Journal of Neurology and Neurobiology

Acute and Subacute Differential Gene Expression in Rat Midbrain Following Blast Exposure Compared to Mechanical Brain Trauma

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Abstract

In this study, we employed two models of traumatic brain injury (TBI) in rats: head-directed blast overpressure exposure (OBI) and mechanical controlled cortical impact (CCI). Two groups of animals served as controls: naïve rats and rats exposed to blast noise. A genomics neurosystems biology approach was used to analyze differential gene expression between experimental groups. Midbrain RNA was isolated 24 hours (acute) and 7 days (subacute) after traumatic insult, probed with Affymetrix array containing 31,100 gene sequences, and quantified by Agilent Technologies. Differentially expressed genes were grouped into functional clusters and the significance of gene expression changes at 24 h and 7 days was further assessed using parametric and nonparametric t- test with Welch correction. Then, gene interactions in pathological pathways following blast exposures vs. mechanical impact were created and analysed using neurosystems biology tools.

According to ANOVA ($p<0.05$), there was a significant difference in expression of 994 genes within blast exposure groups (vs. control) and in 1532 genes within CCI group (vs. control) with an overall overlap of 579 genes. Parametric and nonparametric t-tests revealed significant differences in temporal profile of changes in various genes at 24 hours vs. 7 days in blast exposed animals compared to CCI. Specifically, genes involved in neural development and repair, such as ROBO1 and NEDD4 were up-regulated in CCI, while they were shown to be down-regulated following blast exposure.

These results demonstrate several differences as well as overlaps in the expression of genes between the two brain insults. This will help to reveal specific molecular signatures of each brain insult and assist in developing diagnostics of chronic posttraumatic encephalopathy.

Keywords: Blast brain injury; Controlled cortical impact; Gene expression; Neurorepair; Genomics; Neurosystems biology

Introduction

Despite remarkable progress in understanding traumatic brain injury (TBI), particularly combat related TBI, the diverse pathological pathways and long-term outcome remain to be revealed and delineated [1]. Specifically, the question has to be addressed is to what degree the mechanisms of blast-induced TBI are similar to to mechanical impact type of brain injury, including concussion, and what may be the differences. It is especially important to elucidate the molecular dynamics of changes at different levels-of molecular regulation; this would include gene expression studies (mRNA) and its regulation (miRNA), proteomics and its associated area of metabolomics.

Several studies from our lab and others have been conducted to establish a comprehensive picture of pathology underlying traumatic brain injury, including genomic and proteomic signatures [2-4]. However, there are reports available that analyse side-by-side the genomic changes occurring after blast exposures compared to mechanical insult, such as controlled cortical impact (CCI) or closed head injuries. Also, it appears to be critical to employ neurosystems biology tools to reveal and visualize gene interactions, molecular functions and biological processes involved in such brain insults, as well as to decipher the overlaps or differences in pathways underlying non-penetrating blast-induced injury and mechanical CCI damage.

The main objective of this study was to apply an advanced genomics coupled with neurosystems biology tools to compare altered gene expression patterns of moderate blast exposure with brain injury produced by a mechanical Controlled Cortical Impact (CCI) at different times after challenge. Thus, for this purpose, gene ontology analysis including molecular function and biological process were employed for the differential gene identified; in addition, a a global approach of gene interaction was to see how the genes are related along with a targeted approach to identify downstream targets are being involved due to this interaction.. This allowed us to reveal time-dependent similarities and injury-specific differences between blast and CCI brain insults and reveal critical components of neuroinflammation, neuromediator and neurorepair components.

Materials and Methods

Animal exposure to a controlled blast wave: Modeling blast overpressure exposure was achieved by variable positioning of the target vs. blast generator described in detail previously [5]. The blast pressure data was acquired using PCB piezoelectric blast pressure transducers and LabView 8.2 software. Adult male rats (Sprague-Dawley, Harlan, Indianapolis, IN, USA) were anesthetized with 4% isoflurane in a carrier gas of 1:1 O2/N2O (4 min) [5]. After reaching a deep plane of anesthesia, they were placed into a holder exposing at the distance 5 cm below the exit nozzle of the shock tube. Rats were positioned directly on the shock tube

axis to expose them to the 'composite' blast including the compressed air jet with a peak overpressure of 52 psi, 10 msec duration. The control group of animals underwent anesthesia, placed in the cage aside shock tube and blast was produced thus exposing animals to the noise only.

Controlled Cortical Impact (CCI)

A controlled cortical impact (CCI) procedure was used to model mechanical TBI on rats essentially as previously described [6]. Briefly, rats were anesthetized with 4% isoflurane as above and maintained in 2.5% isoflurane. Core body temperature was monitored continuously and maintained at 37 ± 1°C. Animals were mounted in a stereotactic frame in a prone position and secured by ear and incisor bars. Following a midline cranial incision and reflection of the soft tissues, a unilateral (ipsilateral to site of impact) craniotomy was performed adjacent to the central suture, midway between bregma and lambda. The dura mater was kept intact over the cortex. Brain trauma was produced by impacting the right (ipsilateral) cortex with a 4 mm diameter impact or tip attached to electromagnetic driver at a velocity of 3.5 m/s with a 2.5 mm compression and 200 ms dwell time.

