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14. ABSTRACT Our overarching scientific hypothesis holds that serotonergic influences on brain development, driven by genetics and early experience, induce a variation of normal brain anatomy that makes the brain highly susceptible to the effects of severe stress. After TATRC review in January of 2011, a revised research plan was developed to include a pre/post-deployment study at Fort Hood and anatomical studies of PTSD in collaboration with NIMH, Yale and USUHS. BAMC IRB for the clinical study was approved in December 2012 and the study was approved by HRPO in October 2013, with no changes recommended by HRPO. However, BAMC review of changes in the protocol has further delayed BAMC approval. Post-mortem brain tissue from 19 PTSD, 18 MDD and 36 controls is being studied with several molecular approaches. A subgroup of Control and PTSD cases (N=8) is being studied with anatomical and molecular techniques. Major findings being prepared for publication include 1) Decreased mature dendritic spine density in the straight gyrus of PTSD BA11 (medio-orbital frontal cortex = mOFCtx) involving mushroom spines (Young et al., 2015), 2) Increased density of stubby spines, suggesting that some mature mushroom spines have regressed to a more immature phenotype in PTSD (Young et al., 2015), 3) evidence of major disruption of microRNA levels in suicide and major depression (Carter et al., in revision), 4) Approximately 1 in 6 microRNAs (112/716) are elevated in medial straight gyrus in PTSD, indicating a major change in cell physiology (Carter et al., in revision), 5) BA11 gene expression assays indicate evidence of reductions in neuronal elements and accentuation of astrocyte activity, possible evidence of accelerated aging, 6) Considering that BA11 is a vital link between the limbic system and brainstem centers governing Autonomic nervous system, HPA activity, heart rate, startle and other physiological systems that are dysregulated in PTSD, this data suggests that anatomical and synaptic irregularities in BA11 may underlie a loss of synaptic plasticity that normally allows this region to gate physiological drive into brainstem, resulting in autonomic and HPA axis dysregulation and psychophysiological distress. These changes may contribute to long-term acceleration in whole-body aging.					
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INTRODUCTION:

This research has been funded in two installments (Phase I and Phase II contracts). The research described below is continuing through 2016 with implementation of the phase II contract, when the main body of the data will be available for analysis and publication.

Our overarching scientific hypothesis holds that serotonergic influences on brain development driven by genetics and early experience induce a variation of normal brain anatomy that makes the brain highly susceptible to the effects of severe stress. Nineteen PTSD brains comprise the core of the collection and both molecular and anatomical techniques will be employed to study these brains. One paper was published this year with 2 additional manuscripts submitted and 3 in preparation. Our data suggest that there are strong signals of accelerated aging in PTSD brain, and that this signal is similar to that observed in Major Depression.

BODY:

KEY RESEARCH ACCOMPLISHMENTS:

Administrative:

Work on the post-mortem portion of the proposal have progressed at accelerated pace, as described below. The IRB for Project 1 has completed initial review at BAMC and has received HRPO and Cadaver approval. No known issues.

Project Specific:

Task 1. Collection of PTSD, MDD and control brains

We have been very successful in collecting PTSD cases. A total of 30 PTSD, 30 MDD (psychiatric controls) and 60 normal controls have been diagnosed and comprise our current available cohort, the largest PTSD post-mortem cohort in existence. Our Current testing cohort consists of 19 PTSD, 18 MDD and 38 Controls.

As a related matter to this task, we have assisted in standing up a PTSD Brain Bank for the VA. The Bank has been designed so that PTSD cases at several sites can be shared to increase the scientific usefulness of the collections, including those cases used in the present study. The Official VA announcement is here <http://www.va.gov/opa/pressrel/pressrelease.cfm?id=2715> An additional 40 brains were obtained for the collection this quarter, and will be available to validate present findings.

Task 2. Compare gene expression in the frontal cortex of PTSD and controls.

