on the development of these species-specific behavioral and cognitive processes with the hypothesis that male infants could be more vulnerable than females. So far, four 31-35 days old infant male rhesus macaques and their mothers were separated from their social group and transported at the Yerkes Main station for a maximum of 12 days. After 1-2 days of habituation to the environment, the infants were temporarily separated from their mother to receive MRI-guided neurotoxic injections of the ibotenic acid bilaterally within the amygdala (AMY Group, N=2) or no injections (SHAM Group, N=2). All surgical procedures went without major complications. After a 24-hr recovery in the primate nursery, the infants were reintroduced to their mothers who
accepted them eagerly, except one of the female who did not attend to her offspring the first day. In this case, the infant (from the SHAM Group) was removed and brought back in the nursery. The following morning the pair was returned to the field station to increase chances of reunion in a familiar environment. Intensive behavioral surveillance showed that at the end of the first day back at the field station, the mother was nursing, carrying and protecting him. The two infants from the AMY Group received a second MRI one week after the surgery to verify the location and assess the extent of their lesion. To control for this second separation from the mother, the second SHAM subject was also separated a second time for 4 hours from his mother. After the second MRI or separation, the infants were reunited with their mother, who accepted them again easily, and rapidly each pair was then returned to the field station. Each reintroduction in the social group was closely monitored and all were entirely successful. We have started regular behavioral observations of each pair in the social group, which will continue for the next few months. The last mother-infant pair has just arrived at the main station and the infant will receive a lesion of the amygdalae within the next weeks. Two additional animals in each group will be added in the Spring 2006.

b. Development of a reversible deactivation, via cooling, technique to study higher cognitive function in monkeys. The ability to perceive, decode and use social signals (reinforcers with variable valence) from conspecifics as well as to regulate one’s own behavior adaptively upon changing external (social environment) and internal (motivational state) contingencies are critical for successful social interactions. Growing evidence indicates that the amygdala (AMY) and orbital prefrontal cortex (ORB) are part of a neural network critical for social cognition. However, determining the precise contributions of each structure has proved to be challenging. The goal of the present study is to refine and implement a neural reversible deactivation technique by cooling probes to assess in rhesus monkeys the respective role of the AMY and ORB in the ability to flexibly alter behavioral responses upon changes in the incentive value of a previously learned conditioned reinforcer or in the current motivational states. We hypothesize that the AMY participates in the elaboration of the representation of learned reinforcing stimuli as well as in the inhibition of impulsive behavioral responses whereas the ORB regulates learned behavioral responses with respect to changes in the valence of the reinforcing stimuli or current motivational states. So far, we have acquired all the technical material necessary for the preparation of the miniatures cooling probes for rhesus macaques. The delicate preparation of these probes has been started by Dr. Goursaud who has been successfully trained by an expert (Dr. Clarke, San Diego) to build them. The two subjects that will be implanted with 4 cooling probes (1 in each AMY and 1 in each ORB, bilaterally) have just come out of a quarantine period and will start their training on the devaluation task within the next weeks. As soon as the training will be acquired, all subjects will receive the MRI-guided implantation of the probes. This should be performed by the end of October 2005 when the cooling experiment will start.

c. Distribution of CRH-like peptides in the rhesus monkey brain: Focus in amygdala. Based on the important role of CRFergic pathways originating in central amygdaloid nucleus (CeA) and BNST in anxiety and fear in rodents we are studying these pathways in the primate brain. We have previously reported undetectable levels of CRF expression in the rhesus monkey CeA and restricted expression in the BNST
Thus, we have now analyzed whether other CRF-like peptides (UCN I, UCN II or UCN III) are, expressed in these primate brain regions, instead, and still able to act on CRF receptors. The answer is no. We used mRNA in situ hybridization histochemistry to map the location of the urocortins (I, II, III), not only in amygdala and BNST, but across all rhesus macaque brain. In particular, during last year we performed a more exhaustive mapping study of UCN III mRNA and peptide distribution in the macaque brain. We found particularly high levels of UCN III mRNA in the lateral geniculate of the thalamus, the dentate gyrus of the hippocampus, the paraventricular nucleus of the hypothalamus, and the cerebellar cortex. In addition, low to moderate levels of hybridization were detected throughout the neocortex. In summary, UCN III mRNA in this non-human primate is expressed in areas associated with cognitive and emotional processes, learning and memory, visual processing, and stress regulation, as well as control of movement and posture. Interestingly, UCN III mRNA distribution in macaque brain seems far more “corticalized” than in rat or mouse brain.

d. Effects of infant abuse on fear responses in juvenile rhesus monkeys (cross-collaboratory project with Fear and Affiliation collaboratories). We have previously reported that infant abuse in rhesus monkeys provokes high basal cortisol and behavioral distress at early ages (when abuse rates are high) followed by a compensatory downregulation of HPA function at later ages. We are now analyzing long-term alterations on emotional and HPA axis reactivity as the animals go through adolescence. Ten maltreated and ten matched control macaques were exposed to novel stimuli of varying threatening intensities: 1) Human Intruder paradigm, 2) neutral or rewarding objects and 3) fear-evoking objects. Juveniles with histories of infant abuse, particularly males, exhibited shorter latencies than controls to retrieve a treat adjacent to a fear-evoking object (e.g. snake), or to touch the object, demonstrating inappropriate fear responses in the maltreated group (Grand et al. and Sanchez, 2005). Fear-evoking objects provoked higher ACTH responses than neutral stimuli in all animals. Although no group or sex differences were detected in HPA reactivity to each task, ACTH and cortisol responses were positively correlated with latencies. Analysis of CSF levels of monoamine metabolites will provide information on associations between the high impulsivity detected in maltreated juveniles and possible alterations in monoamine neurotransmission.