Appropriate pre- and post-injury management for both blast and CCI was preformed to minimize pain and discomfort and to insure compliance with guidelines set forth by the University of Florida, Institutional Animal Care and Use Committee and the National Institutes of Health guidelines detailed in the Guide for the Care and Use of Laboratory Animals. In addition, research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the "Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition."

Brain tissue collection: At 24 hours or 7 days post-TBI, animals were euthanized; brain tissues were dissected with Brain Slicer device from the midbrain area (4-5 mm thick) according to the scheme (Figure 1). The central part of section (from about -5 mm to -9 mm posterior of Bregma) was cleared of surrounding cortex, pituitary and other tissues. It was cut in 2 halves and snap-frozen for proteomics (L), or preserved in RNALater (Ambion, Austin, TX) for RNA studies.

RNA isolation, quality characterization and validation were assured (Agilent Bioanalyzer, Santa Clara, CA) and genomic profiling was conducted according QC/QA guided procedures using Affimetrix GeneChip array at Assuragen, Inc. (Austin, TX). The raw fluorescent data were collected after hybridization and array scanning using Affymetrix GCOS v1.3. The data were further normalized and calculated using the Affymetrix MAS 5.0 algorithm. The summarized values were analyzed using one-way ANOVA and differences between paired naïve, noise control, and TBI groups were further determined by parametric and non-parametric t-tests with Welch correction (GraphPad, Inc.). Pathway maps were created using Systems Biology software PathwayStudio (Ariadne Genomics, Rockville, MD). Other Analysis Software: Partek Genomic Solutions 6.2 (Partek Inc., St. Charles, MO); Matlab 7 (Mathworks Inc., MA).

Neurosystems Biology Analysis

The genomics microarray differential expression of the CCI , blast injury cohorts vs. their respective controls were further analyzed using a bioinformatics systems biology approach to assess the altered pathway(s) relevant to differential specific brain injury altered genes and their its contribution to the development of either CCI and/or blast injury. PathwayStudio software (v 10; Ariadne Genomics, Rockville, MD, USA) was applied for the neurosystems biology analysis. This software helps to interpret biological meaning from differential gene expression, build and

analyze pathways, and identify altered cellular processes and molecular functions involved.

Results and Discussion

According to ANOVA (p<0.05), there was a significant difference in expression of 994 genes within blast exposure groups (vs. control) and in 1532 genes within CCI group (vs. control) with overall overlap of 579 genes. Parametric and nonparametric t-tests revealed significant differences in temporal profile of changes in various genes at 24 hours vs. 7 days in blast exposed animals compared to CCI. Moreover, expression of several genes in rats subjected to noise exposure was changed compared to naïve rats.

Several studies have been performed to evaluate gene expression changes in rodent brain following mechanical trauma, including CCI and fluid percussion injury (FPI), mostly cortical and hippocampal tissues [3,7-10]. Less information is available on gene pattern expression following blast in rodents. In a recent study in rats, hippocampal and prefrontal cortex changes have been assessed using a limited gene array with an emphasis on inflammatory components 24 hours after blast [11]. Risling and co-workers studied mechanisms of blast induced TBI in rats with and without head acceleration and in brain penetration injury, including genomic alterations in hippocampus at 24 hours, also with emphasis on neuroinflammation, cell death and synaptic transmission [12]. They noted however an important down-regulation of genes involved in neurogenesis and synaptic transmission. In mice, behavioral changes and accompanied expression of certain genes in hippocampus were compared after blast and mechanical trauma and then validated by Q-PCR [13].

In our study, we employed blast TBI model and compared side-by-side genomic changes in rat midbrain with CCI at 24 hours and 7 days after challenge using a full size Affimetrics GeneChip (31,100 sequences) array provided by Assuragen, Inc. Also, we analyzed significantly changed genes with ANOVA p<0.05 and grouped them into major functional clusters/ groups, (a) systemic/vascular responses; neuroinflammation; proteolysis; (b) neuromediators; signaling; ion channels; and (c) neural development/ repair; In both blast and CCI groups, t-tests were used for assessing significance at 24 h and 7 days vs respective controls and, in some cases CCI control vs blast noise control. Finally, gene interactions following blast and CCI have been mapped and depicted using Systems Biology tool. Pathways reflecting gene-associated molecular functions altered after blast injury were created and compared to CCI.

