Low-level blast exposure induces chronic vascular remodeling, perivascular astrocytic degeneration and vascular-associated neuroinflammation

Miguel A. Gama Sosa, Rita De Gasperi, Dylan Pryor, Georgina S. Perez Garcia, Gissel M. Perez, Rania Abutarboush, Usmah Kawoos, Seth Hogg, Benjamin Ache, William G. Janssen, Allison Sowa, Timothy Tetreault, David G. Cook, Susan J. Tappan, Sam Gandy, Patrick R. Hof, Stephen T. Ahlers and Gregory A. Elder

Abstract

Cerebral vascular injury as a consequence of blast-induced traumatic brain injury is primarily the result of blast wave-induced mechanical disruptions within the neurovascular unit. In rodent models of blast-induced traumatic brain injury, chronic vascular degenerative processes are associated with the development of an age-dependent post-traumatic stress disorder-like phenotype. To investigate the evolution of blast-induced chronic vascular degenerative changes, Long-Evans rats were blast-exposed (3 × 74.5 kPa) and their brains analyzed at different times post-exposure by X-ray microcomputed tomography, immunohistochemistry and electron microscopy. On microcomputed tomography scans, regional cerebral vascular attenuation or occlusion was observed as early as 48 h post-blast, and cerebral vascular disorganization was visible at 6 weeks and more accentuated at 13 months post-blast. Progression of the late-onset pathology was characterized by detachment of the endothelial and smooth muscle cellular elements from the neuropil due to degeneration and loss of arteriolar perivascular astrocytes. Development of this pathology was associated with vascular remodeling and neuroinflammation as increased levels of matrix metalloproteinases (MMP-2 and MMP-9), collagen type IV loss, and microglial activation were observed in the affected vasculature. Blast-induced chronic alterations within the neurovascular unit should affect cerebral blood circulation, glymphatic flow and intramural periarterial drainage, all of which may contribute to development of the blast-induced behavioral phenotype. Our results also identify astrocytic degeneration as a potential target for the development of therapies to treat blast-induced brain injury.

Keywords: Animal model, Blast, Brain, Chronic, Vascular, Neurovascular unit, Rat, Vascular pathology, Astrocyte, Tight junctions

Introduction

Traumatic brain injury (TBI) has been linked to mental health disorders and is considered a risk factor for later development of neurodegenerative disorders [31]. Many veterans from the recent conflicts in Iraq and Afghanistan suffer from chronic neurobehavioral syndromes that include post-traumatic stress disorder (PTSD) [31].
Indeed, a striking feature in veterans from the most recent war theaters has been the overlap between a history of blast-related mild TBI (mTBI) and PTSD [17, 31, 115, 134, 140, 141]. While these symptoms may improve, they frequently persist and may worsen with declines driven mainly by worsening PTSD and depression [83]. Although the mechanisms underlying how blast affects human neurobiology are incompletely understood, much evidence suggests that the cerebrovasculature may be particularly vulnerable to blast [112, 131, 133].

Research in rat models of repetitive low level blast exposure has shown that animals exposed to repetitive low-level blast exhibit chronic cognitive impairment and PTSD-related traits that develop over time [30, 33, 100, 101, 123]. These traits include anxiety, enhanced acoustic startle, altered fear learning and impaired cognition in tests such as novel object recognition. Blast-exposed rats thus model many of the features found in human PTSD. A single predator scent challenge delivered 8 months after the last blast exposure induces additional anxiety-related changes that are still present 45 days later [101] suggesting that besides inducing PTSD-related traits blast exposure sensitizes the brain to react abnormally to subsequent psychological stressors.

The evolution of the resulting blast-induced cognitive and behavioral phenotypes in rats seems to overlap the development of cerebral vascular degenerative processes. [38–40]. Cerebral blood circulation is essential for normal brain metabolic functions, as it provides oxygen, glucose and other essential metabolites as well as removes metabolic wastes [125]. Increased synaptic activity increases blood flow in brain regions where higher oxygen concentrations are required via neurovascular coupling. The neurovascular unit is composed of endothelial cells, associated blood–brain barrier tight junctions, a basal lamina covered with mural cells (pericytes and smooth muscle), neural cells (astrocytes and neurons) and an extracellular matrix (ECM). The neurovascular unit also encompasses the lymphatic and intramural perivascular drainage systems. The lymphatic CSF waste clearance system utilizes perivascular channels, formed by astroglial cells, to promote elimination of soluble proteins and metabolites from the CNS [14, 61, 91]. In this system, CSF enters interstitial spaces after aquaporin 4 (AQP4)-dependent transport through the astroglial cytoplasm, drains into perivenous routes in a direction parallel to the blood flow, and then enters the subarachnoid CSF or bloodstream across cerebral vessels [142]. The intramural perivascular drainage system (IPAD) is a major pathway by which solutes drain from the brain to cervical lymph nodes along the walls of cerebral arteries in a direction counter to the blood flow [1, 56, 136]. In the IPAD system, parenchymal interstitial fluid (ISF) enters the basal membrane of capillaries and is progressively moved onto the basal membrane of the tunica media of intracerebral arterioles and arteries, and onto the walls of leptomeningeal and internal carotid arteries [5, 19, 142].

Direct consequences of blast exposure include acute damage and chronic structural degeneration of the cerebral vasculature. Early blast-induced vascular injury includes apoptosis of vascular structural elements, capillary strictures, vascular occlusion, blood–brain barrier disruption, vascular rupture, breakdown of the choroid plexus, reduced dilator responses to decreased intravascular pressure, reduced cerebral perfusion, and increased cerebral vascular resistance, [38, 39, 63, 66, 75, 113, 114]. These acute events are followed by the development of a chronic secondary pathology characterized by perivascular astrocytic degeneration, luminal collapse, disruption of neurovascular interactions, ECM remodelling, “double-barreled” vessels, intraluminal astrocytic processes, vascular smooth muscle degeneration, vascular occlusion by CD34+ progenitor cells, generalized vascular attenuation, aneurysm formation, vascular fragility and stroke [40]. These combined events can lead to disruptions in cerebral blood circulation that seem to be associated with development of PTSD-related symptoms in blast-exposed individuals [31, 33, 83, 102, 132]. Similarly, CSF and ISF drainage in the brain through the glymphatic and IPAD systems along the external perivascular spaces and laminae may also be affected.

In the present research, we used a combination of X-ray microcomputed tomography (micro-CT) with computerized morphological analyses to determine the evolution of vascular structural alterations in the brain circulation of blast-exposed rats. Micro-CT scans revealed regional cerebral vascular attenuation as early as 48 h post-blast and cerebral vascular attenuation and disorganization at 6 weeks and 13 months post-blast. Immunohistochemical and histological analyses confirmed the presence of previously reported vascular degenerative processes but also revealed at 13 months post-blast the emergence of a vascular pathology characterized by the detachment of the vasculature from the brain parenchyma with affected arterioles at the core of an enlarged paravascular space. This chronic vascular pathology was associated with perivascular-astrocytic degeneration, vascular attenuation, vascular-associated inflammation and vascular remodeling. Our results further document the evolution of blast-induced neurovascular uncoupling due to astrocytic and vascular degenerative processes and provide evidence for a novel pathology that may affect blood circulation and perivascular flow of CSF and ISF through the glymphatic and IPAD systems in the brain.
Materials and methods

Animals
Adult male Long-Evans hooded rats (250–350 g, 10 weeks of age, Charles River Laboratories International, Wilmington, MA, USA) were used. All studies involving animals were reviewed and approved by the Institutional Animal Care and Use Committees of the Walter Reed Army Institute of Research (WRAIR)/Naval Medical Research Center and the James J. Peters VA Medical Center. Studies were conducted in compliance with the Public Health Service policy on the humane care and use of laboratory animals, the NIH Guide for the Care and Use of Laboratory Animals, and all applicable Federal regulations governing the protection of animals in research.

Blast overpressure exposure
The Walter Reed Army Institute of Research (WRAIR) shock tube at the Naval Medical Research Center (NMRC, Silver Springs, MD, USA) was used to expose rats to overpressure injury. This apparatus, which simulates the effects of air blast exposure under experimental conditions, has been used in our prior studies to deliver blast overpressure injury to rats [4, 20–22, 25, 30, 39, 40]. As in previous studies, anesthetized rats were randomly assigned to sham or blast conditions with the head facing the blast exposure without any body shielding, resulting in a full body exposure to the blast wave. The physical characteristics of the blast wave and further details of the blast exposure have been described in detail [4]. Blast-exposed animals received a total of three 74.5-kPa (10.8 psi) exposures, with one exposure administered daily for 3 consecutive days. Control animals were anesthetized and placed in the blast tube but not subjected to a blast exposure. Within 10 days after the last blast exposure, the animals were transferred to the James J. Peters VA Medical Center (Bronx, NY, USA) where all other procedures were performed. Control and experimental cohorts were euthanized at 48 h (n = 6 per group), 6 weeks (n = 6 per group) and 13 months (n = 4 blast-exposed rats and n = 5 control rats) post-blast for observation of acute, subacute and chronic effects, respectively.

Behavioral testing
Some animals used in this study were previously characterized behaviorally [99]. Data from selected animals were used here. Elevated zero maze (EZM) as well as contextual and cued fear conditioning (FC) tests were performed at 40 and 42 weeks post-exposure, respectively [99].

