DEPARTMENT OF
Psychiatry
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

Update on research efforts aimed at understanding the development of psychiatric illness.

Dr. Ned Kalin, Chair of the Department of Psychiatry at the University of Wisconsin–Madison has dedicated his career to understanding the development of psychopathology with the goal of decreasing the suffering of patients with psychiatric diseases. Notably, anxiety and depressive disorders are common in children and can have severe consequences, including suicide. While current treatments can be effective, sadly, many individuals fail to get better.

Below, are new findings that are detailed in a progress report to the NIH that summarize new results from Dr. Kalin's recent grant. This grant examines differences in brain function and brain structure in non-human primates as a model of highly anxious human children who are likely to develop anxiety and depressive disorders. The overall goals of the research are to develop new treatments to prevent the life-long suffering that accompanies anxiety and depressive disorders, and thereby stem the increasing rates of suicide.

The progress described in the report includes data from "nursery/peer-reared" monkeys. The nursery/peer-reared monkeys at the Wisconsin National Primate Research Center were either neglected or abused by their mothers. As part of the standard animal husbandry practice, the baby monkeys were initially housed in incubators located in a clinical nursery (like in a NICU), and fed by human caregivers until they were able to feed themselves. Once able to feed themselves, they lived alongside other young monkeys.

Childhood neglect is all too common in our society. Similarly, maternal rejection occurs in non-human animals living in natural habitats, as well as in zoos and research environments. Nursery/peer rearing is a commonly used method to care for a young monkey when it is rejected by its mother. UW–Madison researchers chose to study nursery/peer-reared monkeys because of the relevance of childhood neglect to psychiatric illnesses.

Dr. Kalin invites interested parties to examine the scientific results of this progress report to learn more about the research.

A. COMPONENT COVER PAGE

Project Title: Project 1: Neural mechanisms mediating adversity's impact on the risk for developing anxiety

Component Project Lead Information:

Kalin, Ned H

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Anxious temperament (AT) identified early in childhood is a significant risk factor for developing anxiety, depression, and comorbid substance abuse. AT children at greatest risk for developing psychopathology remain stable in this disposition across development. Using young nonhuman primates, we characterized the neural circuitry that underlies the AT phenotype (increased threat-related freezing and cortisol accompanied by decreased vocalizations) and identified a key component of the circuit, which is trait-like increased metabolic activity of the central nucleus of the amygdala (Ce). In addition to genetic factors, negative environmental events are important in mediating the development of AT and maintaining its stability as it subsequently develops into psychopathology.

While numerous studies document the marked increased risk for developing psychopathology associated with early adversity or trauma, very little is known about the mechanisms by which early adversity affects development of the structure and function of brain systems that mediate maladaptive anxiety. The proposed studies will focus on the amygdala by examining mechanisms in the Ce of primates that mediate the influences of early adversity on the development of extreme AT. Peer rearing (PR), most akin to parental neglect and orphanage rearing, will be used as the adverse experience because it robustly increases emotional reactivity and anxiety. This manipulation allows individuals to form attachment bonds with peers but deprives them of benefiting from the mature experience and nurturance associated with good parental rearing. Multimodal structural and functional imaging strategies will be repeatedly employed during the first year of life. These data will be used to test the hypothesis that adversity increases AT by maintaining increased amygdala activity via a failure of the normal development of prefrontal cortical (PFC) – amygdala regulatory pathways. Importantly, the primate model provides the opportunity to examine molecular mechanisms in the lateral division of the Ce (CeL) that we hypothesize via altered GABAergic neurons projecting to the medial division of the Ce (CeM) and bed nucleus of the stria terminalis (BST) mediates the effects of adversity on neural circuit level changes underlying AT. At 1 year of age, brains will be collected to perform immunohistochemical (GABA and DARPP32) and molecular analyses (RNA-seq) on CeL tissue. In addition, induced pluripotent stem cells (iPSC) will be made and differen-tiated into GABAergic neurons. The data from mature CeL GABAergic neurons obtained from 1 year old brains will also be used to validate the use of iPSC derived GABAergic neurons for investigating basic cellular mechanisms underlying extreme AT.

Aim 1: Fully characterize, in developing rhesus monkeys, the ontogeny of the AT phenotype. Explore the hy-pothesis that PR will result in a developmental delay in the ability to adaptively regulate anxiety and will be associated with the development of extreme AT.