Systemic/vascular responses, neuroinflammation, proteolysis

Prominent changes in several proteolysis and inflammation genes were found in both blast and CCI groups, including acute phase kininogen 1, cathepsins and chemokines (Table 1A and 2A). However, there are important disparities between blast and CCI groups in these responses. First, several chemokines were markedly up-regulated 24 h and 7 d post-CCI impact, while only C-X-C ligand 1 mRNA expression was modestly increased 24 h post-blast followed by down-regulation at day 7 after blast. Second, expression of TNF superfamily member 11 was highly increased at all times after blast exposure, but was unchanged after CCI. In contrast, MMP-9 gene was up- regulated after CCI throughout post-impact period, but slightly down-regulated at day 7 after initial modest increase at 24 h following blast (Table 1A and 2A).

There are several common regulators and pathways for both blast and CCI groups such as kininogen 1 (KNG1) linking inflammation and hemostasis, cytokines and downstream NF-kB (Figures 1 and 2). The difference is reflected by involvement of estrogen receptor 2, hemoxygenase and several chemokine network in CCI (Figure 2),

while CD69, C-reactive protein and acute phase cytokines together with vasoconstrictor endothelin gene are up-regulated pathways mostly characteristic for blast exposure (Figure 1). As mentioned above, distinct directions and time-course of changes in genes encoding tumor necrosis factor (TNFSF11) and metalloproteinases (MMP-9) has been revealed.

Early up-regulation of genes encoding cytokines and chemokines (IL-1, IL-6, C-X-C-L10) has been demonstrated in several recent studies, including gene expression profiling in hippocampus and cortex after both CCI and blast [7,11 14,15]. In addition to pro-inflammatory molecules, up-regulation of anti-inflammatory IL-10 and tissue inhibitor of

Table 1: Selected mRNA differentially expressed in midbrain after blast exposure. RNA samples were hybridized with fluorescent labelled probes in triplicate using Affimetrix chips and analysed with Agilent Technology scanner equipped with software (see Materials and methods for details). mRNAs were grouped in functional clusters and time-course difference of most important gene expression is presented. Background-corrected and internal control-normalized fluorescence values are shown as Mean+ SEM of at least 3 rats in animal group, each rat sample processed in duplicate.

|	Contol	1 day post-blast		7 days post-blast		P	Fold-Change		
	Mean ± SEM	Mean	± SEM	Mean	± SEM	ANOVA	RefSeq ID	D1 /Control	D7/Control
A. Systemic/vascular; neuroinflammation; proteolysis									
Up-regulated:									
granzyme B	1.11	0.25	4.23	0.27	3.95	0.41	0.003	NM_138517	**3.81**	**3.56**
caspase 9	4.44	0.05	6.02	0.28	6.03	0.25	0.003	NM_031632	**1.36**	**1.36**
kininogen 1 similar to alpha-1 major acute phase protein	2.41	0.34	5.52	1.35	1.69	0.14	0.033	NM_001009628	**2.29**	**0.70**
tumor necrosis factor superfamily member 11	0.40	0.75	3.30	0.34	0.64	0.47	0.017	NM_057149	**8.18**	**1.59**
C-reactive protein	3.37	0.07	4.70	0.03	4.36	0.25	0.002	NM_017096	**1.40**	**1.29**
Down-regulated:									
cathepsin K	6.15	0.74	6.94	0.35	3.73	0.06	0.007	NM_031560	**1.13**	**0.61**
mast cell protease 9	3.97	0.41	1.57	0.38	4.06	0.35	0.006	NM_019323	**0.39**	**1.02**
CD69 antigen	3.86	0.11	4.01	0.29	2.97	0.26	0.039	NM_134327	**1.04**	**0.77**
Biphasic									
Chemokine (C-X-C) ligand 1	2.54	0.30	3.18	0.41	1.67	0.32	0.057	NM_030845	**1.25**	**0.66**
matrix metallopeptidase 9	4.55	0.14	5.08	0.44	3.79	0.11	0.044	NM_031055	**1.12**	**0.83**
B. Neuromediators; Signaling; Ion Channels									
Up-regulated:									
Glutamate receptor ionotropic N-methyl-D-aspartate 3B	2.79	0.17	4.82	0.30	2.95	0.26	0.002	NM_133308	**1.72**	**1.05**
Glutamate receptor ionotropic N-methyl D-aspartate 2A	1.79	0.19	3.15	0.36	2.35	0.28	0.043	NM_012573	**1.76**	**1.31**
gamma-aminobutyric acid (GABA) A receptor pi	2.90	0.69	4.81	0.18	4.85	0.22	0.030	NM_031029	**1.66**	**1.68**
Calcium/calmodulin-dependent protein kinase II delta	2.23	0.30	3.89	0.59	2.53	0.05	0.049	NM_012519	**1.74**	**1.13**
Down-regulated:									
purinergic receptor P2X ligand-gated ion channel 2	6.34	0.37	4.08	0.22	6.34	0.10	0.001	NM_053656	**0.64**	**1.00**
C. Growth factors/Neural Development/Repair									
Up-regulated:									
granulocyte-macrophage colony stimulating factor 2	4.51	0.42	5.56	0.30	6.08	0.23	0.037	XM_340799	**1.23**	**1.35**
Activin receptor-like kinase 7: neuronal expression	3.76	0.11	6.43	0.16	6.23	0.71	0.008	NM_139090	**1.71**	**1.66**
SRY-box containing gene 11	2.98	0.12	4.34	0.27	4.37	0.37	0.018	NM_053349	**1.45**	**1.47**
scratch homolog 1 zinc finger protein	4.70	0.57	6.60	0.25	6.57	0.41	0.032	XM_345848	**1.40**	**1.40**
Achaete-scute complex homolog-like 1	1.12	0.75	3.16	0.08	2.74	0.21	0.041	NM_022384	**2.82**	**2.44**
Down-regulated:									
fibroblast growth factor receptor 2	4.81	0.08	5.50	0.71	1.81	0.35	0.003	XM_341940	**1.14**	**0.38**
dihydropyrimidinase-like 3	5.20	0.41	4.24	0.25	3.51	0.30	0.029	NM_012934	**0.82**	**0.67**
Neural precursor cell expressed developmentally down- regulated gene 4A	3.87	0.54	2.90	0.84	1.05	0.02	0.035	XM_343427	**0.75**	**0.27**
Robo1, roundabout axon guidance receptor, homolog1	3.18	0.74	2.56	0.69	1.74	0.32	0.140	NM_031537	**0.80**	**0.55**