X-ray high-resolution micro-CT scanning
Rats were anesthetized with 150 mg/kg ketamine and 30 mg/kg xylazine and transcardially perfused first with 60 ml of 10 µg/ml heparin in phosphate-buffered saline (PBS), pH 7.2, followed by 250 ml of a 30% solution of the Brite Vu Special Projects contrast agent supplemented with its enhancer (Scarlet Imaging, Murray UT, USA) maintained at 65 °C. The perfused animals were chilled by immersion in an ice-water bath for 2 h to gel the intravascular contrast agent. Brains were then dissected, post-fixed overnight in 4% paraformaldehyde in PBS and maintained in sterile PBS at 4 °C. Brains were scanned at a 7.5-µm voxel size using 60 kV, 166 µA X-ray settings and a 0.25-mm aluminum filter with exposure time of 508 ms per frame, with 3 frames averaged at each projection angle with a Bruker SkyScan 1272 micro-CT (Micro Photonics, Allentown, PA, USA). Three-dimensional reconstruction and morphological profiling of the cerebral vasculature was performed with the Bruker’s NRecon software or the Vesselucida 360 software (v2018.1.1, MBF Bioscience LLC, Williston, VT, USA) using data obtained from the micro-CT scans and reconstructing the respective three-dimensional vascular networks. For quantitative analyses, automatic reconstruction of the vasculature with the Vesselucida 360 software was performed using identical settings for all animals. A voxel scooping algorithm was applied with the following settings: trace and seed sensitivity set to 80, medium seed density with refine filter set to 2, maximum gap tolerance. No manual editing was performed. The parameters determined were total vascular length, volume, surface area, length density, the number of isolated vessels, the longest vessel, the number of branching nodes, number of ending vessels and the estimated total tissue volume.

Histological and immunohistochemical analysis
Coronal sections (50 µm thickness) of the micro-CT scanned brains were prepared with a VT1000S Vibratome (Leica Biosystems, Buffalo Grove, IL, USA). General histology was assessed by hematoxylin–eosin staining of sections. For immunohistochemistry, sections were blocked with 10% normal goat serum in 50 mM Tris HCl, pH 7.6, 0.15 M NaCl, 0.3% Triton-X-100 and incubated overnight with the primary antibodies diluted in blocking solution at room temperature. After washing with PBS (6 times for 10 min each), sections were incubated with the appropriate Alexa (488, 568 and 647)-conjugated secondary antibodies (1:300, ThermoFisher, Waltham, MA, USA) in blocking solution for 2 h. After washing with PBS (6 times for 10 min each), the sections were mounted with Fluorogel mounting medium (Electron Microscopy Sciences, Hatfield, PA, USA). To visualize...
nuclei, sections were incubated in PBS containing 0.1 μg/ml DAPI (4′,6-diamidine-2′-phenylindole dihydrochloride) in the next to last PBS wash. The primary antibodies were a rat monoclonal anti-glial fibrillary acidic protein (GFAP; 1:500, clone 2.2B10, gift of Dr. Virginia Lee, University of Pennsylvania, Philadelphia PA, USA), rabbit anti-GFAP (1:500, G9269, RRID: AB_477035, Sigma-Aldrich, St Louis, MO, USA), mouse anti-α-smooth muscle actin (αSMA, 1:300, A2547, RRID: AB_262054, Sigma-Aldrich), rabbit monoclonal anti-MMP-9 (1:300, 13667S, RRID: AB_2798289, Cell Signaling Technology, Danvers, MA, USA), mouse monoclonal anti-MMP-9 (1:200, NB2-80855, RRID: AB_2811297, Novus, Littleton, CO, USA) and rabbit anti-ionized calcium-binding adaptor molecule 1 (Iba1; 019-19741, RRID: AB_839504, Fujifilm Wako Pure Chemical, Osaka, Japan). Vascular staining was performed with anti-collagen type IV antibodies (rabbit anti-collagen type IV, 1:300, ab6586, RRID: AB_305584, Abcam, Cambridge, MA, USA) either in the absence or presence of a pepsin treatment [37, 42]. For the pepsin treatment, sections were incubated with 1 mg/ml pepsin (Agilent Technologies, Santa Clara, CA, USA) in 3% acetic acid for 50 min at 37 °C. Sections were washed once in 0.1 M sodium borate, pH 8.5, washed 5 times with PBS, blocked and stained with antibodies against collagen IV and GFAP in combination with the respective Alexa-conjugated secondary antibodies as described above. For TUNEL staining (terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling), sections were washed in TBS, permeabilized with 0.1% Triton X-100 in TBS for 1 h and washed extensively with TBS. End labeling of DNA with fluorescein-dUTP was performed using a commercial kit (Roche, Indianapolis, IN, USA). After several washes with PBS, the sections were blocked and stained with antibodies against GFAP and ionized calcium-binding adaptor molecule (Iba1) as described above.

**Electron microscopy**

Electron microscopy was performed using protocols optimized to study the ultrastructure of the vasculature. Anesthetized rats were transcardially perfused with ice-cold 2.0% glutaraldehyde, 2% paraformaldehyde, 0.1 M sodium phosphate (pH 7.3). Brains were removed, postfixed in the same fixative as above and stored at 4 °C until ready for processing. Fixed brains were placed on a rat brain slicer matrix, and coronal slices containing the frontal cortex were excised. Sections were washed in 0.1 M sodium cacodylate buffer, pH 7.2 and postfixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.2. Sections were washed again in cacodylate buffer, dehydrated through graduated ethanol (70–100%) and propylene oxide series, and resin-infiltrated with Epon (Electron Microscopy Sciences, Hatfield, PA, USA). Material was polymerized in a vacuum oven at 60 °C for 48 h. Semi-thin (1 μm) Toluidine blue-stained sections were used to identify the regions of interest. Ultrathin sections (80 nm) were cut with a diamond knife on a Leica UCT ultramicrotome and mounted on copper grids using a Coat-Quick adhesive pen (Electron Microscopy Sciences). Sections were counterstained with uranyl acetate and lead citrate. Frontal cortical sections were imaged on a Hitachi 7700 electron microscope (Hitachi, LTD., Tokyo, Japan) and photographed with an Advantage CCD camera (Advanced Microscopy Techniques, Danvers, MA, USA). Image brightness and contrast were adjusted using Adobe Photoshop 2021 software (version 22.3.1; Adobe, Inc., San Jose, CA, USA).

**Zymography of vascular metalloproteases**

Enriched cerebral vascular fractions were prepared as previously described [40]. Blast-exposed and control rats (6 weeks post-blast) were euthanized by CO2 inhalation. Brains were cleaned of meninges and homogenized in cold 18% dextran in PBS (10 ml/g of tissue) using a Potter-Elvejem homogenizer with a loose-fit Teflon pestle (6–8 strokes at low speed, setting 2 of a Wheaton head stirrer, Millville, NJ, USA). Each homogenate was overlayed over an equal volume of Ficoll-Paque PLUS™ (GE Healthcare Life Sciences, Marlborough, MA, USA) and centrifuged for 30 min at 1500×g and 4 °C. Vascular-enriched pellets were resuspended in PBS, washed twice with PBS, and stored at -80 °C [40]. Enriched vascular fractions were lysed in 10 mM NaPO4, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, and 1% sodium dodecyl sulfate (SDS) supplemented with protease and phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich, St Louis, MO, USA). Lysates were centrifuged at 15,000 ×g for 15 min, and the protein concentration in the supernatants was determined with the BCA reagent (ThermoFisher, Waltham MA, USA). Heat-denatured samples containing 20 μg of protein were electrophoresed through a 10% polyacrylamide gel containing 0.1% gelatin (Novex 10% Zymogram Plus Gelatin Protein Gel, Thermo Fisher Scientific, Waltham, MA). After protein renaturation, incubation, and staining with Brilliant Blue, gelatinase activity was visualized as cleared bands reflective of gelatin hydrolysis.

**Statistical analyses**

Statistical differences were evaluated with unpaired t-tests using Prism 7.0 software (GraphPad, La Jolla, CA). Statistical significance was set at an α level of 0.05.
Results
Cerebral vascular disorganization revealed by micro-CT scanning
The evolution of the blast-induced chronic cerebral vascular pathology was investigated by X-ray high-resolution micro-CT scanning of brains of blast-exposed rats at 48 h, 6 weeks and 13 months post-blast exposure following perfusion with the Brite Vu contrast agent. Figure 1 shows representative maximum intensity projection (MIP) images of the volume-rendered brain vasculature of control and blast-exposed animals at the three time points. In Fig. 2, magnified high resolution micro-CT images are shown which reveal that in addition to large and medium sized arteries, smaller vessels including precapillary arterioles and possibly venules could be imaged. Quantitative analyses of the total cerebral vasculature (Fig. 3) did not show significant differences in major parameters, including length, surface area and volume, of the cerebral vasculature between blast-exposed and controls at any of the time points.

Nevertheless, post-blast imaging analyses of regional optical coronal sections (3.75-mm thickness) revealed stochastic vascular alterations in blast-exposed animals with attenuation, occlusion or disruption of the regular radial patterns visible in the brains of control animals. For example, Fig. 4a shows a rat brain from the 48 h post-blast cohort with vascular alterations in the hippocampal and posterior cortical regions of the left cerebral hemisphere, most likely the result of occlusion or attenuation of the posterior branches of the MCA as well as collateral branches of the posterior cerebral artery (PCA) and the anterior choroidal artery (AChA).

At 6 weeks and 13 months post-blast, major alterations and disorganization of the cerebral vasculature were also observed. At 6 weeks post-blast, half of the blast-exposed rats (3/6) exhibited vascular alterations. Figure 4b shows attenuation of the vasculature irrigating the cingulate, motor, somatosensory, and retrosplenial cortex most likely involving the rostral branches of the MCA. At 13 months post-blast, cerebral vascular alterations were clearly observed in all blast-exposed animals (4/4) and none of the sham control rats (5/5) (Fig. 4c). In these animals, tissue tears, induced by the initial blast wave injury were clearly visible [39] (Fig. 4c). For example, in Fig. 4c lesions extend through the perirhinal or piriform cortical regions as well as the external capsule and amygdala and are similar to those described in previous blast-exposed cohorts [38, 39]. In the animal shown in Fig. 4c, blast-induced injuries resulted in bilateral amygdalar lesions. Interestingly, this animal which had been previously characterized behaviorally [95] was a relative outlier among blast-exposed animals in an elevated zero maze.
(EZM; Fig. 5a–c) making more open arm entries (Fig. 5a) as well as spending more time in the open arms (Fig. 5b) and exhibiting a short cross arm latency (Fig. 5c). In a fear conditioning task (Fig. 5d–f), this animal showed no freezing responses during the training session (Fig. 5d) but exhibited relatively normal freezing during the contextual (Fig. 5e) and cued fear testing (Fig. 5f). In Fig. 5, behavioral data from the control animal illustrated in Fig. 4f is shown for comparison.