Aim 2: Examine the hypothesis that early adversity increases AT by means of maintaining increased amygdala metabolism across development and that dispositionally increased amygdala metabolism is associated with impaired development of PFC-amygdala functional and structural connectivity. Multimodal functional (FDG-PET, rs-fMRI) and structural (DTI, VBM) imaging will be performed repeatedly across development in maternal reared (MR) and PR monkeys. In addition to neural circuit systems level analyses, these data will be used with the post mortem data to understand how molecular changes in a key component of the Ce microcircuit influences the development and function of the regulatory PFC-amygdala macro circuit.

Aim 3: Examine the hypothesis that early adversity enhances AT by decreasing the expression of neuroplasticity genes in CeL GABAergic neurons. The CeL is comprised of > 95% GABAergic medium spiny neurons that project to and regulate CeM and BST. In turn, CeM and BST neurons provide the outflow to downstream stress relevant effector sites. In our ongoing collaboration with the [redacted], RNA deep sequencing (RNA-seq) will be performed in laser captured microdissected CeL neurons. Transcript expression profiles of neuroplasticity genes will be identified that reflect the influences of early adversity and predict individual differ-ences in AT and CeL metabolism.

Aim 4: iPSC cell lines will be made from fibroblasts collected from four high AT and four low AT monkeys. The [redacted] laboratory has been very successful in deriving GABAergic forebrain neurons that share phenotypic characteristics with CeL GABAergic neurons. This will afford an unprecedented opportunity to compare, within the same high and low AT animals, transcription profiles from mature CeL neurons with those from the iPSC derived GABAergic neurons. Functional differences between high and low AT cell lines will be characterized with electrophysiology exploring the hypothesis that GABAergic neurons derived from high AT monkeys will show increased firing activity and increased GABA release. Validation of the iPSC in vitro model will allow for further mechanistic studies.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B.2 Accomplishments Proj 1.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITITES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

For Aim 2 we are obtaining a customized, special MRI coil for infant monkeys that will enable early life scanning to assess the longitudinal trajectories of gray and white matter development as well as metabolic activity. These structural and functional measures will be assessed in relation to the development of individual differences in AT. For Aims 3 and 4 we have identified animals that will serve as our initial pilot subjects to refine the methods for iPS cell line production. Skin biopsies will be collected, fibroblasts will be harvested and iPS cells will be produced. These animals will be sacrificed to compare gene expression profiles in CeL amygdala cells and iPS cells and how early adversity impacts the structure and function as well as gene expression profiles in both cell populations.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

Major Activities – We have made significant progress towards completing the studies described in Aims 1 and 2 of the funded proposal. We examined the effects of maternal/peer rearing in a non-human primate model of AT. Specifically, in 25 nursery/peer-reared monkeys and 25 age-matched, maternally-reared monkeys we assessed differences in measures of anxiety-related behavior, stress hormone and neuropeptide levels, brain structure (diffusion tensor imaging; DTI and deformation based morphometry; DBM), metabolic activity (^{18}F-fluorodeoxyglucose-positron emission tomography; FDG-PET) and resting functional connectivity (fMRI).

Specific Objectives – To assess the effects of early life adversity on behavior, we measured the levels of anxiety-related behavior in the mildly threatening No-Eye-Contact (NEC) condition of the human intruder paradigm. This paradigm is used to characterize individual differences in anxious temperament (AT). At the end of this paradigm we collected plasma to measure the stress-induced levels of the hormone cortisol and the neuropeptide oxytocin and we collected cerebrospinal fluid (CSF) to measure levels of corticotropin-releasing hormone (CRH) and oxytocin. The brain metabolic activity that occurred during NEC exposure was measured using (FDG-PET) and behavior was rated to assess AT. In order to identify structural differences, MRI data was collected, as well as DTI to measure the structural connectivity and fMRI to measure functional connectivity between brain regions.

Significant Results – Data were collected in 50 young rhesus monkeys (25 nursery/peer-reared: mean-age: 1.76 yrs; 7 females; and 25 matched controls: mean-age: 1.82 years; 7 females). During NEC there was significantly more self-directed behavior in the nursery/peer-reared animals compared to the maternally-reared animals (Figure 1). This self-directed behavior consisted primarily of finger and toe sucking. This suggests that the early-life stress leads to aberrant behavioral attempts at self-soothing. Somewhat surprisingly, there was a small but significant decrease in the duration of freezing behavior observed during the NEC (data not shown). This was also associated with alterations in other behaviors indicative of a decrease in anxiety. In addition, assessment of behavior during other conditions of the human intruder paradigm, such as the alone condition, suggested that nursery/peer reared animals were less anxious. This is important because it indicates that across different contexts, even those not associated with the potential threat of a human, nursery/peer-reared animals are less anxious.