Project 5: Electrophysiology In The Bed Nucleus Of The Stria Terminalis (BNST) With Functional Tests In Vivo. PIs: Michael Davis, Don Rainnie

a. Analysis of the network properties of neurons in the BNST and how these neurons are modulated by neurotransmitters and peptides. We have had an ongoing interest in the basic physiological properties of these neurons, and based on these properties have separated them into three cell types that differ in the expression of several active conductances. In the past year, we have further characterized each of these conductances in isolation. We are currently nearing completion of a manuscript reporting these data. Based on our current understanding of these data, we can now determine if neurotransmitters and peptides might modulate these conductances individually. We also have an ongoing interest in determining the direct effects of serotonin (5-HT) on BNST neurons. We have characterized several responses in the past, including a 5-HT2A receptor-mediated depolarization and a 5-HT1A mediated receptor hyperpolarization. In
the past year, we discovered that a small population of BNST neurons express a 5-HT7 receptor-mediated depolarization, which is an extremely novel and important finding. Because these neurons are a much smaller percentage of the total population of BNST neurons, 5-HT7 activation may target a very interesting and specific population of BNST neurons. We have also been using immunohistochemical techniques to investigate 5-HT receptor expression with subregions of the BNST, in order to help determine how different populations of neurons are modulated by 5-HT. In addition to investigating the effects of 5-HT in the BNST, we are interested in the modulation of 5-HT responses by stress and stress-hormones. We have shown that corticotropin-releasing factor (CRF) changes the profile of BNST 5-HT responses to favor inhibition, and in the past year have shown that one-week of isolation-housing has the same effect. These data are some of the first showing that a simple behavioral manipulation can change the properties of BNST neurons.

We are also currently in the process of investigating whether chronic treatment with corticosterone can change the profile of BNST 5-HT responses towards excitation. We are also investigating the effects of BNST serotonergic activation on startle behavior, which has been argued to be a behavioral measure of anxiety. We have shown that the 5-HT agonist, 5-CT, decreases baseline startle levels, but until recently it was unclear which 5-HT receptor mediated this effect. Recently we blocked the effects of 5-CT with the 5-HT1A antagonist, WAY100635, showing that 5-HT1A receptors mediate an anxiolytic action within the BNST.

**Project 6:** Conditioned Defeat in Hamsters (cross-collaboratory projects with Aggression Collaboratory). PI: Kim Huhman

a. **Effects of 5HT infused into the dorsal raphe nucleus on social defeat.** Previous research on learned helplessness has shown that the behavioral consequences of uncontrollable stress are mediated in part by 5-HT1a autoreceptors in the dorsal raphe nucleus (DRN). We examined whether a 5-HT1a receptor agonist (flesinoxan) given into the DRN would reduce conditioned defeat in hamsters. Consistent with the learned helplessness literature, we found that flesinoxan given into the DRN reduced the acquisition and expression of conditioned defeat.

b. **Role of CRF receptors in social defeat.** Previous research has shown that corticotropin-releasing factor (CRF) can modulate stress-induced changes in behavior. Some studies have implicated CRF type1 receptors (R1) in the acquisition of stress-induced changes in behavior, while others have demonstrated a role for CRF R2. We have shown that a CRF R2 antagonist, but not a CRF R1 antagonist, given into the lateral ventricle reduces the acquisition of conditioned defeat.

**Project 7:** Neuroimaging And Memory Encoding. PIs: Anna Bollini, Stephan Hamann, Marise Parent, Elaine Walker,

a. **Neuroimaging the effects of cortisol and glucose on declarative memory for neutral and emotional stimuli.** The goals of this venture grant were to determine the separate effects of acute administration of cortisol or glucose on (a) brain function, as assessed by functional MRI (fMRI) and (b) declarative memory for neutral and emotional stimuli, as assessed by cognitive tests. A third goal was to determine the relationship between the cognitive and neural effects of cortisol and glucose using correlational
analyses. To date, we have (a) finished collecting all of the data, (b) completed most of the analyses of the FMRI and behavioral memory data for the cortisol portion of the project and are currently analyzing the glucose data, and (c) preparing manuscripts based on these data. Thus, far, various portions of the data have been presented at four scientific meetings.