Perivascular astrocytic degeneration, loss, and mislocalization

We previously described the blast-induced degeneration of astrocytes surrounding the cerebral vasculature as already ongoing at 6 weeks post-exposure [40]. To evaluate the astroglial coverage of the brain vasculature at 13 months post-blast, sections of control and blast-exposed animals were analyzed first by electron microscopy (Fig. 6). Electron micrographs showed long-term blast-induced effects on perivascular astrocytes including swelling and degeneration of astrocytic endfeet in the brain cortical vasculature. The processes of astrocytic endfeet in an advanced degenerating phase were devoid of cellular organelles (Fig. 6). Some small vessels and arterioles had enlarged paravascular spaces that reflected the loss of perivascular astrocytes bridging the parenchymal neuropil and the vessel. Apoptotic perivascular astrocytes and microglia were detected by Tunel staining associated with vasculature with enlarged paravascular spaces (Fig. 7). These observations are in agreement with the development of an unusual vascular pathology observed in two additional animals from the 13 months post-blast cohort (Fig. 8). This pathology, characterized mainly by the presence of vascular segments within enlarged paravascular spaces (Fig. 8), often affected mainly one cerebral hemisphere. The brain regions affected generally included the cerebellum, hypothalamus, thalamus, neocortex, hippocampus, basal ganglia, piriform cortex and amygdala (Fig. 8a). Vascular reconstructions of micro CT optical sections showed that brain regions with enlarged paravascular spaces also showed drastic reductions of micro-CT-traced vessels. As an example, Fig. 8a, b shows that enlarged paravascular spaces in the hypothalamus, ventral thalamus and, most strikingly, the amygdala and piriform cortex have a clear reduction of micro-CT-traced vessels. The lack of vascular tracing within regions with enlarged paravascular spaces is indicative of vascular hypoperfusion or attenuation and could be a consequence of lack of vascular tone regulation due to perivascular astrocytic degeneration. In behavioral testing this animal exhibited an anxiety phenotype in an EZM that was similar to other blast-exposed animals. (Fig. 5a–c). In fear conditioning, it exhibited a training curve (Fig. 5d) relatively similar to other blast-exposed animals but showed increased freezing in contextual (Fig. 5e) and cued (Fig. 5f) fear testing. Histochemical, immunohistochemical and electron microscopic analyses showed that the vasculature enclosed within the enlarged paravascular spaces involved mainly arterial vessels (Fig. 8c–f) and in some of these vessels the adventitial layer could be found detached (Fig. 8d, f).

As previously reported [3, 38, 40], some of the arterioles presented increased tortuosity and structural smooth muscle alterations as strictures and tortuosity could result in loss of smooth muscle (Fig. 9e). GFAP and collagen type IV immunostaining revealed collagen IV degradation associated with loss of perivascular astrocytes in the affected vasculature (Figs. 7, 9, 10). Compared to unaffected brain regions, loss of vascular collagen IV immunostaining was seen in areas with enlarged paravascular spaces (Fig. 9a–f). Although perivascular astrocytes were lost in the affected vasculature and immediate surrounding parenchyma, remains of...
GFAP+ astrocytic feet could be seen remaining attached in some vessels (Fig. 9c).

Remodeling of the adventitial ECM
In addition to the loss of perivascular astrocytes, some vessels showed mislocalization of astrocytes. We previously reported the presence of intravascular GFAP+ astrocytic processes in the cerebral vasculature of animals at 6 weeks post-blast exposure [40]. At 13 months post-blast, confocal microscopy identified astrocytes under the adventitial ECM scaffold (Fig. 10f) and in the lumen (Fig. 11c, d). This implies ECM remodeling by adventitial fibroblasts along with perivascular astrocyte repositioning within the vascular internal structural layers. The presence of intraluminal astrocytes or astrocytic processes may identify non-functional degenerating vasculature into which perivascular astrocytes and/or their processes could extend.

Adventitial fibroblasts become involved in vascular remodeling via their regulation of ECM degradation mediated by MMPs and tissue inhibitors of metalloproteinases (TIMPs). The presence of vascular protease activity in rats at 10 months post-blast has previously been demonstrated by the immunostaining of the cerebral vasculature with collagen type IV antibodies without prior protease treatment [38]. Similarly, at 13 months post-blast, the cerebral vasculature was extensively stained with anti-collagen type IV
antibodies in the absence of a protease treatment. In age-matched controls, collagen type IV vascular staining was very limited (Fig. 12a, b).

Increased levels of brain MMP-9, MMP-9 mRNA, and plasma MMP-9 have been observed acutely after a 130-kPa blast exposure in association with blood–brain barrier dysfunction [66]. Gelatin zymography of purified cerebral vascular fractions showed that levels of MMP-2 and MMP-9 could also be increased at 6 weeks post-blast exposure (Fig. 13a). At 13 months post-blast, the cerebral vasculature showed a reduction in collagen type IV immunoreactivity in some regions (Fig. 9), smooth muscle expression of MMP-9 (Fig. 13b–e) and thickening of the vascular laminae (Fig. 14). In addition, vascular IgG leakage was detected at this age suggesting that the peripheral circulation might be an additional source of proteases affecting the vasculature (Fig. 13f, g). These results illustrate the developing chronic vascular remodeling of the ECM in the vascular scaffold after blast exposure.

**Vascular-associated neuroinflammation**

Microglial activation is a brain pathology hallmark that occurs in response to injury through damage-associated molecular patterns and removal of immunosuppressive signals. Electron microscopic observations at 13 months post-blast identified periarteriolar microglia with intracellular cholesterol crystals next to degenerating astrocytic endfeet (Fig. 15a, b, d).

At 13 months post-blast, activated microglia were predominantly Iba1+ types 3 and 4 microglia (relative large soma and short ramifications) in areas neighboring affected vessels (Figs. 16, 17). Electron microscopy also showed that these vascular-associated microglia harbored dilated endoplasmic reticulum (Fig. 15e). Activated microglia tended to cluster together and were frequently observed in contact with the affected vasculature. Figure 16a–c shows a tortuous tract of the pericallosal artery within the corpus callosum with clustered Iba1+ activated microglia. Similarly, Fig. 17 also shows activated microglia next to affected vasculature. Evidence for perivascular microglial phagocytosis of astrocytes was obtained in confocal optical sections as intracellular
GFAP+ material was found within perivascular Iba1+ activated microglia (Figs. 16d–f, 17c, d). Small perivascular patches of activated microglia expressing high levels of MHCII (MHCII+, characteristic of M1 microglia), were also observed in the brain of a blast-exposed animal. Some perivascular microglia were visibly undergoing apoptosis as seen by their TUNEL staining (Fig. 7) and presence of apoptotic blebs (Fig. 18). Furthermore, Iba1+ processes from activated perivascular microglia seemed to transverse the nucleus of neighboring Lochkern-like vascular cells (Fig. 18).

Discussion

**A rat model of low-level blast exposure**

We have been studying a rat model developed to mimic blast exposures associated with mild human TBI [4]. Because multiple blast exposures were common among veterans returning from Iraq and Afghanistan [31], we used a design in which rats received three blast exposures, delivered one per day on 3 consecutive days. Under the blast exposure conditions utilized here (74.5 kPa, 10.8 psi), there is no post-exposure apnea, mortality is rare and there are no pulmonary hemorrhages or other lung pathology. During the blast overpressure exposures head motion is restricted to minimize rotation/acceleration injury. Control (sham-exposed) animals are treated identically including receiving anesthesia and being placed in the blast tube, but are not blast-exposed. The lack of histological evidence for coup/contrecoup injuries or generalized brain tissue damage supports the mild nature of the injury and lack of significant rotation/acceleration injury [4, 30].

**Blast exposure affects the cerebral vasculature**

The high metabolic demand of the brain requires tight coordination between neuronal activity and blood flow [10, 23, 29, 48, 58]. Because gliovascular and

---

**Fig. 5** Correlation of behavioral traits with blast-induced cerebrovascular lesions in rats exposed to repetitive low-level blast at 13 months post-exposure. Elevated zero maze (EZM, a–c) and fear conditioning (FC, d–f) testing was performed as part of the studies described in ref [99]. EZM testing was performed at 40 weeks post-blast exposure. Shown for EZM are a open arm entries, b time spent in the open arms and c latency to cross between two open arms. Labeled in color are results from selected rats whose cerebral vasculature is illustrated in Figs. 4 (Fig. 4c, blast-exposed, red square; Fig. 4f, control, aqua circle) and 8 (Fig. 8a, b, blast-exposed, green square). FC testing was performed 42 weeks post-blast. Results are shown for the d training phase, e contextual fear memory testing (assessed 24 h after training) and f cued fear memory testing (assessed another 24 h later). Results of selected blast-exposed rats (Figs. 4c, red and 8a, b, green), are compared to the mean ± standard error measurements of blast and control cohorts determined previously [99]. Statistically significant differences between blast-exposed and control cohorts are indicated (*p<0.05; **p<0.01, unpaired t tests) [99]
neurovascular interactions control cerebral blood flow at multiple levels, their disruption following blast exposure would be expected to affect cerebral autoregulation. Morphological, biochemical and functional studies of humans and animal models have identified the vasculature as a primary target for blast-induced tissue damage [2, 6, 32, 38–41, 45, 50, 54, 63, 66, 75, 82, 112–114, 116, 130–133, 138]. Considerable evidence supports the concept of a thoracic effect whereby pressure waves transmitted through the systemic circulation damage the brain providing an additional mechanism for why blood vessels and perivascular cellular elements may be particularly susceptible to blast injury [116, 117]. A summary timeline of cerebral pathological alterations in acute, subacute and chronic cohorts are shown in Fig. 19.