We have added additional MDD cases to the analysis this past quarter for a total of 19 PTSD cases and 36 controls. A separate biostatistician at JHU has blindly repeated our analysis. The analysis reveals that the preliminary findings of accelerated aging from last report were largely supported with the larger N, in both PTSD and MDD samples.

BA11 Gene expression in PTSD. We have performed differential gene expression assays on medial orbital frontal cortex tissue (BA11) from 19 individuals with PTSD, 18 MDD and 36 controls using ANCOVA analysis, controlling for age, race, PMI, pH and gender. Blind reanalysis by colleagues at JHU using different normalization procedures yielded virtually identical results to that below and this final product is being submitted for publication. In addition, we found that many similarities between PTSD and MDD groups.

The top 200 transcripts with the greatest differential effect size (in both directions) and FDR less than 0.05 were examined in DAVID using an overall BA11 gene expression background (N=10,015). The top 200 genes that were less abundant in PTSD were found to be enriched in neuronal elements while genes that were more abundant in PTSD were enriched in astrocyte markers. Examples of neuronal transcript reductions include synaptophysin (FDR corrected $p < 0.0012$) and the GABR4 receptor (FDR $p < 0.000006$). Gene set analysis (Toppgene) of less abundant genes in PTSD also found enrichments in genes that are down-regulated in oligodendrocytes (4.44×10^{-18}) and striking enrichments in genes that are down-regulated in interneurons (1.35×10^{-53}). Genes that decreased in abundance during aging were also less abundant in PTSD (4.7×10^{-16}). The number of significantly reduced abundance genes identified in PTSD greatly outnumbered gene transcripts that were elevated ($>10:1$). David analysis found that genes that were more abundant in PTSD were enriched in genes that are up-regulated in astrocytomas (Table 3), and gene set analyses found enrichment in genes sets up-regulated in astrocyte cell lines (Table 4). Metallothionins, excreted by astrocytes in the brain to combat cellular stressors, were identified by DAVID analysis as the most positively enriched class of transcripts in PTSD (FDR $p < 0.00001$). All nine metallothionins expressed in brain had numerically increased numbers of transcripts in PTSD, with 5 significantly elevated (FDR $p < 0.05$) and 2 elevated at the trend level (0.05-0.1). Important astrocyte genes involved in glutamate removal from the

synapse and thought to be involved in a variety of neuropathologies, slc1a2 (GLT-1, EAAT2) and slc2A3 (Glast, EAAT3) were also found to be elevated in PTSD. Finally, transcripts for APOE were elevated in PTSD, a significant finding considering recent indications that APOE genotypes confer susceptibility for PTSD. The MDD group showed similar signals as PTSD, and we are contrasting the two groups to look for potential differences.

There are multiple interpretations possible for these observations. The most likely possibility is that the findings are primarily driven by changes in the ratios of neurons and astrocytes. Second, the data could indicate physiological phenomenon where neurons in BA11 are quiescent/impaired and astrocytes are activated, indicating up- and down- gene regulation.

Interpretation: Based on the data from our recently published study, the largest post-mortem PTSD study to date, and the results of the BA11 gene expression data, we are pursuing an explanation that involves robust accelerated aging (senescence) in PTSD and MDD Orbitofrontal Cortex.

Task 3. Compare anatomical markers in frontal cortex/hippocampus of PTSD, MDD and controls, with 5HTTLPR and other genetic variants as cofactors.

BA11 spine density in PTSD. This paper is now published (<http://dx.doi.org/10.1016/j.ynstr.2015.07.002>). We concluded that there is an increased stubby spine density in BA11 (mOFCtx) and a trend for decreased mushroom spine density in PTSD (BA11), consistent with animal models. A subset of mature mushroom spines appear to have been partially replaced by stubby spines. Stubby spines are less sophisticated than mushroom spines because they do not have necks, where modulatory neurotransmission can be applied to alter excitatory glutamatergic activity. This is the first confirmation that changes in dendritic spines may be a “final common pathway” for extreme stress in humans, as has been observed in animal models. Considering that BA11 is a vital link between the limbic system and brainstem centers governing HPA activity, heart rate, startle and other physiological systems that are dysregulated in PTSD, our data suggest that synaptic irregularities in BA11 may underlie a loss of synaptic plasticity that normally allows this region to gate physiological drive into the brainstem, resulting in HPA axis dysregulation and psychophysiological distress. This anatomical data is consistent with the above molecular evidence suggesting neuronal deficits in PTSD.

