Assessment of Diet and Stress Effects in Rodent Models of Operationally-Relevant Brain Insults using a Multi-Omics Approach

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Abstract

US Soldiers engaged in combat operations often suffer traumatic brain injuries (TBI), as well as later development of post-traumatic stress disorder (PTSD). These insults can have an overlap of symptoms, which complicates diagnosis. Systems level and high throughput approaches are key to understand the primary molecular mediators, as well as to discriminate between these afflictions. There is also a need to find effective pharmacological therapies. Nutritional countermeasures are a potential approach for preventing blast TBI and PTSD-related debilitations. Dietary omega-3 polyunsaturated fatty acids are crucial components of neuronal membranes and can be converted to potent anti-inflammatory metabolites. Thus, we explored the possibility that an omega-3 poor diet increases the vulnerability to TBI and PTSD. To examine this, adult male rats were maintained on a standard house chow or custom-made diet deficient in omega-3s. After anesthesia, animals were exposed to a simulated blast over pressure wave followed by a weight drop head-concussion to induce TBI. Separate rats were subjected to forced immersion under water to induce traumatic stress. Shams received anesthesia and/or handling. Animals were assessed out to 14 days post-insult, using behavioral testing and brain histopathology. Likewise, gene expression was evaluated in the blood and brain, using transcriptomic assays, and intestinal (fecal) microbiome populations determined, using 16S rRNA sequencing. Our results found TBI and traumatic stress exposures in rats, within 14 days, produced accompanying neurobehavioral and neuropathological impairments. Brain and blood showed distinct genomic transcript profiles for the two insults, with most differentially enriched for inflammation response and oxidative stress related networks. For the intestinal microbiome, the insults lead to imbalances in beneficial bacterial species. In particular, the genomic and microbiome changes were exacerbated by the omega-3 deficient diet. Thus, our findings suggest that poor dietary conditions can markedly influence the resistance of Warfighters to TBI and PTSD. This work was intramurally funded by the USAMRDC / MOMRP.

Background

The high incidence of mild TBI and PTSD among combat personnel has prompted recognition of the need to establish the means to increase resilience to these insults to hasten safe return-to-duty and minimize subsequent debilitations (1-3). Using rats, we attempted to establish whether an omega-3 polyunsaturated fatty acid (ω-3 PUFA) deficient diet promotes vulnerability to TBI and traumatic stress. Long chain PUFAs are vital neuronal components, ligands for transcription factors, and precursors to bioactive mediators of inflammation (4-6). ω-6 and ω-3 PUFAs compete with each other in biochemical pathways, as based upon their respective ratios and levels in the diet (7-10). The present-day Western diet is relatively deficient in ω-3 PUFAs and very high in ω-6 PUFAs, i.e., 10-20:1. This imbalance leads to adverse consequences as ω-3 PUFAs (e.g. docosahexaenoic acid; DHA) have potent anti-inflammatory, inflammation resolving, and neuroprotective properties; whereas, ω-6 PUFAs (e.g. arachidonic acid) primarily stimulate inflammation responses. Diet-induced changes in PUFA composition can also modify membrane fluidity and signal transduction (8). All of these mechanisms can influence the relative vulnerability or resilience of a warfighter to blast induced TBI and traumatic stress. Such diet-derived vulnerabilities, should be readily and safely correctible through dietary supplements (e.g. fish oil), so the results of this research are highly translatable.

References

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Methods

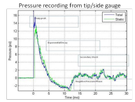
In our study, rats are fed one of two isocaloric rodent chows specially formulated to compare different ω-6 and ω-3 PUFA nutritional compositions, i.e., 1% of energy (en%) or 8 en% as derived from linoleic acid (LA; ω-6 PUFA), along with 1 en% as α-linolenic acid (ω-3 PUFA), but devoid of long chain ω-3 PUFAs (e.g. DHA) (11). Separate rats are given a standard animal-facility diet (house chow), which is well-balanced in all PUFAs, including DHA. The animals are then exposed to high fidelity simulated blast waves plus a weight drop skull concussion (12) or an underwater trauma stressor (13). The resulting changes in the brain are characterized mainly by behavioral and pathophysiological assessments.