In the present study, we investigated the evolution of blast-induced vascular disease using a rat model. Notably, the general structure of the circulatory system of rats is comparable to that of humans, with the brain deriving its arterial blood supply from the extracranial carotid and vertebral arteries. The internal carotid arteries and their branches supply the anterior two-thirds of the cerebral
The vertebral arteries that fuse into the basilar artery and its branches supply the remaining posterior and medial regions of the hemispheres, most of the diencephalon, brainstem, cerebellum, and cervical spinal cord. Blast exposure leads to prolonged impairment in vasoreactivity as well as changes in the structure, integrity and phenotype of cerebral blood vessels and astrocytes [2, 40, 63, 66]. Localized blast-induced vascular injury may be a determinate of the brain areas where functional alterations will occur.

**Blast-induced vascular alterations**

Micro-CT is becoming a widely used method for the accurate resolution of three-dimensional cerebral vascular structures and quantitative volumetric measurements [28, 145]. By applying this methodology in combination with computerized morphometry, we performed quantitative analyses of the brain vasculature of blast-exposed and control rats at different time points post-exposure reflecting acute, subacute and chronic periods. Statistical analyses did not show significant differences in the vascular length, surface area and volume between blast-exposed and control groups at any time point when parameters were analyzed across the whole brain. However, micro-CT analysis allowed the visualization of regional blast-induced alterations of the cerebral vasculature. For example, a rat brain examined 48 h post-exposure showed vascular attenuation in the left hemisphere, affecting the hippocampus as well as the retrosplenial,
visual, auditory, perirhinal and piriform cortical regions as well as part of the amygdala. This involved, most likely alterations in the anterior and posterior circulations, maybe through occlusion or attenuation of the posterior branches of the MCA as well as collateral branches of the PCA and the AChA. At 6 weeks and 13 months post-blast exposure, animals also presented regional alterations and disorganization in the cerebral vasculature. Lesions in the brain (tissue tears) associated with vascular alterations (vascular disorganization and hypoperfusion) correlated with behavioral observations as a rat with bilateral amygdalar lesions, cortical hypoperfusion and hippocampal vascular disorganization failed to express the characteristic chronic anxiety-related behaviors of blast-exposed animals.

We previously described blast-induced vascular attenuation/occlusion due to amorphous cellular debris, vascular collapse due to the negative pressure generated by the fast movement of blast-displaced blood, vascular “pinching” where opposite sides of the vascular walls adhere to each other, occlusion by CD34+ cells, tortuosity and structural alterations in the arterial muscle layer [3, 38, 40].

Dissociation of the vasculature from the neuropil and vascular remodeling

Electron microscopy and morphological analyses of brain samples from the 13 months post-exposure cohort showed development of a unique pathology characterized by dissociation of arterioles from the brain parenchyma (neuropil) and perivascular astrocytic degeneration. The arterial/arteriolar wall is a three-layered structure composed of an intima (endothelial cells), media (smooth muscle cells) and adventitia (fibroblasts, nerves, progenitor and immune...
The adventitia is lined with astrocytic endfeet and serves as an anchor for nerve endings [36, 87, 129]. Each layer contributes in unique ways to maintaining vascular homeostasis and regulating vascular responses to environmental conditions, stress or injury [129]. The adventitia is the principal vascular injury sensing tissue in which local fibroblasts (mesenchymal progenitors) play a central role in vascular remodeling by modulating smooth muscle cells and interacting with endothelial cells through soluble factors [53, 87, 129]. Adventitial fibroblasts produce and organize elements of the ECM (fibrillar collagens type I and II, collagen type IV, fibronectin, tenascin, osteopontin, thrombospondin), which provide mechanical strength to the vessel [108, 127, 129]. They are also involved in vascular remodeling via the regulation of matrix metalloprotease degradation of the ECM through TIMPs [18, 78, 129, 144].

Acute increases in cerebral MMP-9 associated with blood–brain barrier disruptions have been observed 2–3 h to 1 day post-blast exposure [66]. Blast-induced brain injury, as well as other forms of traumatic brain injury, have shown biphasic patterns of blood–brain barrier disruptions, where the barrier re-establishes itself after the acute phase and reopens at a later point in time [9, 11, 55, 66, 81, 106, 119]. Our results show that blast injury induces a chronic remodelling of the arteriolar laminae as evidenced by increased levels of metalloproteinases MMP-2 and MMP-9 observed at 6 weeks post-blast as well as decreased levels of

Fig. 9 Loss of vascular collagen type IV in brain regions with enlarged paravascular spaces in rats at 13 months post-blast exposure. Coronal brain sections of a blast-exposed rat were treated with pepsin to unmask the collagen type IV epitopes and stained with rabbit polyclonal antibodies against collagen type IV (red). a Lack of collagen type IV immunostaining (red) in the piriform cortical region exhibiting large paravascular spaces (arrows). Note the normal collagen IV staining (red) in the adjacent areas (asterisk). The inset in a corresponds to a section from a control animal showing normal collagen type IV staining (arrow) of the vasculature in the piriform cortex and neighboring regions. b Normal vascular collagen type IV staining of the hippocampal vasculature in the same blast-exposed animal (coll IV, red; GFAP, green). c, d Loss of collagen IV immunostaining in small vessels associated with hypothalamic enlarged paravascular spaces. GFAP+ perivascular astrocytic endfeet remained attached (GFAP, green, arrows). e Loss of collagen type IV in the arterial adventitia in regions with enlarged paravascular spaces (α-SMA, green). Arrows in e show arterial regions devoid of collagen IV (red) around a vascular stricture (*). Also note the loss of smooth muscle (α-SMA+, green) around the stricture. f Loss of adventitial collagen IV (arrows) in hippocampal arterioles (coll IV, red; α-SMA, green). DAPI, blue. Scale bar, 140 μm a, 20 μm for b–f.
vascular collagen type IV in the affected vasculature at 13 months post-exposure.

Matrix metalloproteases are responsible for degradation of ECM components, including basement membrane collagen, interstitial collagen, fibronectin and various proteoglycans [129]. Smooth muscle cells produce metalloproteases [62, 88, 93]. Vascular infiltrating inflammatory cells, endothelial cells and fibroblasts are also known to produce metalloproteases [79]. Excessive expression of vascular MMP-2 and MMP-9 gelatinase activity decreases the elasticity and modifies the structure and mechanical properties of the vessel by degrading the elastic and collagenous fibers of the tunica media and adventitia in addition to the basal lamina.

Acutely, activation of metalloproteases induces vasoconstriction via the transactivation of epidermal growth factor receptors and the production of reactive oxygen species (ROS) [51, 87, 88, 94]. Chronically,
metalloproteases and ROS can induce remodeling processes in response to prolonged vasoconstriction [87, 88]. The observed thickened basal and elastic laminae in the cerebral vasculature of blast-exposed animals could be a reflection of this phenomenon (Fig. 14). Altogether, these alterations affecting the composition and physical characteristics of the basal membrane associated with the arterial vascular smooth muscle would predict hypofunctionality of ISF flow via the IPAD system[5]. The presence of extravascular IgG also attests to vascular fragility resulting in leakage, which in turn may trigger more neuroinflammation and carry proteases involved in ECM degradation into the CNS from the periphery.

**Chronic astrocytic degeneration**

Astrocytes are multifunctional brain cells that provide ionic and trophic support to neurons, regulate intracerebral circulation and have an important role in cognitive functioning [13, 72, 77, 85, 86, 92, 96, 135]. Perivascular astrocytic degeneration is visible at 72 h through 6 weeks post-blast with no overt alterations in neuronal somatodendritic morphology [40, 81]. Similarly, decreased levels of GFAP have been observed in rats 24 h post-blast exposure [117]. Blast exposure induces epigenetic alterations with decreases in astrocytic histone acetylation and altered gene expression [8].

**Fig. 11** Microglia/macrophage and astrocytic cells in the vascular lumen. a Confocal optical section (0.56 μm) showing an Iba1+ microglia/macrophage (arrow) inside the vascular lumen (Iba 1, red; GFAP, green). Panel c shows an astrocytic cell (arrow) and its process inside the lumen of a small vessel (GFAP, green; coll IV, red). Panels b and d correspond to three-dimensional stack reconstructions of the fields shown in panels a and c. DAPI, blue. Scale bar, 15 μm for a, b, 20 μm for c, d.
In our rat model, late chronic vascular alterations induced by blast exposures were previously documented at 6–10 months following exposure [32, 38–40]. In the present study at 13 months post-blast, astrocytic degeneration and perivascular apoptosis was observed to be associated with enlarged paravascular spaces and dissociation of the vasculature from the parenchymal neuropil. Perivascular astrocytic endfeet from the blast-exposed animals were swollen with degenerating organelles, including elongated and swollen mitochondria and cellular debris. It is known that mitochondrial swelling causes rupture of the outer membrane and release of pro-apoptotic proteins such as cytochrome c into the cytosol [103]. Immunohistochemical analyses and confocal microscopic observations showed evidence for phagocytosis of perivascular astrocytes by activated microglia (Figs. 16, 17).

At advanced degenerative stages, the vasculature was dislocated from the parenchyma as bridging perivascular astrocytes were lost, suggesting that blast induces chronic perivascular astrocytic degeneration. Several studies have documented astrogliosis following blast injury [8, 26, 27, 32, 43, 65, 98]. In addition, a distinct type of glial scar and cortical perivascular astrocytosis have been described in humans exposed to blast injury [122]. Our study shows widespread, blast-induced loss of perivascular astrocytes. At 13 months post-exposure, loss of perivascular astrocytic coverage was clearly visible in GFAP- and collagen IV-immunolabeled sections and confirmed by electron microscopic observations. The loss of perivascular astrocytes would affect CSF flow through the glymphatic system. The dramatic enlargement of the paravascular space associated with perivascular astrocytic loss in major regions of the brain at 13 months post-blast may also...
indicate the continuous expansion of initial blast-induced neurovascular disconnections across the vasculature through the mechanical action of heartbeat-induced arterial peristaltic pulsations [68]. Supporting a possible similar effect in humans, Piantino et al. [104] found evidence for enlarged perivascular spaces by MRI in living Iraq/Afghanistan veterans who had been exposed to blast. These enlarged perivascular spaces (termed PVS burden) had a significant positive correlation with number of blast-induced TBIs [104]. They were also associated...
with poor sleep and persistent post-concussive symptom severity. Thus, multiple lines of evidence in animals and humans support the notion that the perivascular space is disturbed and enlarged after blast injury.