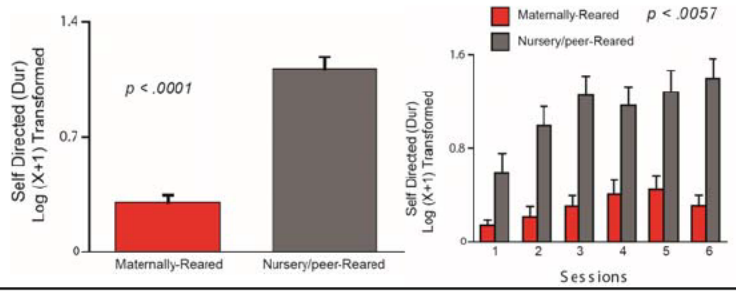

Figure 1. Increase in self-directed behaviors in nursery/peer-reared animals compared to maternally-reared. The graph on the right shows the duration of the self-directed behaviors assessed in the six 5-minute epochs that comprise the 30-min NEC session. The graph on the left shows the duration of self-directed

We also assessed CSF levels of CRH and oxytocin in the two groups of monkeys. There were significantly lower levels of oxytocin in the CSF of nursery/peer-reared monkeys compared to the maternally-reared monkeys (Figure 2), which is in agreement with previously published work (Winslow et al, *Neuropsychopharmacology* 2003). This effect was selective to CSF as plasma levels of oxytocin did not differ between the nursery/peer-reared (367.4 ± 84.7 pg/ml) and maternally-reared animals (305.2 ± 63.4 pg/ml; p=0.52; N=25). In addition, the CSF CRH levels did not differ between nursery/peer-reared (38.9 ± 3.4 pg/ml) and maternally-reared monkeys (37.3 ± 3.9 pg/ml; p=0.74; N=25).

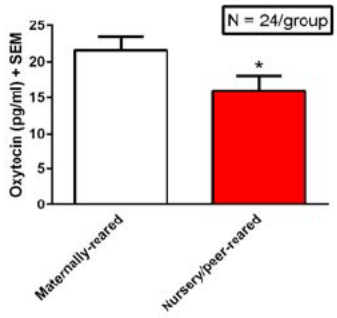

Figure 2. A history of nursery/peer rearing results in lower levels of oxytocin in the CSF compared to maternally-reared monkeys. *, p < 0.05,

There was also no significant difference in plasma cortisol levels sampled immediately following NEC (nursery/peer-reared 62.6 ± 2.4 µg/dL; maternally-reared 62.0 ± 2.3 µg/dL).

Functional brain activity was also assessed in the monkeys using FDG-PET to measure brain metabolism that occurred during NEC. These results demonstrated significantly lower levels of

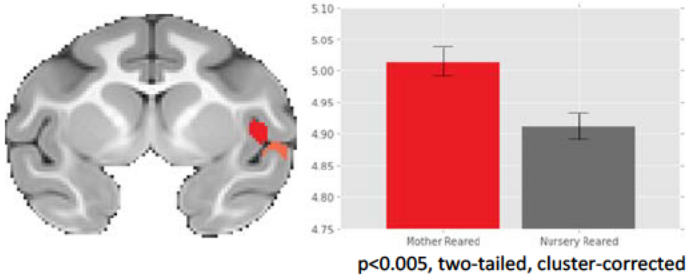

Figure 3. Decreased anterior insular cortex metabolism in nursery/peer-reared monkeys compared to maternally-reared, age-matched controls.

metabolic activity in the anterior insula (Figure 3). Interestingly, across both groups, the metabolic activity in this brain region was correlated with the amount of freezing diplayed in the NEC (Figure 4).

DBM was used to assess volumetric differences between groups and the size of several brain regions were revealed to be different between the nursery/peer-reared and the maternally-reared monkeys (Figure 5). Specifically, the superior temporal gyrus and medial orbitofrontal cortex were significantly larger in the nursery/peer-reared animals; whereas, the dorsolateral prefrontal cortex and a regional of the dorsal amygdala/anterior commisure were smaller in the nursery/peer-reared animals.

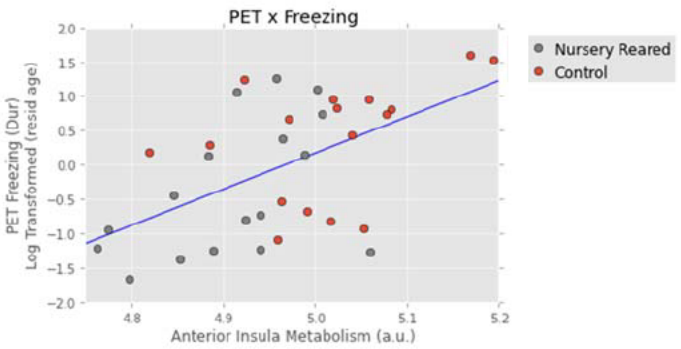

Figure 4. Anterior insular cortex metabolism as assessed by FDG-PET was correlated with freezing behavior measured during the NEC. T=2.471, p = 0.01, controlling for group, sex and age.