b. **Blood glucose levels correlate with medial temporal lobe and prefrontal brain activation during encoding for words.** Glucose administration preferentially enhances hippocampal-dependent memory, such as verbal declarative memory in humans. Research in rodents has shown that the hippocampus is particularly sensitive to the effects of increases in circulating blood glucose levels, and that direct infusions of glucose into the hippocampus enhance hippocampal-dependent memory. The present study tested whether glucose administration would influence regional brain activity observed during encoding of verbal information in humans. Thirteen healthy male college students participated in a single-blind cross-over design consisting of two sessions approximately 1 week apart. At each session participants ingested a lemonade beverage that was sweetened with either placebo (saccharin, 23.7 mg in 8 oz) or glucose (dextrose anhydrous, 50 g). Fifteen minutes later their brain activity was assessed using functional magnetic resonance imaging (fMRI) at 3T while they performed an intentional encoding task for word pairs. Blood glucose levels were measured before, and 15 and 75 min after the beverage was consumed. Recognition memory was tested 24 hr later. The results indicated that glucose significantly elevated blood glucose levels at both time points, but did not influence recognition memory. As expected, increased activity in medial temporal lobe and left prefrontal brain regions was observed during verbal encoding compared to activity during a low-level control task. Interestingly, there was a significant correlation between the magnitude of the increase in blood glucose levels and the degree of activation observed in these same brain areas. These findings are consistent with the hypothesis that elevations in blood glucose selectively influence brain areas important to declarative memory.

c. **Stress-level cortisol inhibits neural activity related to working memory and episodic memory in humans: an fMRI study.** Acute stress-level elevation of cortisol in humans has been shown to impair working memory and episodic memory in a dose-dependent manner. To investigate the neural basis for this effect, we administered a stress-level oral dose of hydrocortisone (100 mg) and assessed working memory and episodic (face recognition) performance and task-related brain activity in 14 healthy young adult males. A placebo-controlled, double-blinded, within-subject crossover design was used in which subjects performed the same task on two different days (using a counterbalanced order and comparable stimuli), two hours after either hydrocortisone or placebo administration. Brain activity was scanned at 3T while subjects performed a working memory task (alternating blocks of two-back and zero-back tasks) and in a separate run encoded novel faces. To isolate cortisol effects on encoding, recognition memory for the faces was assessed one day after scanning. Cortisol elevation was primarily associated with robust decreases in task-related activity in dorsolateral prefrontal and parietal cortex during high working memory load, relative to the placebo session. Similar cortisol-related decreases were observed for face encoding, with additional decreases in the right hippocampus and fusiform gyrus. For both tasks, in contrast to the robust cortisol-related decreases in task-related brain activity, cortisol-
related increases in task-related brain activity were markedly weaker and were limited in extent. Behavioral data collected during scanning revealed decreased performance in both tasks. These findings suggest that acute stress-level cortisol elevation may influence working memory and nonverbal episodic memory encoding by reducing task-related brain activation.

Reproduction Collaboratory

The research in the **Reproduction Collaboratory** has been developed as a direct result of interactions among faculty that have occurred in our collaboratory meetings and all of the projects involve multiple laboratories. The Reproduction Collaboratory has recently agreed that sex differences in behavior and neural function are an overarching theme in our research. Thus, during the coming year of support the Reproduction Collaboratory will be working to integrate this theme into research in each of the specific research areas that we have developed during the first years of support. We anticipate that this will lead to integration with research in other collaboratory areas as well as a more programmatic development of venture grants within the Reproduction Collaboratory.

Progress is described on four of our active venture grants.

**Project 1:** Sex discrimination across the menstrual cycle: A comparative study in chimpanzees and rhesus monkeys. **PI:** Agnès Lacrueuse

Fluctuations of ovarian hormones across the menstrual cycle influence a variety of behaviors in primates, including social and cognitive behaviors. In the social domain, female rhesus monkeys exhibit heightened interest for males and increased agonistic interactions with other females during periods of high estrogen levels. In the present studies, we examine whether increased interest for males compared to females during the peri-ovulatory period of the cycle is also found at the level of face perception. Furthermore, we investigate the existence of similar mechanisms in chimpanzees, a species closer to humans.

In the first experiment, we tested four intact female rhesus monkeys on a computerized touchscreen system on two face tasks involving neutral portraits of male and female rhesus monkeys, chimpanzees and humans. In the visual preference task (VP), monkeys had to press a button to view a face image. The image remained on the screen as long as the button was depressed. Pressing duration was measured as an index of the monkey’s viewing preference. In the Face-Delayed Recognition Span Test (Face-DRST), monkeys were rewarded for touching the new face in an increasing number of serially presented faces. The pattern of responses was analyzed to evaluate whether subjects attended to or avoided certain faces according to sex. Blood collection was performed every other day to measure levels of estradiol and progesterone. Two of the four females were cycling at the time of testing. As predicted, these two individuals looked longer at conspecifics’ male faces during the peri-ovulatory period and tended to commit more errors on the DRST when choosing male, rather than female monkey faces. Such effects were absent for heterospecific faces and the two noncycling subjects. These data suggest that ovarian hormones influence females’ preferences for specific faces, with heightened preference for male faces during the periovulatory period of the cycle.

The studies in the chimpanzee are ongoing. Three females are tested across two menstrual cycles on the VP, the face-DRST and a face categorization task requiring the
animals to categorize male and female faces of chimpanzees, rhesus monkeys, and humans. Daily ratings of anogenital swellings are used to monitor menstrual cycle phases.

The data should determine whether heightened interest for stimuli of significant reproductive relevance during periods of high conception risk is a widespread phenomenon among primates that may help guiding social and sexual behavior.