One curious observation is the presence of intraluminal astrocytic processes within blood vessels (Fig. 10). This phenomenon has been repeatedly observed in this and previous studies [40]. We suspect that the intraluminal astrocytic processes appear in hypofunctional damaged vessels into which astrocytic cells and processes could extend. Based on earlier work by six weeks after blast exposure, there is loss of GFAP in isolated vascular fractions with swelling and degeneration of astrocytic endfeet, creating an effective gliovascular disconnection [40]. However, vascular fractions from animals taken at 8 months post-blast exposure show normal levels of GFAP, suggesting some attempt at restablishing astrocytic perivascular connections [40] although the connections remain highly abnormal (Fig. 6). The unusual accumulation of perivascular astrocytes in some affected vein-like vessels at 13 months post-exposure further supports a late-onset astrocytic recovery, that although abnormal is occuring in some vessels (Fig. 10).

Reductions in cerebral blood flow result in release of vasoactive metabolites that induce vasodilation [23]. Blood flow is also controlled by hypercapnia and hypoxia, which induce dilation of cerebral arteries and arterioles, resulting in increased blood flow. On the other hand, hypocapnia causes vasoconstriction and decreased blood flow. Arterial autoregulation of blood flow is further maintained by contraction of arterial smooth muscle, which is also disturbed in this model [40]. Unlike pial arteries and arterioles, parenchymal arterioles are in close association with astrocytes and to a lesser extent with neurons [23]. Cortical vessels are innervated from subcortical brain regions with the majority of neural varicosities targeting the astrocytic endfeet surrounding arterioles [74].

Although direct signaling from neurons to blood vessels contributes to the regulation of cerebral blood flow, astrocytes play an important role in mediating neurovascular coupling [7, 60, 70]. Glutamate released from neurons acts on astrocytic metabotropic glutamate receptors to evoke Ca\(^{2+}\)-dependent release of vasoactive metabolites of arachidonic acid from astrocytic endfeet. These astrocytic mechanisms, regulated...
by pO2 and lactate, induce either vascular dilation via prostaglandin E2 (PGE2) and epoxyeicosatrienoic acids (EETs) or vasoconstriction via 20-hydroxyeicosatetraenoic acid (20-HETE). Under normoxic conditions, astrocytic Ca2+ signaling results in vasodilation. Under hyperoxia, however, vasoconstriction is favored. Release of K+ from astrocytic endfeet upon active neuronal stimulation may also contribute to vasodilation [35]. Direct stimulation of astrocytes raises calcium levels in the endfeet to induce the release of K+, 20-HETE and PGE2, which in turn causes dilation of neighboring arterioles [34, 90, 146].

Neuronal activity may also regulate capillary blood flow through Ca2+-dependent astrocytic mechanisms [15, 89] that actively dilate and constrict pericytes in response to vasoactive agents. Pericyte-induced relaxation has been shown to be mediated by activation of PGE2 receptors and by nitric oxide inhibition of the synthesis of the vasoconstrictor 20-HETE [47, 84]. Pericyte degeneration leads to neurovascular uncoupling and limits oxygen supply to the brain [71].

Control of local blood flow to match neuronal demand results, in part, from the release of neurotransmitters that stimulate receptors on smooth muscle, endothelium, pericytes or astrocytes to induce

Fig. 16 Perivascular microglia in the brain of blast-exposed animals. Panels a–c show clustered Iba1+ microglia (red, arrow in b) with amoeboid morphology in the perivascular area of a tortuous large vessel (pericallosal artery) in the corpus callosum of a rat brain at 13 months post-blast exposure. Panel d shows an orthogonal projection of a section through a thalamic artery stained for perivascular astrocytes (GFAP+, green) and microglia (Iba1+, red). Co-localization of astrocytic GFAP+ material within a perivascular microglial cell, suggests phagocytic ingestion of perivascular astrocytes by microglia. Scale bar, 100 µm a, 40 µm b, 20 µm c, d
constriction or dilation through multiple signaling pathways [7, 23, 29, 48, 49, 58–60, 109, 121]. Therefore, any disruption within the neurovascular unit by blast exposure is likely to result in altered cerebral perfusion. As confirmed here, blast exposure results in structural alterations of the arterial medial smooth muscle layer. In addition to disrupted gliovascular and neurovascular signaling and cerebral circulation, lymphatic flow and IPAD drainage could also be impaired by altered vascular smooth muscle structure[5, 142]. In the human context failure of the lymphatic and IPAD systems may translate in the long term to Abeta deposition, development of cerebral amyloid angiopathy (CAA) and Alzheimer’s disease [5].

Microglia activation associated with vascular degeneration

Microglia are small macrophage-like glial cells of the central nervous system that constitute ~ 10% of the cells in the brain [57, 76, 107]. They are in contact with synaptic elements (axon terminals, dendritic spines, astrocytic processes and synaptic clefts) where they maintain homeostasis, remove tissue debris and pathogens, and remodel synapses [16]. Upon neural injury, pathological states or chronic stress, microglia are activated and undergo morphological, proliferative and physiological changes that increase phagocytic activity and secretion of proinflammatory molecules [107]. This results in propagation of neuroinflammatory and neurodegenerative responses.

Fig. 17 Perivascular inflammation in the brain of a blast-exposed animal. Panel a shows a brain region with patches of clustered activated microglia (Iba1+, red) next to a vascularized region in between the deep cerebral white matter, choroid plexus and hippocampal fimbria. Insert in a shows higher magnification of a 0.56 µm optical section of the area indicated by arrow. Note the tortuous vessel (arrow). Panel b denotes higher magnification of an artery (identified by * in panel a) with perivascular activated microglia (Iba1+, red). Panels c, d show GFAP+ intracellular material (green) in perivascular activated microglia (Iba1, red; GFAP, green). Scale bar, 200 µm a; 10 µm b–d.
Microglial morphologies are associated with different states of activation and include ramified, primed, reactive, and ameboid microglia (Types 1–4, respectively) [69, 73, 120, 124, 128, 139]. Functional microglial activation states include the pro-inflammatory M1 activation associated with enhanced neurotoxicity and ECM damage, anti-inflammatory and neuroprotective M2a activation that induces phagocytosis, and the microglia-deactivating M2c activation involved in wound healing [143].

In a previous study we found no morphological or biochemical evidence of inflammation in the brains of rats 6 weeks post-blast exposure [41]. However, neuroinflammation was reported in an animal 16 weeks post-blast exposure with evidence of vascular rupture [41]. In the present study we present evidence that rats 13 months post-blast exposure exhibited neuroinflammation associated with vascular alterations including the presence of perivascular activated MHCII+ microglia. Expression of the major histocompatibility class II (MHCII) in microglia is linked to the inflammatory response in neurodegenerative diseases [118]. Our results indicate that this vascular-associated neuroinflammation develops after the subacute phase (6 weeks post-blast) into the chronic stage. In association with degenerating perivascular astrocytic endfeet, we also identified microglia with

Fig. 18 Perivascular microglial activation. MHCII expression is associated with microglial activation. Shown are perivascular amoeboid Iba1+ microglia (red). Some microglia are also expressing MHCII (green) and extend transcellular processes across the nuclei of Lochkern-like cells. a, Merged images, b Iba1 staining (red); c MHCII staining (green); and d DAPI staining (blue). Insert in d shows an enhanced view of a Lochkern-like cell. Arrows indicate the location of the Lochkern-like cells with hollow nuclei and the transcellular microglial processes (Iba1+ and MHCII+). Cellular blebs indicate that MHCII+ microglia appear to be undergoing apoptosis. Scale bar, 15 μm a–d; 5 μm insert in d.
dilated endoplasmic reticulum, mitochondrial alterations and cholesterol crystals in lysosomal vacuoles. Cholesterol crystals have been shown to induce destabilization of lysosomes, causing leakage of the protease cathepsin B, leading to activation of the innate NLRP3 inflammasome complex, resulting in caspase-1–mediated activation and secretion of proinflammatory cytokines [44, 110]. Following blast-induced vascular fragility and leakage, cholesterol-rich debris (including myelin) may be engulfed by microglia and infiltrating macrophages into phagosomes, which fuse with lysosomes to form phagolysosomes. Compromised reverse cholesterol transport, which normally mediates efflux of cholesterol will result in cholesterol overload, leading to formation of lysosomal membrane-damaging cholesterol crystals, release of lysosomal enzymes, inflammasome activation and cytokine secretion [80].

It has been shown that local inflammation associated with vascular degeneration may be due to elevated permeability of the blood–brain barrier. This results in clustering of activated microglia which contact the affected vasculature [16, 24, 46, 52, 126]. The dilated endoplasmic reticulum identifies these cells as dark microglia [16, 126].

Interestingly, rare activated microglia neighboring blood vessels contained cells with hollow nuclei (Lochkern-like cells [12, 105]) through which microglial processes seemed to transverse. Although the significance of this unusual observation remains unknown, transcellular migration of microglia has been extensively described in the retina after neuronal damage [64, 95, 111, 137]. This process is involved in the rapid recruitment and migration of immune and inflammatory cells to inflammatory sites without the loss of barrier integrity. Microglial transcellular and paracellular transport within the neurovascular unit [52] may be increased by the chronic vascular degenerative events induced by blast exposure.