Table 2: Time course of selected mRNA expression in midbrain after CCI

RNA samples were hybridized with fluorescent labelled probes in triplicate using Affimetrix chips and analysed with Agilent Technology scanner equipped with software (see Materials and methods for details). mRNAs were grouped in functional clusters and time-course difference of most important gene expression is presented. Background-corrected and internal control-normalized fluorescence values are shown as Mean+ SEM, of at least 3 rats in animal group, each rat sample processed in duplicate.

	Contol		1 day post-CCI		7 days post-CCI		P	Fold-Change		
	Mean	*± SEM*	*Mean*	*± SEM*	*Mean*	*± SEM*	*ANOVA*	*RefSeq ID*	*D1/Control*	*D7/Contro*
A. Systemic/vascular; neuroinflammation;										
Up-regulated:										
chemokine (C-X-C motif) ligand 1	2.07	0.54	6.68	0.31	3.95	1.07	0.011	NM_030845	**3.23**	**1.91**
chemokine (C-C motif) ligand 6	1.90	0.14	6.56	0.39	5.74	1.13	0.007	NM_001004202	**3.45**	**3.02**
chemokine (C-C motif) ligand 7	3.62	0.17	9.11	0.96	6.38	0.23	0.002	NM_001007612	**2.52**	**1.76**
tissue inhibitor of metalloproteinase 1	7.68	0.06	11.69	0.59	9.69	0.66	0.004	NM_053819	**1.52**	**1.26**
CD68 antigen	6.17	0.42	7.66	0.13	8.22	0.24	0.0060	XM_213372	**1.24**	**1.33**
interleukin 1 receptor, type II	1.95	0.08	6.29	0.40	3.05	1.13	0.011	NM_053953	**3.23**	**1.56**
kininogen 1 similar to alpha-1 major acute phase protein	1.95	0.09	6.28	0.82	4.23	0.66	0.007	NM_001009628	**3.22**	**2.17**
matrix metallopeptidase 9	5.39	0.32	7.61	0.51	6.63	0.51	0.037	NM_031055	**1.41**	**1.23**
interleukin 8 receptor, beta	3.53	0.23	5.55	0.44	4.69	0.55	0.042	NM_017183	**1.57**	**1.33**
Down-regulated:										
cathepsin Q	4.26	0.61	4.79	0.13	2.37	0.13	0.008	NM_139262	**1.12**	**0.56**
tumor necrosis factor superfamily, member 11	4.86	0.49	4.03	1.25	4.96	0.40	0.6	NM_057149	**0.83**	**1.02**
B. Neuromediators; Signaling; Ion Channels										
Up-regulated:										
S100 calcium binding protein A11 (calizzarin)	8.61	0.08	10.31	0.16	9.60	0.36	0.006	NM_001004095	**1.20**	**1.11**
S100 calcium binding protein A8 (calgranulin A)	2.88	1.06	7.31	0.39	5.45	0.63	0.008	NM_053587	**2.54**	**1.89**
S100 calcium-binding protein A4	8.67	0.14	10.32	0.04	10.64	0.56	0.012	NM_012618	**1.19**	**1.23**
purinergic receptor P2X ligand-gated ion channel 2	3.54	0.09	3.51	0.34	4.60	0.30	0.027	NM_053656	**0.99**	**1.30**
synaptotagmin 3	5.60	0.13	6.55	0.18	6.44	0.04	0.004	NM_019122	**1.17**	**1.15**
gamma-aminobutyric acid A receptor pi	4.04	0.48	4.70	0.24	4.82	0.03	0.230	NM_031029	**1.17**	**1.19**
Biphasic/not changed:										
arrestin, beta 1	2.42	0.31	3.64	0.12	1.69	0.18	0.002	NM_012910	**1.50**	**0.70**
Glutamate receptor ionotropic N-methyl-D-aspartate 3B	3.34	0.19	3.10	0.38	3.22	0.80	0.70	NM_133308	**0.93**	**0.97**
Glutamate receptor ionotropic N-methyl D-aspartate 2A	2.81	0.16	2.30	0.31	2.77	0.11	0.400	NM_012573	**0.82**	**0.99**
C. Growth factors/Neural Development/Repair										
Up-regulated:										
Robo1, roundabout axon guidance receptor, homolog1	2.72	0.73	6.14	0.26	5.16	0.34	0.007	NM_031537	**2.26**	**1.90**
Platelet-derived growth factor receptor-like	3.90	0.17	4.08	0.16	5.30	0.42	0.023	NM_001011921	**1.05**	**1.36**
Fibroblast growth factor receptor 2	4.51	0.39	5.91	0.47	5.42	0.31	0.300	XM_341940	**1.3**	**1.2**
Down-regulated:										
granulocyte-macrophage colony stimulating factor 2	5.71	0.20	5.24	0.23	4.86	0.32	0.152	XM_340799	**0.9**	**0.8**
Achaete-scute complex homolog-like 1	2.82	0.17	2.80	0.12	1.93	0.23	0.019	NM_022384	**0.99**	**0.68**
Dihydropyrimidinase-like 3 (CRMP-4)	4.58	0.25	4.38	0.32	3.89	0.19	0.10	NM_012934	**0.96**	**0.85**
SRY-box containing gene 11	4.54	0.61	3.56	0.47	4.12	0.95	0.6	NM_053349	**0.78**	**0.91**
Biphasic:										
Neural precursor cell expressed developmentally down-regulated gene 4A	2.78	0.60	2.35	0.31	3.80	0.41	0.20	XM_343427	**0.85**	**1.37**
Insulin-like growth factor 1 receptor	4.86	0.21	5.86	0.46	3.85	0.32	0.017	NM_052807	**1.21**	**0.79**