Project 2: Visual Communication in Northern Cardinals. PIs: Donna Maney, Chris Showalter (Fernbank Faculty)

The male Northern Cardinal (Cardinalis cardinalis) exhibits some of the most striking plumage coloration of any bird species and yet the functional significance of this trait is not well understood. The aim of this study was to look for a link between male plumage coloration and male quality. We hypothesized that brighter, redder males would be healthier - as seen by greater mass, longer tarsi, longer wings and lower heterophil to lymphocyte ratios - than duller, less red males. We tested this hypothesis by trapping birds at six sample sites and taking the four measurements outlined above as well as obtaining an image of each bird’s breast with a flatbed scanner. Color analysis of these images showed that birds scoring higher on a principal component analysis of hue, saturation and brightness tended to have shorter tarsi — a measure of body size.

A second goal of this study was to determine whether using a flatbed scanner is an appropriate substitution for using a spectrophotometer – the current standard for measuring color and brightness in the field. In addition to being costly, our spectrophotometer (USB2000; Ocean Optics, Dunedin Fl.) necessitated at least three hands to operate smoothly. A cheaper, easier method to measure color and brightness would be a welcome methodological advance. To test the two methods against each other, a sample of paint chips spanning the spectrum was scanned with both a scanner (CanoScan LiDE 35, Canon) and the spectrophotometer. Scanner data were transformed using Adobe PhotoshopCS and compared with the spec data. We found that both machines give comparable data. These results suggest that using a scanner may be a viable alternative to using a spectrophotometer, a switch that would save both money and manpower.

Project 3: Behavioral effects of neonatal amygdala lesions in monkeys living in a semi-naturalistic environment. (cross-collaboratory project with Fear Collaboratory) PIs: J. Bachevalier, K. Wallen

Previous research has demonstrated the involvement of the amygdala in social cognition. However, little is known about its role in the early development and the long-term maintenance of these cognitive processes. This study proposes to investigate the effects of neonatal damage to the amygdala in the emergence and maturation of behavioral and cognitive processes in non-human primates living in a stable social group (Yerkes Field Station). Specifically, to assess the role of the amygdala in the development of 1) status achievement (independence from mother and acquisition of social rank in the group), 2) fearfulness (toward novel objects and peers) and affiliation (mother-infant attachment and establishment and maintenance of relationships with peers) and 3) maturation of cognitive processes related to social skills (perception and use of social signals) and control of goal-directed behaviors. Our hypothesis is that bilateral
neonatal lesion of the amygdala will affect the emergence and/or maturation of species-
typical behavior and that this may be due to more basic deficits in the development of
cognitive functions. This pilot study proposes to use males but a long-term goal will be to
add females to assess sex differences in the effect of neonatal lesion of the amygdala on
the development of these species-specific behavioral and cognitive processes with the
hypothesis that male infants could be more vulnerable than females.

Four 31-35 days old infant male rhesus macaques and their mothers were
separated from their social group and transported at the Yerkes Main station for a
maximum of 12 days. After 1-2 days of habituation to the environment, the infants were
temporarily separated from their mother to receive MRI-guided neurotoxic injections of
the ibotenic acid bilaterally within the amygdala (AMY Group, N=2) or no injections
(SHAM Group, N=2). All surgical procedures went without major complications. After a
24-hr recovery in the primate nursery, the infants were reintroduced to their mothers who
accepted them eagerly, except one of the female who did not attend to her offspring the
first day. In this case, the infant (from the SHAM Group) was removed and brought back
in the nursery. The following morning the pair was returned to the field station to
increase chances of reunion in a familiar environment. Intensive behavioral surveillance
showed that at the end of the first day back at the field station, the mother was nursing,
carrying and protecting him. The two infants from the AMY Group received a second
MRI one week after the surgery to verify the location and assess the extent of their lesion.
To control for this second separation from the mother, the second SHAM subject was
also separated a second time for 4 hours from his mother. After the second MRI or
separation, the infants were reunited with their mother, who accepted them again easily,
and rapidly each pair was then returned to the field station. Each reintroduction in the
social group was closely monitored and all were entirely successful. We have started
regular behavioral observations of each pair in the social group, which will continue for
the next few months. The last mother-infant pair has just arrived at the main station and
the infant will receive a lesion of the amygdala within the next weeks. Two additional
animals in each group will be added in the Spring 2006. Dr Goursaud (CBN postdoctoral
fellow) from the Bachevalier’s lab has been involved in all separation/reunion phases and
MRI/surgical procedures. She will now be trained by the Wallen’s lab in field behavioral
observations.

Project 4: Measuring Attachment Security in Rhesus Monkeys. (cross-collaboratory
project with Fear and Affiliation collaboratories) PIs: Mar Sanchez, Kim Wallen