Microglia are also known to have a critical role in vascular repair and maintenance of vascular integrity in the central nervous system [46]. Chronic mild hypoxia in the spinal cord induces transient vascular leakage associated with activated microglia clustering next to the affected vasculature, particularly in the white matter. Microglial depletion in this system leads to exaggerated vascular leakage associated with astrocyte-vascular uncoupling and loss of endothelial tight junction proteins. Microglial vascular repair depends on fibrinogen–Mac1 receptor interactions [46]. Similarly, in our rat model of blast-induced traumatic brain injury, at 13 months

---

**Fig. 19** Summary timeline of cerebral pathological alterations in a rat model of blast-induced mild TBI. Shown are observations in blast-exposed animals from acute, subacute and chronic cohorts, all of which received 3 x 74.5 kPa exposures [2, 39–41, 63, 66, 67]. Acute, 24-72 h post-blast; Subacute, 6 weeks post-blast (normal behavioral phenotype); Chronic, 16–52 weeks post-blast, (PTSD-like behavioral phenotype)
post-exposure we observed clustered activated microglia next to affected vasculature in the brain white matter. Some of these cells exhibited intracellular GFAP+ material as evidence for microglial phagocytosis of astrocytes. TUNEL analysis showed perivascular apoptosis of astrocytes and astrocytic-associated microglia. The brains of blast-exposed animals at this late time point also presented vascular leakage as evidenced by the presence of extravascular IgG. Microglial repair function may prevent further major leakage. Apoptotic loss of perivascula r microglia may result in loss of vascular repair and increased vascular fragility.

Conclusions
Our results show that blast exposure affects cellular interactions within the neurovascular unit and that astrocytes play a central role bridging the vasculature and the parenchymal neuropil. The cognitive and behavioral alterations that develop following blast exposure in both humans and animal models [30, 31, 33, 83, 99, 102] may be caused by the resulting disruptions in blood circulation, glymphatic CSF flow and intramural periarterial ISF drainage that develop as the result of altered neurovascular structure, astrocytic degeneration, vascular remodeling, vascular smooth muscle degeneration, vascular fragility, vascular leakage and neuroinflammation. Furthermore, impaired microglial function may impede vascular repair and accelerate the neurovascular degenerative processes associated with blast-induced traumatic brain injury.

Acknowledgements
We sincerely thank the anonymous reviewers whose constructive suggestions improved this manuscript considerably. We also thank Dr. Virginia Lee for generously providing the rat anti-GFAP antibody. The work described in this paper was supported by the Department of Veterans Affairs, Veterans Health Administration, Rehabilitation Research and Development Service Awards 1H01BX0004067-01 (GE), 1K08NS06659-01 (GE), 1 I21 RX003459-01 (MAGS), 1 I21 RX002069-01 (MAGS) and 1 I21 RX002876-01 (MAGS), by the Department of Defense work unit number 0000B999.0000.000.A1503, the Alzheimer’s Drug Discovery Foundation (SG) and by NIA P50 AG011508 and P30 AG066514 to Mary Sano (SG, PRH). MAGS, RDG, DP, GMP, GE, SG, RA, UK, DGC and STA are employees of the U.S. Government. This work was prepared as part of their official duties. Title 17 U.S.C. §105 provides that “Copyright protection under this title is not available for any work of the United States Government.” Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person’s official duties. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, Department of Veterans Affairs, nor the U.S. Government.

Authors’ contributions
MAGS, RDG, DP, GPG, SG, WGI, DC, SG, SJT, PRH and GE: design of experiments, analysis and interpretation of data, design of neuropathological characterization, biochemical assays and manuscript writing; DP and GMP: execution of neuropathological characterization; GPG and GMP: behavior tests; WGI and AS: electron microscopy; SH and BA: micro-CT scanning; TT and SJT: morphological and automated quantitative analyses of the cerebral vasculature; STA, RMM, RA and UK: design and conduct of blast experiments, interpretation of data and manuscript writing. All authors read and approved the manuscript.

Availability of data and materials
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

Author details
1 General Medical Research Service, James J. Peters Department of Veterans Affairs Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468, USA. 2 Department of Psychiatry, Icahn School of Medicine at Mount Sinai, One Gustave Levy Place, New York, NY 10029, USA. 3 Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. 4 Research and Development Service, James J. Peters Department of Veterans Affairs Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468, USA. 5 Department of Neurology, Icahn School of Medicine at Mount Sinai, One Gustave Levy Place, New York, NY 10029, USA. 6 Nash Family Department of Neurosurgery, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. 7 Department of Neurotrauma, Operational and Undersea Medicine Directorate, Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA. 8 The Henry M. Jackson Foundation for the Advancement of Military Medicine Inc, Bethesda, MD, USA. 9 Micro Photonics, Inc, 1550 Pond Road, Suite 110, Allentown, PA 18104, USA. 10 MBF Bioscience LLC, 185 Allen Brook Lane, Williston, VT 05495, USA. 11 Geriatric Research Education and Clinical Center, VA Puget Sound Health Care System, 1660 S Columbia Way, Seattle, WA 98108, USA. 12 Department of Medicine, University of Washington, 1595 NE Pacific St, Seattle, WA 98195, USA. 13 Mount Sinai Alzheimer’s Disease Research Center and the Ronald M. Loeber Center for Alzheimer’s Disease, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. 14 Department of Geriatrics and Palliative Care, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. 15 Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. 16 Neurology Service, James J. Peters Department of Veterans Affairs Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468, USA.

Received: 25 May 2021 Accepted: 29 September 2021
Published online: 15 October 2021

References
1. Abbott NJ (2004) Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. Neurochem Int 45:545–552. https://doi.org/10.1016/j.neuint.2003.11.006
2. Abutarboush R, Gu M, Kawoos U, Mullah SH, Chen Y, Goodrich SY, Lashof-Sullivan M, McCarron RM, Statz JK, Bell RS et al (2019) Exposure to blast overpressure impairs cerebral microvascular responses and alters vascular and astrocytic structure. J Neurotrauma 36:3138–3157. https://doi.org/10.1089/neu.2019.6423
3. Ackermans NL, Varghese M, Wicinski B, Torres J, De Gasperi R, Pryor D, Elder GA, Gama Sosa MA, Reidenberg JS, Williams TM et al (2021) Unconventional animal models for traumatic brain injury and chronic traumatic encephalopathy. J Neurosci Res. https://doi.org/10.1002/jnr.24930
4. Ahlers ST, Vasserman-Stokes E, Shaughnessy MC, Hall AA, Shear DA, Chavko M, McCarron RM, Stone JR (2012) Assessment of the effects of acute and repeated exposure to blast overpressure in rodents: toward a greater understanding of blast and the potential ramifications for injury in humans exposed to blast. Front Neurol 3:32
5. Aldea R, Weller RO, Wilcock DM, Carter RO, Richardson G (2019) Cerebrovascular smooth muscle cells as the drivers of intramural periarterial drainage of the brain. Front Aging Neurosci 11:1. https://doi.org/10.3389/fnagi.2019.00001
6. Alford PW, Dabiri BE, Goss JA, Hempill MA, Brigham MD, Parker KK (2011) Blast-induced phenotypic switching in cerebral vasospasm.
et al. Acta Neuropathol Commun (2021) 9:167

8. Bailey ZS, Grinter MB, VandeVord PJ (2016) Astrocyte reactivity following blast exposure involves aberrant histone acetylation. Front Mol Neurosci 9:64. https://doi.org/10.3389/fnmol.2016.00064

10. Ballabh P, Braun A, Nedergaard M (2004) The blood-brain barrier: an overview: structure, regulation, and clinical implications. Neurobiol Dis 16:1–13. https://doi.org/10.1016/j.nbd.2003.12.016

11. Baskaya MK, Rao AM, Dogan A, Donaldson D, Dempsey RJ (1997) The biphasic opening of the blood-brain barrier in the cortex and hippocampus after traumatic brain injury in rats. Neurosci Lett 226:33–36. https://doi.org/10.1016/S0304-3940(97)00239-5

12. Bayle MV (2016) Nuclear vacuolization. Giant Lorchern-like cells. J Oral Maxillofac Pathol:20:339–341. https://doi.org/10.4103/0973-029X.190895

13. Ben Menachem-Zidon O, Avital A, Ben-Menahem Y, Goshen I, Kreisel V, Elder GA, Ge Y, Haghighi F (2020) Blast-related mild TBI alters anxiety-like behavior and transcriptional signatures in the rat amygdala. Front Behav Neurosci 14:160. https://doi.org/10.3389/fnbeh.2020.00160

15. Biesecker KR, Srivenc AL, Shimoda AM, Agarwal A, Bergles DE, Kofuji P, Newman TA, Page AM, Nicoll JA, Perry V, Bandl M, Janssen PL, Perez GM, Searcy C, Stone JR, Dickstein DL, Hof PR, Ahlers ST et al (2017) The glymphatic system and waste clearance with brain aging. Acta Neuropathol Commun 7:6. https://doi.org/10.1186/s40478-018-0647-5

16. Blezard J, Choi I, Wang Z, Umalik M, Mendelev N, Tschiffely AE, Ahlers ST (2014) Effects of low-level blast exposure on the nervous system: is there really a controversy? Front Neurol 5:269. https://doi.org/10.3389/fneur.2014.00269

17. Blaze J, Choi I, Wang Z, Umalik M, Mendelev N, Tschiffely AE, Ahlers ST (2019) The glymphatic system and waste clearance with brain aging: a review. Gerontology 65:106–119. https://doi.org/10.1159/000490349

18. Buxbaum JD et al (2008) Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroinflammation. Neuropharmacol Appl Neurobiol 34:131–144. https://doi.org/10.1111/j.1475-6366.2007.00362.x

19. Chavko M, Koller WA, Prusaczyk WK, McCarron RM (2006) Lung injury and recovery following blast exposure. J Trauma 60:754–762. https://doi.org/10.1097/01.ta.0000218343.74554.28

20. Chavko M, Koller WA, Prusaczyk WK, McCarron RM (2007) Measurement of blast wave by a miniature fiber optic pressure transducer in the rat brain. J Neurosci Methods 159:277–281

21. Chavko M, Prusaczyk WK, McCarron RM (2006) Lung injury and recovery after exposure to blast overpressure. J Trauma 61:933–942

22. Chavko M, Watanabe T, Adeeb S, Lankasky J, Ahlers S, McCarron R (2011) Transfer of pressure wave through the body and its impact on the brain. In: Nato symposium on a survey of blast injury across a full landscape of military science, city

23. Cicolla MJ (2009) The cerebral circulation. Morgan & Claypool Life Sciences, San Rafael, CA. Available from: https://www.ncbi.nlm.nih.gov/books/NBK53087/