Nonlinear tensor normalization (DTI-TK) was performed and fractional anisotropy (FA), a measure of white-matter integrity, was computed. The effect of early adversity on FA was tested using voxel-wise group t-tests. Additionally, regressions were performed between FA and stress-related cortisol levels. In nursery/peer-reared animals, FA was significantly (p<.005, two-tailed uncorrected) higher in the anterior uncinate fasciculus (UNC) and internal capsule (IC) (Blue in Figure 6). In contrast, FA was lower within the ventral amygdalo-fugal pathway (VAF) and paraventricular thalamus (PV) (Red in Figure 6). Interestingly, FA in IC, VAF and PV significantly correlated with stress-related cortisol (p<.05, r>.26; graph not shown). These results demonstrate that nursery/peer rearing was associated with differential microstructural alterations within white-matter tracts connecting the amygdala to other limbic and prefrontal regions. Nursery/peer-reared animals showed decreased FA in VAF, which connects the amygdala to the bed nucleus of stria terminalis, and increased FA within UNC, which links the amygdala to prefrontal cortex.

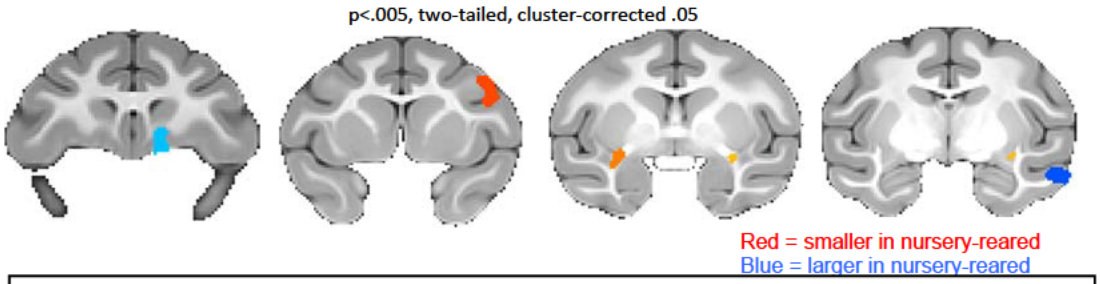

Figure 5. Influence of early-life adversity on local brain volumes.

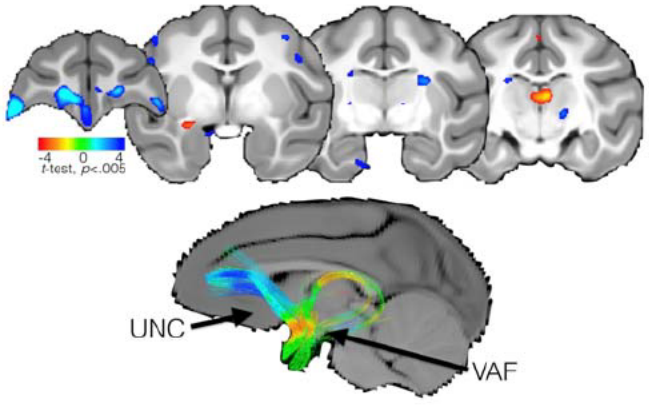

Figure 6. Influence of early life adversity on FA.

Key outcomes – These studies identify several interesting and potentially important differences associated with the early life adversity of nursery/peer rearing. Compared to maternally-reared monkeys, nursery/peer-reared monkeys displayed significantly more self-directed behaviors during the potentially threatening NEC condition and this was associated with lower amounts of freezing behavior. In addition, CSF oxytocin levels were decreased in the nursery/peer-reared monkeys. Early-life adversity was also associated with significantly less metabolic activity in the anterior insular cortex, a region that in humans is associated with uncertain anticipation and interoceptive somatic representations. There were also significant differences in local brain volume between the two groups of monkeys. Lastly, work examining structural brain connectivity suggests that white matter tracts connecting the amygdala to the prefrontal cortex are altered by the experience of early-life adversity. Taken together, these results indicate significant effects of early life adversity on behavior and brain metabolic activity. The findings of alterations in brain volume and white matter connectivity highlight a potential mechanism by which the effects of early adversity may influence the risk to develop psychopathology.