The goal of this proposal is to develop a methodology that bridges the research on
mother-infant relationships in humans and nonhuman primates. The specific aims are:
Aim 1: to adapt a widely used method of assessing attachment security in human
children, the Attachment Q-Sort, for use with captive rhesus monkey mother-infant pairs.
Attachment security measures the balance between exploration and comfort-seeking
behavior in infants/children and is associated with developmental outcomes.
Aim 2: to determine whether measures of attachment security from the non-human
primate version of the Attachment Q-Sort (AQS) are associated with behavioral, socio-
emotional and neurobiological development.
The human Attachment Q-sort (AQS) has been previously adapted for use with Japanese
macaques, and the authors reviewed the appropriateness of this instrument to measure
attachment security in infants of that species, supporting the instrument’s content validity. The main goal of our studies is to assess its discriminant and predictive validity in rhesus macaque infants at the Yerkes National Primate Research Center, through funding received from this CBN venture grant. One of our co-investigators, Dr. James Warfield, who adapted the Japanese macaque AQS for use with free-ranging rhesus monkeys at Cayo Santiago, has worked with us since August 2005 to adapt this AQS methodology for use with captive rhesus monkeys at the Yerkes colony. We have generated a “Yerkes infant attachment Q-set”, consisting of 95 items, and its corresponding criterion sort. Our primate coders (blind to attachment theory) have finished training with Dr. Warfield, reached reliability and are currently coding the experimental tapes. Their current Q-security scores correlations with Dr. Warfield’s range from 0.7-0.88.

Throughout the process of achieving reliability, we have already selected several “training tapes” that will be provided together with this instrument to the primatology community (one of our aims within this venture grant). We are also finishing up a “training tutorial”. Our long-term aim is to analyze whether infant abuse or maternal separation (our current models of early adverse care in rhesus monkeys) affect attachment scores in infant rhesus monkeys, and whether attachment security early in life is associated with the infants’ socioemotional development and functioning of stress-emotion system. Preliminary results from studies funded by this CBN venture grant and based on a random sample of tapes sorted by Dr. Warfield in the 0-6 months age block, abused infants had lower attachment security scores (0.19) than controls (0.39) towards their mothers.

Two new venture grants awarded this year will extend the work of the Reproduction collaboratory into primate cognition and energy utilization. As they have just been awarded they are only starting their research, but are promising new areas for the CBN and reproduction. They are:

**Project 5:** An ethological approach to cognition in monkeys: Inference of social rank. PIs: Robert Hampton, Mark Wilson, Andrew Fischer (Behavioral Technology Core) 

Social rank is an important influence on reproduction and reproductive maturity. This project will develop a novel, ethologically-grounded test of nonhuman primate social cognition. Monkeys will be trained to select the dominant individual from a pair of monkeys displayed in video clips on a touch screen. Monkeys will then be tested for the ability to deduce a linear dominance hierarchy based on several such pairs. Mark Wilson (Reproduction) will collaborate by arranging social interactions for filming. Andrew Fischer (Behavioral Technology Core) will provide programming and engineering expertise for production of automated computerized testing equipment. This project will enable us to prepare a grant proposal for extramural funding to study the neural basis of social cognition using imaging, lesion, and single unit recording techniques.

**Project 6:** Developing a model to study the adverse effects of metabolism in primates. PIs: Mark Wilson, Tim Bartness, Ruth Harris, Andrew Fischer (Behavioral Technology Core).

Rates of obesity continue to rise in children which have adverse effects on growth and development and place them on a trajectory for secondary health problems as adults.
New data from rodent models indicates that stress induces the consumption of highly palatable, calorically dense foods (comfort food) at the expense of normal chow. Consumption of this chow attenuates the neuroendocrine response to stress and, not surprisingly, increases fat mass. Non-stressed animals show no preference for the comfort food. We propose a pilot project to validate a monkey model to study the development of obesity and its adverse consequences on growth, metabolism, and development. We will use the normal social structure of rhesus monkey groups as the natural stressor as social subordination produce a chronic stressor in this species. The overriding goal of this venture project is to determine whether social stress associated with social subordination results in the preferential consumption of a highly palatable food over monkey chow. Groups of juvenile females eating normal monkey chow will be compared to those who have the choice of eating chow or the comfort food. Food intake will be monitored electronically. Behavioral, metabolic, neuroendocrine, and morphometric data will be collected during the 20-week study. These data will allow us to develop a model to better understand sex differences in the development of obesity in children and its adverse effects on growth, sexual maturation, metabolism, behavior.

2. What are the major impediments for conducting research in the center? Perhaps the main impediment has been finding creative and convenient ways to foster research collaborations across institutions that are located in different parts of Atlanta. Despite the geographical nearness of these institutions, many obstacles such as traffic and parking can make it difficult for busy scientists to work together effectively. The center has developed a videoconferencing system among the participating institutions that has provided a means for groups of investigators at different locations to meet regularly to discuss their research collaborations. In addition, graduate students and postdocs that are supported by the center play a pivotal role in fostering these cross-laboratory and cross-institutional collaborations. Students and postdocs have more flexibility to move between labs and have the time and ability to learn and transfer techniques and knowledge between labs. While providing a creative and useful way to carry out cross-lab and cross-institutional collaborations, having students and postdocs work in more than one lab also broadens their education and training tremendously. Thus, the impediments to conducting the center’s research have been addressed in innovative ways that provide other benefits to those involved.

3. What percentage of the center’s funding has been paid out of Fund Code 10? List amounts for the last five years. The CBSH and CBN has received between $100,000 and $120,000 in Fund Code 10 monies over each of the last five years. This represents approximately 2% of the Centers annual budget.