24. da Fonseca AC, Matias D, Garcia C, Amaral R, Geraldo LH, Freitas C, Lima FR (2014) The impact of microglial activation on blood-brain barrier in brain diseases. Front Cell Neurosci 8:362. https://doi.org/10.3389/fncel.2014.00362

25. De Gasperi R, Gama Sosa MA, Kim SH, Steele JW, Shaughness MC, Maudlin-Jeronimo E, Hall AA, Dekosky ST, McCarron RM, Nambari MP et al (2012) Acute blast injury reduces brain abeta in two rodent species. Front Neurol 3:177. https://doi.org/10.3389/fneur.2012.00177

26. de Lanerolle NC, Bandak F, Kang D, Li AY, Du F, Swauger P, Parks S, Ling G, Kim JH (2011) Characteristics of an explosive blast-induced brain injury in an experimental model. J Neurotrauma Exp Neurol 70:1046–1057

27. Dickerson MR, Bailey ZS, Murphy SF, Urban MJ, VandeVord PJ (2020) Gial activation in the thalamus contributes to vestibulomotor deficits following blast-induced neurotrauma. Front Neurol 11:618. https://doi.org/10.3389/fneur.2020.00618

28. Dorr NP, De Gasperi R, Gama Sosa MA, Shaughness MC, Maudlin-Jeronimo E, Hall AA, McCarron RM, Ahlers ST (2012) Blast exposure induces post-traumatic stress disorder-related traits in a rat model of mild traumatic brain injury. J Neurotrauma 29:2564–2575. https://doi.org/10.1089/neu.2012.2510

29. Elder GA, Ehrlich ME, Gandy S (2019) Relationship of traumatic brain injury to chronic mental health problems and dementia in military veterans. Neurosci Lett 707:134294. https://doi.org/10.1016/j.neulet.2019.134294

30. Elder GA, Stone JR, Ahlers ST (2014) Effects of low-level blast exposure on the nervous system: is there really a controversy? Front Neurol 5:269. https://doi.org/10.3389/fneur.2014.00269

31. Elder GA, Stone JR, Ahlers ST (2014) Effects of low-level blast exposure on the nervous system: is there really a controversy? Front Neurol 5:269. https://doi.org/10.3389/fneur.2014.00269

32. Elder GA, Gama Sosa MA, De Gasperi R, Stone JR, Dickstein DL, Haghighi F, Hof PR, Ahlers ST (2013) Vascular and inflammatory factors in the pathophysiology of blast-induced brain injury. Front Neurol 6:48. https://doi.org/10.3389/fneur.2015.00048

33. Elder GA, Stone JR, Ahlers ST (2014) Effects of low-level blast exposure on the nervous system: is there really a controversy? Front Neurol 5:269. https://doi.org/10.3389/fneur.2014.00269

34.Filosa JA, Bonev AD, Nelson MT (2004) Calcium dynamics in cortical astrocytes and arterioles during neurovascular coupling. Circ Res 95:e73–e81. https://doi.org/10.1161/01.RES.0000148636.60732.2e

35. Filosa JA, Bonev AD, Straub SV, Meredith AL, Wilkerson MK, Aldrich RW, Nelson MT (2006) Local potassium signaling couples neuronal activity to vasodilation in the brain. Nat Neurosci 9:1397–1403. https://doi.org/10.1038/nn1779

36. Filosa JA, Ildings JA (2013) Astrocyte regulation of cerebral vascular tone. Am J Physiol Heart Circ Physiol 305:H609-619. https://doi.org/10.1152/aphys.00359.2013

37. Francios S, De Gasperi R, Dickstein DL, English DF, Rocher AB, Janssen WG, Christoffel D, Sosa MA, Hof PR, Buixbaum JD et al (2007) Pepsin pretreatment allows collagen IV immunostaining of blood vessels in adult mouse brain. J Neurosci Methods 163:76–82

38. Gama Sosa MA, De Gasperi R, Janssen PL, Yue FJ, Anazodo PC, Pricop PE, Paulino AJ, Wicinski B, Shaughness MC, Maudlin-Jeronimo E et al (2014) Selective vulnerability of the cerebral vasculature to blast injury in a rat model of mild traumatic brain injury. Acta Neurochophol Commun 2:67. https://doi.org/10.1186/2051-5960-2-67

39. Gama Sosa MA, De Gasperi R, Paulino AJ, Pricop PE, Shaughness MC, Maudlin-Jeronimo E, Janssen PL, Yue FJ, Dorr NP et al (2013) Blast overpressure induces shear-related injuries in the brain of rats exposed to a mild traumatic brain injury. Acta Neurochophol Commun 1:51. https://doi.org/10.1186/2051-5960-1-51

40. Gama Sosa MA, De Gasperi R, Perez Garcia GS, Perez GM, Searcy C, Vargas D, Spencer A, Janssen PL, Tschiffely AE, McCarron RM et al (2019) Low-level blast exposure disrupts glivascular and neurovascular connections and induces a chronic vascular pathology in rat brain. Acta Neurochophol Commun 7:6. https://doi.org/10.1186/s40478-018-0647-5

41. Gama Sosa MA, De Gasperi R, Perez Garcia GS, Sosa H, Searcy C, Vargas D, Janssen PL, Perez GM, Tschiffely AE, Janssen WG et al (2017) Lack of chronic neuroinflammation in the absence of focal hemorrhage in an adult mouse brain. J Neurosci Methods 263:163–72

42. Goodrich JA, Kim JH, Situ R, Taylor W, Westmoreland T, Du F, Parks S, Ling G, Hwang JY, Rapuano A et al (2016) Neuronal and glial changes in the brain resulting from explosive blast in an experimental...
44. Grebe A, Latz E (2013) Cholesterol crystals and inflammation. Curr Rheumatol Rep 15:313. https://doi.org/10.1007/s11926-012-0313-z

45. Hald ES, Alford PW (2014) Smooth muscle phenotype switching in blast traumatic brain injury-induced cerebral vasospasm. Transl Stroke Res 5:385–393. https://doi.org/10.1007/s12975-013-0300-3

46. Halder SK, Milner R (2019) A critical role for microglia in maintaining vascular integrity in the hypoxic spinal cord. Proc Natl Acad Sci U S A 116:2603–26037. https://doi.org/10.1073/pnas.1902781116

47. Hall CN, Reynolds C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, O'Tarrell FM, Buchanan AM, Lauritzen M, Attwell D (2014) Capillary pericytes regulate cerebral blood flow in health and disease. Nature 508:55–60. https://doi.org/10.1038/nature13165

48. Hamel E (1985) (2006) Perivascular nerves and the regulation of cerebrovascular tone. J Appl Physiol 100:1059–1064. https://doi.org/10.1152/japplphys.00094.2005

49. Hamilton NB, Attwell D, Hall CN (2010) Pericyte-mediated regulation of capillary diameter: a component of neurovascular coupling in health and disease. Front Neuroenergetics. https://doi.org/10.3389/fneur.2010.00005

50. Han X, Chai Z, Ping X, Song LJ, Ma C, Ruan Y, Jin X (2020) In vivo two-photon imaging reveals acute cerebral vascular spasm and microthrombosis after mild traumatic brain injury in mice. Front Neurosci 14:210. https://doi.org/10.3389/fnstr.2020.00210

51. Hao L, Du M, Lopez-Campistrous A, Fernandez-Patron C (2004) Agonist-induced activation of matrix metalloproteinase-7 promotes vasoconstriction through the epidermal growth factor-receptor pathway. Circ Res 94:68–76. https://doi.org/10.1161/01.RES.0000109413.57726.91

52. Haruvyaka K, Kegami A, Tachibana Y, Ohno N, Konishi H, Hashimoto A, Matsumoto M, Kato D, Ono R, Kyama H et al (2019) Dual microglia effects on blood brain barrier permeability induced by systemic inflammation. Nat Commun 10:5816. https://doi.org/10.1038/s41467-019-13812-z

53. Haurani MJ, Pagano PJ (2007) Advenitial fibroblast reactive oxygen species act as autocrine and paracrine mediators of remodeling: bellwether for vascular disease? Cardiovasc Res 75:679–689. https://doi.org/10.1016/j.cardiores.2007.06.016

54. Hernandez A, Tan C, Plattner F, Logsdon AF, Pozo K, Youssf MA, Singh T, Turner RC, Luke-Wold BP, Huber JD et al (2018) Exposure to mild blast forces induces neuropathological effects, neurophysiological deficits and biochemical changes. Mol Brain 11:44. https://doi.org/10.1186/s12987-018-0332-6

55. Hicks RR, Baldwin SA, Scheff SW (1997) Serum extravasation and cytoskeletal alterations following traumatic brain injury in rats. Comparison of lateral fluid percussion and cortical impact models. Mol Chem Neuropathol 32:1–16. https://doi.org/10.1016/S0306-3672(97)80007-2

56. Hiladky SB, Bannard MA (2018) Elimination of substances from the brain parenchyma, egress via perivascular pathways and via the blood-brain barrier. Fluids Barriers CNS 15:30. https://doi.org/10.1186/s12987-018-0113-6

57. Hoogland IC, Houbolt C, van Westerloo DJ, van Gool WA, van de Beek ER, Banks WA, Cook DG (2018) Blast exposure elicits blood-brain barrier permeability in a rat model. PLoS ONE 11:e0167510. https://doi.org/10.1371/journal.pone.0167510

58. Kacza J, Seeger J (1997) Transcellular labelling of activated retinal microglia following transaction of the optic nerve. Inflamm Res 46:430–433. https://doi.org/10.1007/s0001100500219

59. Kallauri S, Desai A, Feng K, Tummala S, Saif T, Zhang L, Cavanaugh JM, King Al (2017) Neuronal injury and glial changes are hallmarksofs of open field blast exposure in swine frontal lobe. PLoS ONE 12:e0169293. https://doi.org/10.1371/journal.pone.0169293

60. Kawoos U, Abutarboush R, Gu M, Chen Y, Statz JK, Goodrich AH, Ayers ST (2021) Blast-induced temporal alterations in blood-brain barrier permeability in a rodent model. Sci Rep 11:5096. https://doi.org/10.1038/s41598-021-84730-8