Citation: Svetlov SI, Prima V, Zhang Z , Curley KC, Kobeissy F, et al. (2015) Acute and Subacute Differential Gene Expression in Rat Midbrain Following Blast Exposure Compared to Mechanical Brain Trauma. J Neurol Neurobiol Volume1.1: http://dx.doi.org/10.16966/2379-7150.103
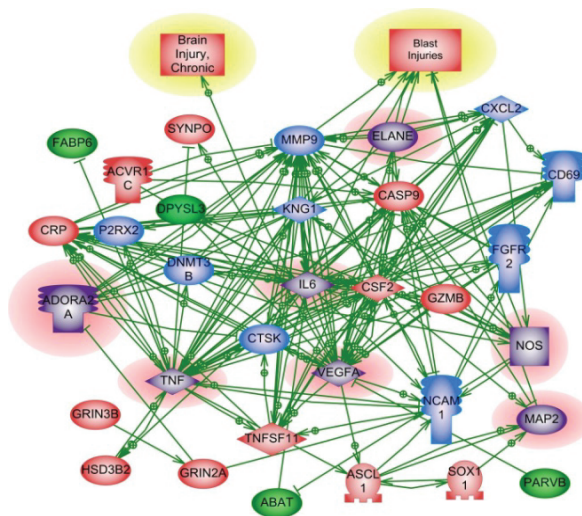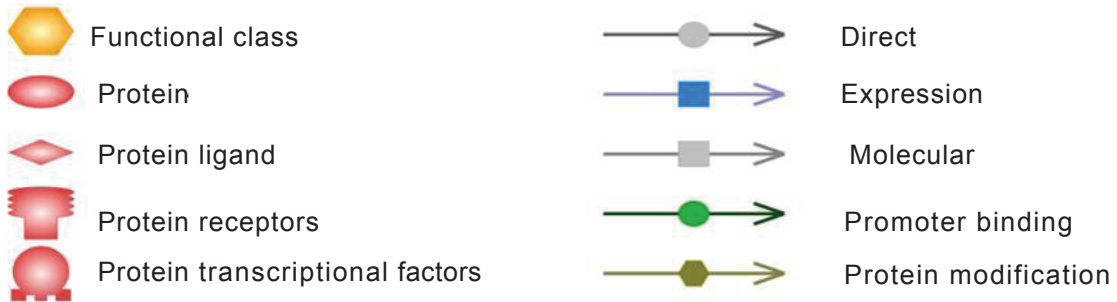
Figure 1: Systems biology maps of gene interactions following blast brain injury

Pathways critical components that do not overlap in blast and CCI groups are shown in frames. CASP9-caspase 9; CRP- C reactive protein; IL1R2-interleukin 1 receptor type 2; ESR2-estrogen receptor type 2; HMOX1- heme oxygenase 1; CXL1-Chemokine (C-X-C) ligand 1; CXCR2- chemokine receptor. KNG1- kininogen 1; TNFSF11- tumor necrosis factor superfamily member 11. Please see text for a detailed description. The upregulated genes are shown in red and downregulated genes are in blue. Yellow highlights indicate biphasic gene regulation (see table),

metalloproteinase-1 (TIMP-1) has been shown in several reports [11,12]. In this paper, we show important differences in blast vs. CCI responses, which may have both diagnostic/prognostic significance and, more important therapeutic strategies for TBI of different genesis.