4. Attach a list of all research activities and other activities (e.g. workshops/programs/conferences/seminars/symposia/etc.) of the center.
   CBN events in 2004-2005
   November 9 and 10, 2004: Seminars by Robert Johnston
   Dept. of Psychology, Cornell University
   The Atlanta Room at The Commerce Club, 34 Broad Street
"Individual recognition: a model system for social-cognitive neuroscience"

Lecture room #2, Nabritt, Mapp, McBay Bldg., Morehouse College
"Understanding individual recognition: behavioral and neural approaches"

**January 25 and 26, 2005: Seminars by John Wingfield**
Professor of Biology, University of Washington at Seattle
**Yerkes Research Center Seminar Room**
"Control of reproduction in diverse habitats: integrating environmental and social cues"
**Room 233 Science Center, Spelman College**
"Control of Life Cycles in an Era of Global Climate Change"

**January 31, 2005: Seminar by Alan Watts**
Associate Professor of Neuroscience, Physiology and Biophysics
University of Southern California
**The Atlanta Room, Commerce Club Bldg., GSU**
“CRH gene expression: what does it really do for CRH neuroendocrine neurons?”

**February 5, 2005 9 am – 1 pm – Annual Brain Bee**
Fernbank Museum of Natural History
Regional competition for high school students; winner goes to national competition

**February 15 and 16, 2005: Seminar by Ralph Adolphs**
Professor of Psychology and Neuroscience, Cal. Tech.
**Yerkes Research Center Seminar Room**
"The Role of the Amygdala in Emotion and Social Cognition"
**Room 233 Science Center, Spelman College**
"Emotion and the Human Brain"

**March 1, 2005: Seminar by Anne Murphy**
Associate Professor of Biology, GSU
**Teasley Hall Auditorium Agnes Scott College**
“Sex, drugs and pain: How men and women are different”

**March 17, 2005: Seminar by Sarah Pallas**
Associate Professor of Biology, GSU
**Hall Auditorium Agnes Scott College**
“The remarkable plasticity of the young brain”

**March 18-19, 2005 – Annual Neuroscience EXPO**
Zoo Atlanta
Friday by invitation only for middle school students
Saturday open to the general public

**March 21, 2005: Showing of movie Memento**
Fernbank Museum of Natural History
Movie screening and seminar on memory and amnesia by Stuart Zola

**March 22 and 23, 2005: Seminars by Marc Breedlove**
Barnett Rosenberg Professor of Neuroscience,
Depts. Psychology and Zoology, Michigan State University
The Atlanta Room, Commerce Club Bldg., GSU
“Why Sex Really Matters”
Room 233 Science Center, Spelman College
“Eyeless in Gaza: Meandering into Science”

**March 30, 2005: Systems Core Workshop**
8:30 – noon, Georgia State University

**March 29, 2005: Seminar by Harold Gouzoules**
Professor of Psychology, Emory University
Teasley Hall Auditorium Agnes Scott College
"Primatological flip-flopping: the evolution of language from communication in monkeys and apes"

**April 7, 2005: Seminar by Joanne Chu**
Assistant Professor of Biology, Spelman College
Teasley Hall Auditorium Agnes Scott College
“The neurobiology of social motivation in amphibians”

**April 21, 2005 – Workshop on “How to Become an Independent Investigator”**
for postdocs and graduate students, Emory University

**May 7, 2005 – CBN Annual Retreat**
8:30 – 3 pm, Morehouse School of Medicine

*May 21, 2005 - CBN/ACSFN Spring Symposium*

“Impact of early life stressors on behavior: Nature vs. nurture”
Speakers:
Michael Meaney (Douglas Hospital Research Center, Montreal)
“Influence of maternal care”
Terese Kosten (Yale)
“Impact of neonatal stress on adult drug use and abuse”
Charlie Nemeroff (Emory)
“Early life experience and depression in humans”
Jap Koolhaas (Rijksuniversitit; Netherlands)
“Early life experience and aggression”
Rick Richardson (Univ. New South Wales, Australia)
“Developmental aspects of fear”

**September 22nd - Agnes Scott College, Teasley Lecture Hall, Science Center**
“The neurobiology of social status”
Donald Edwards, Ph.D., Professor, Dept. of Biology
Georgia State University

September 27th – CBN Seminar, Yerkes VRC Seminar Room
"Unraveling the multiple signal transduction pathways that participate in hormonal regulation of female reproductive behavior"
Anne Etgen, Ph.D., Professor, Dept. of Psychiatry and Pediatrics
Albert Einstein Medical School

September 28th – CBN Undergraduate Seminar, Spelman College, Room 130, Tapley Hall
“Are estrogens good for brain cells?”
Anne Etgen, Ph.D., Professor, Dept. of Psychiatry and Pediatrics
Albert Einstein Medical School

October 4th – Agnes Scott College, Teasley Lecture Hall, Science Center
“The neural basis and evolution of sluggish behaviors”
Paul Katz, Ph.D., Associate Professor, Dept. of Biology
Georgia State University

October 14th – Imaging Core Workshop
1 – 5 pm – Emory University, 3rd Floor Conference Room, Whitehead Research Bldg.