Molecular studies of microRNAs (miRs).

We have performed MiRNA studies of BA11 in PTSD and found that approximately 1 in every 6 (112/716) brain-expressed miRs were significantly elevated in PTSD at a nominal p value of 0.05, while only 4 were significantly decreased. This is strong evidence ($t < 4.2 \times 10^{-17}$, fisher's exact test) for a global up-regulation of miRs in PTSD. Unlike MDD, we found that this increase was not linked to changes in DICER transcript levels. In the past quarter, we extended our analysis of miR to focus on gender-specific changes in the MD nucleus. We found several miR that are differentially altered according to gender, such as MiR -582-3p was elevated in female controls, but significantly suppressed in SKZ females. In the next quarter, we will extend these gender analyses to our PTSD cases.

In a second set of findings, we observed evidence for systematic changes in miR populations in the mediodorsal thalamus (MD) due to 5HTTLPR genetic effects. The MD provides powerful glutamatergic input to the frontal cortex and is an important node in limbic circuitry. MiR levels and 5HTTLPR genotype with rs23456 following the “triallelic” convention were assessed in RNA/DNA extracted from frozen MD specimens of the Stanley Foundation Research Consortium (12 MDD, 12 SKZ and 14 controls) using PCR and Exiqon Human miRnome V1.M RT-PCR panels, resulting in an N = 577 MD-expressed miRs. A common pattern was that 5HTTLPR SS alleles were associated with reduced miR levels compared to SL genotype across many miR species, as evidenced by a significant reduction in total miR load ($p < 0.027$), controlling for diagnosis, age, gender and PMI. The most reliably affected SL>SS miRs (abundant miRs with p's <0.02) included miRs-7d*, 1979, 320a, 432, 532-5p, 664 and 92b. Interestingly Mir-432, which targets ELK1 (a transcription factor binding to 5HTTLPR), has previously been found to be decreased in SKZ blood. The 7 SS-reduced miRs targeted PTPRD, CNIH, FAM19b, and TOB1 mRNAs as a group. Several of these genes have been previously associated with either SKZ (CNIH, TOB1 and PTPRD) or MDD (TOB1). MiR-377 had

the most reliable evidence for elevated levels compared to SL/LL. Several miRs expressed at relatively low levels displayed significantly altered but skewed distributions suggestive of selective miR editing in SS or LL genotypes, with loss of probe annealing potentially due to edited miR sequences. There was no relationship between 5HTTLPR genotype and MD mRNA levels for DGCR8, Drosha or Dicer transcripts. The present data support a pathophysiological phenotype consisting primarily of reduced miR levels in the MD nucleus of the thalamus associated with the 5HTTLPR-SS genotype. The Mir data was presented at the Society for Neuroscience Annual Meeting and is being prepared for publication.

REPORTABLE OUTCOMES:

1 papers published:

2) **BA11 FKBP5 expression levels correlate with dendritic spine density in postmortem PTSD and controls** <http://dx.doi.org/10.1016/j.ynstr.2015.07.002>

Abstracts:

Gender-specific changes in microRNA's in depression and schizophrenia, Military Health System Research Symposium (MHSRS) 2015.

MiR level reductions in MD thalamus in 5HTTLPR-SS genotype, Society for Neuroscience Annual Meeting, 2015. (<http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=ce01c3b2-ea91-494d-a504-5dde14eb7d79&cKey=bee526d1-1eaa-4169-85d6-33cf14da1b28&mKey=d0ff4555-8574-4fbb-b9d4-04eec8ba0c84>)

CONCLUSION: No scientific conclusions have been made at this point in time.