Adult male Sprague Dawley rat (30 day-old; ~150 g)

Dietary conditions: Rats are fed house chow, 1% LA, or 8% LA diets for at least 6 weeks; and then exposed to blast plus weight drop or underwater trauma insults.

Blast wave exposure: Rats are blasted once at 20 psi (face-on), under anesthesia.

Blast!



Weight drop exposure: Rats are given a weight drop skull concussion, after blasting; 500 g from 125 cm.

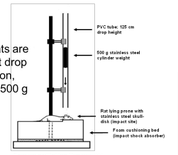
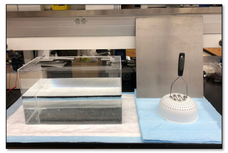


Figure was modified from Marmarou, 1994

Underwater trauma exposure: Rats are subjected once to an underwater trauma stressor (tank, lid, and plunger shown) using a 30 sec free-swim followed by a 30 sec immersion. Shams just swim for 1 min.



Rotarod: Test for balance and coordination. Rats were pre-trained for 2 days on rotarod device (Columbus Instruments) at 10 rpm for 45 sec. Animals were tested using 5 - 35 rpm over 60 sec at baseline and then 2 - 14 days post-blast + weight drop. Latency to fall was reported.

Elevated plus maze: Test for "anxiety" behavior. Animals were placed in center of an elevated plus maze device (Harvard Apparatus) and allowed to freely explore the two open and closed arms (i.e., risky and safe) for 5 min at 8 days post-underwater trauma. Time spent on arms was reported, as the open to closed arm ratio.

Food and tissue fatty acid compositions: Total lipids were extracted from rat food, liver, or brain samples using chloroform / methanol / water. Fatty acid methyl esters (FAME) were prepared by reacting lipids with BF_3 in methanol. FAME were analyzed by GC/MS (Agilent Technologies) and fatty acid species were reported as a percentage of total and/or concentration (i.e., linoleic acid, mg/g food).

Brain histopathology: Brains of PFA-perfused rats at 14 days post-blast + weight drop and underwater trauma were prepared by coronal sectioning and then silver staining or senescence detector (i.e., β-galactosidase expression), respectively (FD Neurotechnologies and Biovision). Sections were examined by microscopy for axonal fiber tract degeneration in the optic tracts and senescent (aged) neurons in the hippocampus and amygdala. Intensity of the coloration, which corresponds to the degree of neuronal damage present, was reported.

Genomic and microbiome arrays: Whole brain and fecal pellet samples from rats at 14 days post-blast + weight drop or underwater trauma were collected. Brain gene expression changes were determined by analyzing extracted total RNA on whole rat genome microarrays (GE_4x44Kv2 two-color; Agilent Technologies). Intestinal (fecal) bacterial populations were characterized by identification of 16S ribosomal RNA using Illumina MiSeq. Microbiome data analysis was conducted by a standard metagenomics pipeline, and reported as Principle Coordinate Analysis (pCoA) plots.

Results

Liver and brain DHA and ω-6 / ω-3 ratios were impaired by feeding rats 1 en% and 8 en% LA diets for 6 weeks:

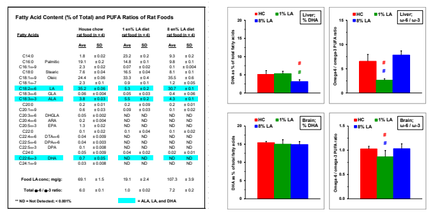


Figure 1. Fatty acid compositions of rat foods, i.e., house chow and 1 en% & 8 en% as from linoleic acid; LA (n = 4). Also, shown are bar graphs for the % DHA content and ω-6 / ω-3 ratios of rat liver and brain after consuming these diets for 6 weeks (mean ± SD, n= 5). #p ≤ 0.05; significant between diets, as by t-test.

Blast plus weight drop exposure leads to balance and coordination impairments in rats fed house chow and 1 & 8 en% LA diet, as by rotorod testing at out to 14 days post-insult:

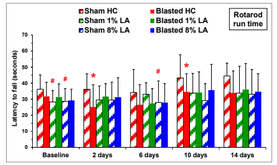


Figure 2. Bar graphs for rotarod testing (latency times), i.e. for balance and coordination, of blast plus weight drop exposed rats at baseline and 2 - 14 days post-insult (mean ± SD, n = 10). *p ≤ 0.05; significant difference from shams, as by t-test. #p ≤ 0.05; significant difference between diets.