October 18th – CBN Seminar, GSU, Brown Room, 18th Floor, Commerce Club Bldg.
“To Be Fit and Fat: Physiological and Molecular Consequences of Obesity in Mammalian Hibernators”
Greg Florant, Ph.D., Associate Professor, Dept. of Biology
Colorado State University

October 19th – CBN Undergraduate Seminar, Spelman College, Room 130, Tapley Hall
"How I got involved in Science: Snakes, Birds, and Marmots"
Greg Florant, Ph.D., Associate Professor, Dept. of Biology
Colorado State University

October 21 – 22, 9 am – 5 pm – CBN Educational Conference, Yerkes Neuroscience and VRC Seminar Rooms
“Considering Best Practices in Science Education: A Conference of Educators, Scientists and Students”

5. Attach separate bibliographies of refereed and nonrefereed publications which have resulted from research activities of the center. List publications for three years only. Due to the large number of these publications, these listed are only from the past fiscal year of the center. Others available upon request.


**Hammock, Elizabeth A.D., Miranda M. Lim, Hemanth P. Nair and Larry J. Young.** (2004) Vasopressin 1a receptor levels are associated with a regulatory microsatellite and behavior. Genes, Brain and Behavior. 4:289-301.


and bed nucleus of the stria terminalis corticotropin-releasing factor in behavioral
responses to social defeat. Behavioral Neuroscience, 118, 1052-1061.

conditioning in mice, measured simultaneously with fear-potentiated startle and freezing.

Jovanovic T, Keyes M, Fiallos A, Myers KM, Davis M, Duncan EJ. (2005) Fear
potentiation and fear inhibition in a human fear-potentiated startle paradigm. Biological
Psychiatry 57:1559-64.

glucose are not restricted to spatial working memory. Neurobiology of Learning and
Memory, 83, 168-172.

Kicklighter, C.E., S. Shabani, P.M. Johnson, and C.D. Derby (2005) Sea hares use

Levita, L. Hammack, S.E., Mania, I., Li, X-Y, Davis, M. Rainnie, D.G,(2004) 5-
HT1A-like Receptor activation in the bed nucleus of the stria terminalis:
electrophysiological and acoustic startle effects, Neuroscience 128:583-596.

functional MRI study of attention shift in human verbal working memory. NeuroImage

for acetylcholinesterase staining of brain sections previously processed for receptor
autoradiography. Biotechnic and Histochemistry. 79:11-16.

Lim, Miranda M., Isadora F. Bielsky and Larry J. Young. (2005). Neuropeptides and the

Lim, Miranda M., Elizabeth A.D. Hammock, Larry J. Young. (2004). Vole species as an
animal model for the evolution of social behavior: from genes to brain to behavior. Acta
Zoologica Sinica. 50:479-489.

Lim, Miranda M., Hemanth Nair, and Larry J. Young (2005). Species and sex
differences in brain distribution of corticotropin-releasing factor receptor subtypes 1 and
2 in monogamous and promiscuous vole species. Journal of Comparative Neurology.


Zhao, Z., & Davis, M. Fear-potentiated startle in rats is mediated by neurons in the deep layers of the superior colliculus/deep mesencephalic nucleus of the rostral midbrain through the glutamatenon-NMDA receptors. Journal of Neuroscience, 2004, 24, 10326-34.

6. Attach a list of grants submitted in the last three academic years and list all sources of funding. Click here for the format to use. For funded grants, give title, funding source, amount, type of grant (research or instruction), GSU project number, and period funded. Specify the amount of funds received from each category (research or instruction) for each of the last three years.

**Venture Grants awarded during 3-year period to Center members:**

**May 2005:**

PL: Laura Carruth - Brain Camp for Kids: Neuroscience in Action! (EDUCATION).

PL: Dolores Bradley, Kai McCormack - Building the concentration in neuroscience at Spelman College (EDUCATION).

PL: Mike Davis, Kerry Ressler - Extinction of performance anxiety using D-cycloserine together with Viagra (FEAR).

PL: Mike Davis, Kerry Ressler, Shella Kielholz - Effects of fear conditioning on Manganese-enhanced circuit tracing in an identified neural circuit (FEAR/IMAGING CORE).

PI: Kyle Frantz, Laura Carruth, Ericka Reid - Retention in Research for Women and Minorities (EDUCATION).

PI: Liz Hammock, Larry Young, Dwight Lawson (Zoo), Tara Stoinski (Zoo) - Evolution of gene structure and social behavior in primates (AFFILIATION/ZOO).

PI: Robert Hampton, Mark Wilson - An ethological approach to cognition in monkeys: Inference of social (AGGRESSION/REPRODUCTION).


PI: Mark Wilson, Tim Bartness, Ruth Harris (UGA), Donna Toufexis, Andrew Fischer - Developing a model to study the adverse effects of metabolism (REPRODUCTION/BEHAVIORAL CORE).