Neuromediators; Signaling; Ion Channels: Very important features of gene expression in this cluster was prominent up-regulation of S100 calcium binding protein A11 (calizzarin), S100 calcium binding protein A8 (calgranulin A), and S100 calcium-binding protein A4 after CCI, but not after blast (Table 2B). Significant up-regulation of GABA A receptor pi gene was observed in blast-exposed rats groups compared with noise control (Table 1B). There was a modest, but not significant (p=0.23) increase in this gene expression in CCI group vs. control (Table 2B). Similarly, remarkable up-regulation of glutamate ionotropic N-methyl-D-aspartate receptors was found in blast-exposed groups vs control but not in CCI treated rats (Table 1B and 2B). Differential gene interactions of this cluster, including NCAM and VEGF-A, after blast, compared to S100A family after CCI is shown in Figures 1 and 2, respectively.

Roles for S100 protein family have been demonstrated and S100B glial protein, a component of Ca^{2+}/calmodulin signaling, has been extensively studied in TBI, including its clinical diagnostic value [16-18]. However, S100 significance has been evaluated in mostly mechanical trauma

models (CCI, closed head injury, FPI) and in patients with TBI, and no attention to other components was given. Our data highlights the involvement of several S100 family genes, including calgranulin A and calizzarin pathogenesis of mechanical cortical impact, and shows a little, if any, impairment of S100 following blast exposure. Important, our data demonstrate up-regulation of glutamate ionotropic receptor genes 2A (24 h and 7 d) and 3B (24 h) over noise control, which has not been previously reported for blast exposures (Table 1B).

Growth factors/Neural Development/Repair. Significant differences between exposure groups were found in genes involved in neural development and repair. Activin receptor-like kinase 7 (neuronal expression) gene was significantly up-regulated following blast exposure vs. control group, but not in CCI-subjected group naïve control. A comparable disparity is observed with Achaete-scute complex homolog-like 1 and SRY-box containing gene 11 which increased after blast exposure but did not change or even down-regulated in CCI vs. naïve control (Tables 1C and 2C).

Important, expression of neural precursor cell expressed developmentally down-regulated gene 4A (Nedd4a) was significantly decreased after blast, but not changed and even slightly (but not significantly) increased after CCI compared to naïve rats. Dihydropyrimidinase-like 3
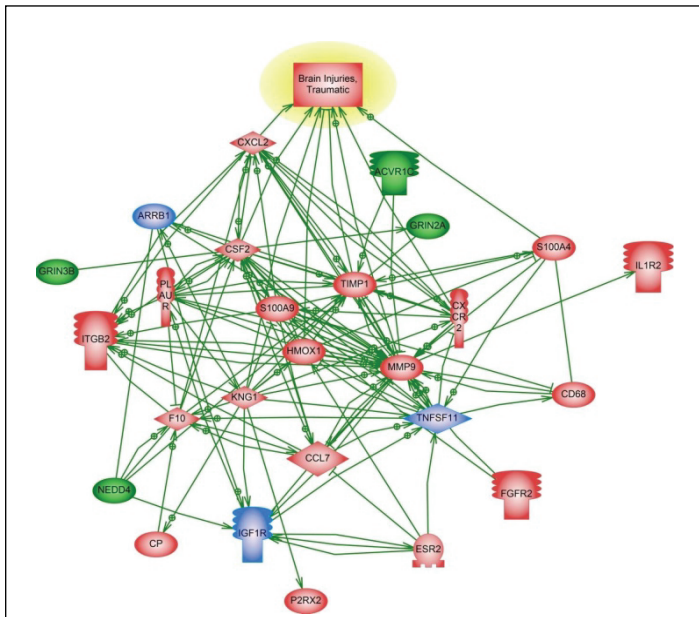
Figure 2: Gene interaction pathways after mechanical controlled cortical impact.