Underwater trauma exposure leads to "anxiety" like impairments in rats fed house chow and 1 & 8 en% LA diets, as by elevated plus maze testing at 8 days post-insult:

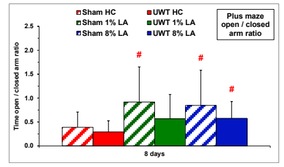


Figure 3. Bar graphs for elevated plus maze testing (open to closed arm time ratios), i.e., for "anxiety", of underwater trauma (UWT) exposed rats at 8 days post-insult (mean ± SD, n = 14). #p ≤ 0.05; significant difference between diets, as by t-test.

Blast plus weight drop and underwater trauma exposures lead to axonal degeneration and senescence (i.e., aging), respectively, of brain neurons in rats fed house chow and 1 & 8 en% LA diets, at out to 14 days post-insult:

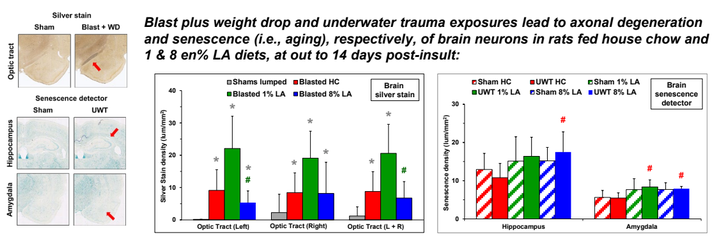


Figure 4. Histopathology (silver stain and senescence detector; 10x) of brains from sham (lumped) and blast plus weight drop and sham (each diet) and underwater trauma exposed rats at 14 days post-insult. Bar graphs for coloration intensity of brain regions (mean ± SD, n = 4). *p ≤ 0.05; significant difference from shams, as by t-test. #p ≤ 0.05; significant difference between diets.

Blast plus weight drop and underwater trauma exposures lead to brain and blood genomic changes in rats fed house chow and 1 en% LA diets, at 14 days post insult:

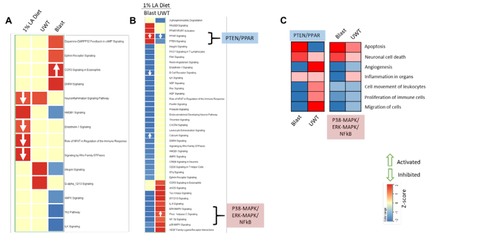


Figure 5. (A) Blast plus weight drop and underwater trauma effects on brain and blood: Heatmap generated using networks altered in brain selected based on Z score. The networks shared by the blood is shown by the arrows. The data is normalized to the sham house chow group. (B) Heatmap, as described above, for 1 en% LA diet effects on brain and blood. (C) Brain gene networks activities shifts selected from Figure 5B.

Blast plus weight drop and underwater trauma exposures lead to intestinal (fecal) microbiome changes in rats fed house chow and 1 & 8 en% LA diets, at 14 days post insult:

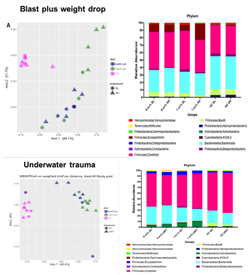


Figure 6. Principal Coordinate Analysis (PCoA) plots based on unweighted samples. Bacterial species taxonomic distributions (Ribosome Database Project) RDP level 3 (phylum-class taxa) showing average relative abundance across all study samples.

Conclusion

Overall, our findings in rats suggest that poor dietary conditions, i.e., omega-3 fatty acid deficiency, can markedly influence the vulnerability or resilience of the Warfighter to TBI and PTSD. Of great interest, it is known from the work of others that microbiome imbalances similar to what we observed can disrupt the normal hormone communications between the gut and healthy brain. The underlying changes in brain and blood molecular pathway mechanisms that we found can help facilitate and provide a knowledge-driven unbiased panel of signatures to discriminate between these two battlefield afflictions and is an essential tool for designing precise care management.