**Dec 2004:**
PI: Jocelyne Bachevalier, KimWallen - Behavioral effects of neonatal amygdala lesions in monkeys living in a semi-naturalistic environment (REPRO/FEAR)

PI: Stuart Zola, Jocelyne Bachevalier - Amygdala-orbital frontal interaction and reward expectancy (FEAR)

PI: Kerry Ressler, Byron Ford - Neuregulin-mediated synaptic plasticity in the acquisition of conditioned fear* (FEAR/MOLECULAR CORE)

**May 2004:**
PI: Balch/Wallen - $30,000 awarded to Ga. Tech, Account active.
Automatic tracking and analysis of monkey proximity initiation in an outdoor social group (REPRO/ITI/BEH CORE)

PI: Herberholz/Edwards/Derby - $$25,000 awarded to GSU, Account active.
The effects of conspecific odor on the behavior of socially experienced crayfish (AGGRESSION)

PI: Maney/Showalter - $24,152 awarded to Emory, Account active.
Vocal and visual communication in the Northern Cardinal, *Cardinalis cardinalis* (AFFILIATION/REPRO)

PI: Sanchez/Wallen - $$22,278 awarded to Emory, Account active.
Measuring attachment security in rhesus monkeys (REPRO/AFFILIATION)

**Dec 2003:**
PI: Toufexis/Wilson/Davis - The effect of estrogen and tamoxifen on fear learning in the female rat (FEAR-REPRO)
PI: Rainnie, Davis, Levita - The role of NPY and NPY-expressing interneurons in the basolateral amygdala: An electrophysiological and behavioral study (FEAR)

PI: Baro, Edwards, Jackson - Differences in octopamine receptor distribution in dominant and subordinate anthropods (AGGRESSION)

PI: Askew, Stahl, Fernandez, Albers - Effects of V1a antagonist infusion in the lateral septum on agonistic behavior and dominance status in a food competition procedure (AGGRESSION)

PI: Parr, Preuss, Rilling - Neural correlates of social recognition in chimpanzees and macaques: A pilot study using PET (AFFILIATION)

PI: Rilling, Hu, Preuss - Comparative Diffusion Tensor Imaging (DTI) and magnetic resonance spectroscopy (MRS) in monkeys, apes and humans (AFFILIATION – IMAGING CORE)

PI: Lacreuse, Martin-Malivel, Brown - Sex discrimination across the menstrual cycle: A comparative study in chimpanzees and rhesus monkeys (REPRO – BEHAVIORAL TECH CORE)

PI: Pazol, Patisaul, Wilson, Wallen - Medroxyprogesterone acetate: Mechanisms of estrogen antagonism (REPRO)

PI: Stoinski, Lennard, Powell - Using Animal Behavior to Educate about Science (Zoo Atlanta) (EDUCATION)

PI: Carruth, Bean - Taking the Genomic Revolution into High School Classrooms (Fernbank) (EDUCATION)

May 2003:
PI - Joanne Chu/Matthew Grober - The role of early immediate genes in the regulation of reproductive behavior in non-mammalian vertebrates (REPRO)

PIs - Chuck Derby/Don Edwards - The role of olfaction in establishing social status in crayfish (AGGRESSION)

PIs - Erica Duncan/Mike Davis - Fear potentiation, conditional discrimination, and fear inhibition in posttraumatic stress disorder (PTSD) (FEAR)

PI - Matthew Grober - The effects of inter- vs. intra-specific aggression on the neuroendocrine stress axis (AGGRESSION)

PIs - Jens Herberholz/Don Edwards/Xiaoping Hu - Magnetic resonance imaging of the crayfish brain (AGGRESSION – IMAGING CORE)
PIs - Kim Huhman/Kerry Ressler - Examining the role of BDNF in mediating conditioned defeat in hamsters using lentiviral vectors (FEAR)

PI - Duane Jackson/Don Edwards - Biogenic amines and the behavioral biology of war among termites (AGGRESSION)

PI - Aras Petrulis/Laura Carruth/Andrew Clancy/Kim Huhman - The function of c-fos in sexual behavior (REPRO)

PIs - Carol Upshaw/Joanne Chu/Elliot Albers – The neuroendocrine regulation of phonotaxis in Hyla (AFFILIATION)

**Dec. 2002:**
PI – Timothy Bartness/Andrew Clancy/Ruth Harris - Do gonadal fat lipid levels control reproductive status and behavior? (REPRO)

PI – Andrew Clancy/Tim Bartness - Androgen and estrogen sensitive neurons and the neural circuit for male mating (REPRO)

PI – Andrew Clancy/Laura Carruth/Aras Petrulis/Deb Baro/Matthew Grober - Estrogen and male mating (REPRO)

PI – Fernando Gonzalez/Elliott Albers - Food competition and physiological concomitants of dominance in the rat (AGGRESSION)

PI – Stephan Hamann/Kim Wallen/Mark Wilson/Xiaoping Hu - Acute androgen effects on human fMRI response to sexual stimuli (REPRO – IMAGING CORE)

PIs - Kerry Ressler/Larry Young – A novel tool to visualize the “labeled-line” representation of olfactory memories (FEAR-AFFILIATION)

PI – Jeanne Stahl, Fernando Gonzalez, Elliott Albers, Brown - Dominance and submission in competition for space, food, and sex (AGGRESSION – BEHAVIORAL TECH CORE)

PI – Jim Winslow/Pat Whitten/Mar Sanchez - Dopaminergic development and social affiliation (AFFILIATION-REPRO)

### D. Center Personnel

**List all personnel funded through the center for the prior fiscal year. Use this format. Faculty who receive course releases or full or partial summer pay should be counted as center members.** Center faculty are not directly funded by center money except for venture grant projects, collaboratory or core money. Some postdocs and
graduate students are fully or partially supported with center funds. For simplicity, participants who have received any center funds during the past fiscal year are noted with "*" beside their names.

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* indicates that the individual is a graduate student.