Symbols/ interactions and non-overlapping components are similar as shown in Fig. 2. NCAM1-neural cell adhesion molecule 1; GRIN3B-KCNQ1-voltage-gated potassium channel; GRIN3B-Glutamate receptor ionotropic N-methyl-D-aspartate 3B; GRIN2A- Glutamate receptor ionotropic N-methyl-D-aspartate 2A; P2RX2- Purinergic receptor P2X, ligand-gated ion channel 2; GABA-gamma aminobutiric acid; S100A-calcium binding proteins; PLAUR- plasminogen activator, urokinase receptor. Please see text for details.
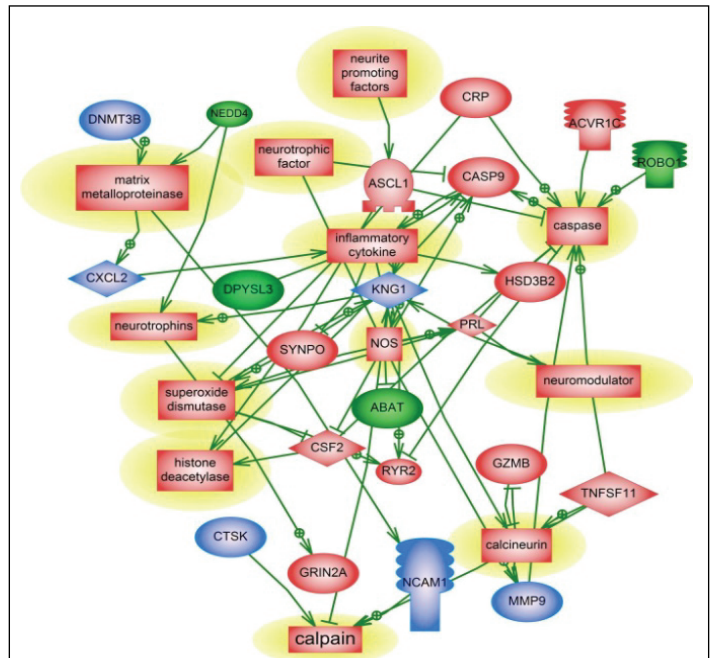
Figure 3: Gene-associated molecular function implicated with blast injury: systems biology map of molecular components and associated pathology. Symbols and interactions are same as in Figure 2. FGFR2-fibroblast growth factor receptor 2; CSF2- granulocyte-macrophage colony-stimulating factor; IGF1R- insulin growth factor 1 receptor; ACVR1C- activin receptor-like kinase 7; ROBO1- roundabout axon guidance receptor 1; NEDD4-neural precursor cell expressed developmentally down-regulated gene 4; DPYSL3-dihydropyrimidinase-like 3 (CRMP-4). Please see text for detailed description and discussion.

(CRMP-4) was down regulated compared respective controls in both blast and CCI groups.

The most intriguing pattern was observed in expression roundabout axon guidance receptor 1 (Robo1) gene. It was very significantly up-regulated after CCI, while showing a distinct trend to down-regulate after blast exposures (Table 1C and 2C).

The pathways mapping gene interactions of this cluster in blast vs CCI are included in Figures 1 and 2 Blast exposure triggered activation of GM-CSF and downstream transcriptional activators, including SOX11 leading to induction of repair processes exemplified by tubulin, WNT (negatively) and RPS6K ribosomal protein kinase genes as well as TGF-beta related pathways, including up-regulation of ALK7 (ACVR1C)/ SMAD transcriptional regulators.

Gene-associated molecular functions

Pathways of molecular interactions in blast and CCI are shown in Figure 3. As indicated, the most important differences found in S100A family genes which were up-regulated in CCI cohort and involved in downstream regulation of ubiquitin, proteinase activated receptor, Rho, GPCR, JAK signaling. In blast-induced group, functional regulation pathways demonstrate potential NCAM1- dependent processes with glutamate receptor, CAMK and calmodulin signaling, and GABA/GABA A receptor activation (Table 1B and Figure 3). The pathology/disease maps generated using this cluster components are very similar in blast and CCI groups and overlap with cluster 1 (systemic/vascular/neuroinflammation/proteolysis), although involve some different components- S100A for CCI and NCAM for blast (Table 2B and Figures 3 and 4).
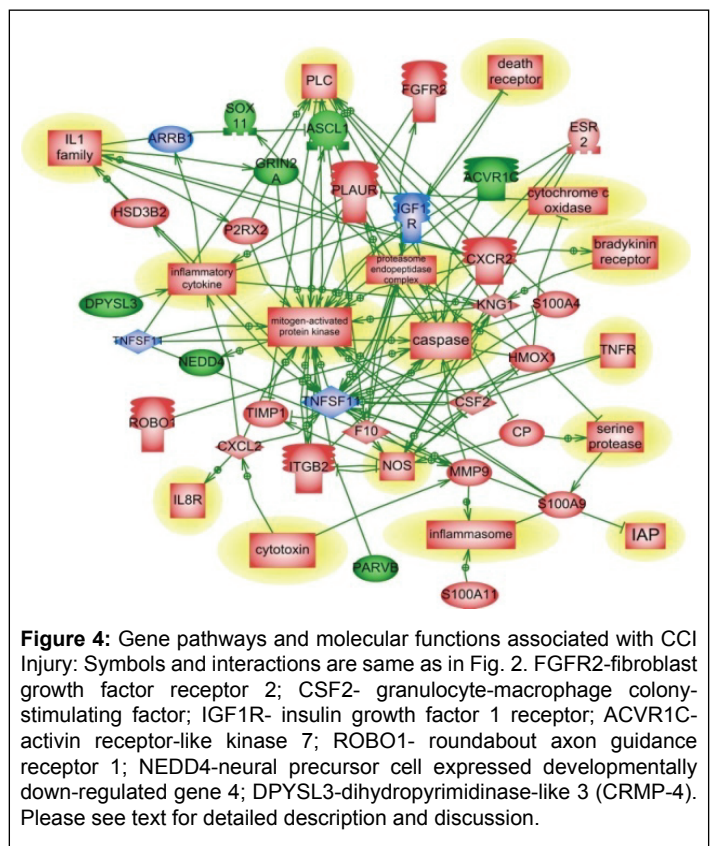
Figure 4: Gene pathways and molecular functions associated with CCI Injury: Symbols and interactions are same as in Fig. 2. FGFR2-fibroblast growth factor receptor 2; CSF2- granulocyte-macrophage colony-stimulating factor; IGF1R- insulin growth factor 1 receptor; ACVR1C-activin receptor-like kinase 7; ROBO1- roundabout axon guidance receptor 1; NEDD4-neural precursor cell expressed developmentally down-regulated gene 4; DPYSL3-dihydropyrimidinase-like 3 (CRMP-4). Please see text for detailed description and discussion.

Genes and molecular processes involved in neurorepair are still unclear, thus elucidating mechanisms, molecular components and biomarkers of post-injury outcome will have immense clinical importance. FGF pathways with downstream MAP kinase family are negatively involved in gene regulation following blast exposure (Table 1C, Figure 1). After CCI, the central point of gene regulation in midbrain appears to be IGF1 and EGF family molecules, including IGF1 receptor and EGF ligand genes connecting similar to blast effector genes such as tubulin, S6 kinase, histones and WNT as shown in Table 2C and Figures 2 and 4.

The functional roles for ROBO1 and NEDD4 in CNS is not clear at present, but it appears from the map that down-regulation of ROBO1 and NEDD4 genes can facilitate PI3 kinase and RAS and stimulate repair (Figures 3 and 4), while up-regulation of ROBO1 and NEDD4 after CCI can negatively influence neural repair.

It has been shown that ROBO1 modulates neurogenesis in the developing cortex [19]. ROBO1 is involved in axonal guidance of dopaminergic neurons by neuropilin-2 [20], up-regulation of which we have shown previously [21]. However, the opposite regulation of ROBO1 after blast compared to CCI may reflect the spatial specificity in the expression and function of ROBO1 in forebrain and neocortex [22-24], which is differentially affected by blast wave in midbrain than by CCI insult directly to the cortex. However, the roles for ROBO1 and related Slit system in brain repair after TBI has not been investigated. Also, roles for Activin receptor family, including ALK kinases have not been studied following TBI, although ALK1 signaling produced lower TBI-induced increases in GFAP mRNA levels, indicating attenuation in astroglial and neuronal responses to injury [25].

ACVR1C was significantly up-regulated after blast compared to noise control, while the CCI values were not equally increased compared to naïve rats. Similarly to ROBO1, NEDD4a was down-regulated in midbrain after blast, but was up-regulated at 7 day after CCI. NEDD4 (WW) was shown to be expressed in surviving neurons after cortical injury [26], and differential expression of these genes in midbrain may reflect spatial disparities. Taken together, our data reveal for the first time that ALK7, ROBO1 and NEDD4 are involved in post-blast and post CCI, possibly brain repair. In a recent paper, Heinzelmann and co-workers reported down- regulation of several growth factors and receptors genes, such as EGFR recepror and tensin-1 in peripheral blood collected from patients with post-blast syndrome [27]. Specific mechanisms involving these important components in neural injury and recovery after TBI remain to be investigated.

Conclusion

To the best of knowledge, this is the first genomics study that assesses altered gene expression side-by-side in 2 well-defined and characterized brain insults- the TBI modeled via blast overpressure vs the CCI open head injury. For this purpose, a comprehensive study utilizing systems biology-based analysis of transcriptomics in rat midbrain has been conducted to compare blast exposure and mechanical cortical impact brain insult. Our data show both an overlap and significant time-dependent differences in gene expression changes among these groups, particularly in genes of neural development and repair. Novel components, such as ROBO1 and NEDD4 have been shown to be expressed in opposing manner following TBI induced by blast compared to CCI, suggesting different mechanisms may be involved in neurorepair dynamics. The results obtained suggest further in-depth studies are required to elucidate differences in mechanisms of brain injury produced by blast compared to mechanical impact. This will help to reveal potential biomarkers and develop diagnostics of chronic posttraumatic encephalopathy.

Required disclaimer:

The views and opinions expressed in this manuscript are those of the author(s) and do not reflect official policy or position of the Department of Defence or the U.S. Government.

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