61. Kedarasetti RT, Drew PJ, Costanzo F (2020) Arterial pulsations drive oscillatory flow of CSF but not directional pumping. Sci Rep 10:101102. https://doi.org/10.1038/s41598-020-66887-w

62. Khratkin H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiol Rev 91:461–553. https://doi.org/10.1152/physrev.00011.2010

63. Kim J, Seo J, Kim S, Kang S, Park J, Lee H, Cho Y, Cho J, Kim Y et al (2017) Neuronal injury and glial changes are hallmarksofs of open field blast exposure in swine frontal lobe. PLoS ONE 12:e0169293. https://doi.org/10.1371/journal.pone.0169293

64. Koh J, Seeger J (1997) Transcellular labelling of activated retinal microglia following transaction of the optic nerve. Inflamm Res 46:430–433. https://doi.org/10.1007/s0001100500219

65. Kacza J, Seeger J (1997) Transcellular labelling of activated retinal microglia following transaction of the optic nerve. Inflamm Res 46:430–433. https://doi.org/10.1007/s0001100500219
et al. acta neuropathol commun           (2021) 9:167

84. Mackie AR, Losordo DW (2011) CD34-positive stem cells: in the treat-
85. MacVicar BA, Newman EA (2015) Astrocyte regulation of blood flow
89. Mishra A, Reynolds JP, Chen Y, Gourine AV, Rusakov DA, Attwell D (2016)
90. Mulligan SJ, MacVicar BA (2004) Calcium transients in astrocyte endfeet
92. Nedergaard M, Ransom B, Goldman SA (2003) New roles for astrocytes:
93. Newby AC (2006) Matrix metalloproteinases regulate migration, prolif-
94. Odenbach J, Wang X, Cooper S, Chow FL, Oka T, Lopaschuk G, Kassiri
95. Omri S, Behar-Cohen F, de Kozak Y, Sennlaub F, Verissimo LM, Jonet L,
96. Ota Y, Zanetti AT, Hallock RM (2013) The role of astrocytes in the
102. Petrie EC, Cross DJ, Yarnyk VL, Richards T, Martin NM, Pagulayan K, Hoff
103. Petronilli V, Penzo D, Scorrano L, Bernardi P, Di Lisa F (2001) The
106. Price L, Wilson C, Grant G (2016) Blood-brain barrier pathophysiology
107. Prinz M, Masuda T, Wheeler MA, Quintana FJ (2021) Microglia and
109. Puro DG (2007) Physiology and pathobiology of the pericyte-con-
110. Rajamaki K, Lappalainen J, Oorni K, Valimaki E, Kovanen
116. Rubio JE, Unnikrishnan G, Sajja V, Van Albert S, Rossetti F, Skotak M, Alay
118. Schetters ST, Gomez-Nicola D, Garcia-Vallejo JJ, Van Kooym Y (2017)
119. Schetters TTG, Gomez-Nicola D, Garcia-Vallejo JJ, Van Kooym Y (2017)

synthase mediates cerebellar dysfunction in mice exposed to repetitive
blast-induced mild traumatic brain injury. Behav Brain Res 340:117–125. https://doi.
10.1016/j.bbr.2016.09.061
101. Perez-Garcia G, Gama Sosa MA, De Gasperi R, Lashof-Sullivan M,
Maudelin-Jerominio E, Stone JR, Haghigi F, Ahlers ST, Elder GA (2018)
Chronic post-traumatic stress disorder-related traits in a rat model of
low-level blast exposure. Behav Brain Res 340:117–125. https://doi.org/10.1016/j.bbr.2016.09.061
Perez-Garcia G, Gama Sosa MA, De Gasperi R, Lashof-Sullivan M,
Maudelin-Jerominio E, Stone JR, Haghigi F, Ahlers ST, Elder GA (2018)

119. Shapira Y, Setton D, Artru AA, Shohami E (1993) Blood-brain barrier permeability, cerebral edema, and neurologic function after closed head injury in rats. Anesth Analg 77:141–148. https://doi.org/10.1213/00000539-199307000-00028

120. Sheng JG, Mrak RE, Griffin WS (1997) Neuritic plaque evolution in Alzheimer’s disease is accompanied by transition of activated microglia from primed to enlarged to phagocytic forms. Acta Neuropathol 94:1–5

121. Shepro D, Morel NM (1993) Pericyte physiology. FASEB J 7:1031–1038

122. Shively SB, Horkayne-Szakaly I, Jones RV, Kelly JP, Armstrong RC, Perl DP (2016) Characterisation of interface astroglial scarring in the human brain after blast exposure: a post-mortem case series. Lancet Neurol 15:944–953. https://doi.org/10.1016/S1474-4422(16)30057-4

123. Skotak M, Townsend MT, Ramarao KV, Chandra N (2019) A Comprehensive review of experimental rodent models of repeated blast TBI. Front Neurol 10:1015. https://doi.org/10.3389/fneur.2019.01015

124. Soltys Z, Ziaja M, Pawlinski R, Sekowicz Z, Janeczko K (2001) Morphology of reactive microglia in the injured cerebral cortex. Fractal analysis and complementary quantitative methods. J Neurosci Res 63:90–97. https://doi.org/10.1002/1097-4547(200101)63:1<90::AID-JNNR11>3.0.CO;2-9

125. Squire LR (2013) Fundamental neuroscience. Elsevier, Amsterdam

126. St-Pierre MK, Simoncicova E, Bogi E, Tremblay ME (2020) Shedding light and complementary quantitative methods. J Neurosci Res 63:90–97. https://doi.org/10.1002/1097-4422(1984)246:6<835:SMM%3E2.3.CO;2-9

127. Stence LR (2013) Fundamental neuroscience. Elsevier, Amsterdam

128. Stence LR, Waite M, Dailey ME (2001) Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices. Glia 33:256–266

129. Steffensen LB, Rasmussen LM (2018) A role for collagen type IV in cardiovascular disease? Am J Physiol Heart Circ Physiol 315:H610–H625. https://doi.org/10.1152/ajpheart.00700.2018

130. Stone JR, Avants BB, Tustison NJ, Wassermann EM, Gill J, Polejeva E, Dell KC, Carr W, Yamelli AM, LoPresti ML et al (2020) Functional and structural neuroimagining correlates of repetitive low-level blast exposure in career bracers. J Neurotrauma 37:2468–2481. https://doi.org/10.1089/neu.2020.7141

131. Sullivan DR (2019) A cerebrovascular hypothesis of neurodegeneration in mTBI. J Head Trauma Rehabil 34:E18–E27. https://doi.org/10.1097/HTR.0000000000000449

132. Sullivan DR, Miller MW, Wolf EJ, Logue MW, Robinson ME, Fortier CB, Fonda JR, Wang DJ, Milberg WP, McGilnchy RE et al (2020) Cerebral perfusion is associated with blast exposure in military personnel without moderate or severe TBI. J Cereb Blood Flow Metab. https://doi.org/10.1002/jcn.20351

133. Sullivan DR, Miller MW, Wolf EJ, Logue MW, Robinson ME, Fortier CB, Fonda JR, Wang DJ, Milberg WP, McGilnchy RE et al (2020) Cerebral perfusion is associated with blast exposure in military personnel without moderate or severe TBI. J Cereb Blood Flow Metab 41:886–900. https://doi.org/10.1002/jcn.20351

134. Sullivan EV (2012) War-related PTSD, blast injury, and anosognosia. Neuropsychol Rev 22:1–2. https://doi.org/10.1007/s11065-012-9188-z

135. Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistratti P, Alberini CM (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. Cell 144:810–823. https://doi.org/10.1016/j.cell.2011.02.018

136. Szentistvanyi I, Patlak CS, Ellis RA, Csern HF (1984) Drainage of interstitial fluid from different regions of rat brain. Am J Physiol 246:F835-844. https://doi.org/10.1152/ajpnyr.1984.246.6.F835

137. Thanos S, Pavlidis C, Mey J, Thiel HU (1992) Specific transcellular staining of microglia in the adult rat after traumatic degeneration of carboxy-anine-filled retinal ganglion cells. Exp Eye Res 55:101–117. https://doi.org/10.1016/0014-4835(92)90098-d

138. Toklu HZ, Muller-Delp J, Yang Z, Okray S, Sakarya Y, Strang K, Ghosp, P, Delp MD, Scarpace PJ, Wang KW et al (2015) The functional and structural changes in the basilar artery due to overpressure blast injury. J Cereb Blood Flow Metab 35:1950–1956. https://doi.org/10.1038/jcbfm.2015.151

139. Torres-Platas SG, Comeau S, Rachalski A, Bo GD, Cuceanu C, Turecki G, Giro B, Mechawar N (2014) Morphometric characterization of microglial phenotypes in human cerebral cortex. J Neuroinflammation 11:12. https://doi.org/10.1186/1742-2094-11-12

140. Troyanskaya M, Pastorek NJ, Scheibiel RS, Petersen NJ, McCulloch K, Wilde EA, Hensk HK, Levin HS (2015) Combat exposure, PTSD symptoms, and cognition following blast-related traumatic brain injury in OEF/OIF/OND service members and Veterans. Mil Med 180:285–289. https://doi.org/10.25755/MILMED-D-14-00256

141. Trudeau DL, Anderson J, Hansen LM, Shagalov DN, Schmoller J, Nugent S, Barton S (1998) Findings of mild traumatic brain injury in combat veterans with PTSD and a history of blast concussion. J Neuropsychiatry Clin Neurosci 10:308–313. https://doi.org/10.1176/jnp.10.3.308

142. Ueno M, Chiba Y, Murakami R, Matsumoto K, Fujihara R, Uemura N, Ymanse K, Partama M (2019) Disturbance of intracerebral fluid clearance and blood-brain barrier in vascular cognitive impairment. Int J Mol Sci. https://doi.org/10.3390/ijms20102600

143. Varnum MM, Ikezu T (2012) The classification of microglial activation phenotypes on neurodegeneration and regeneration in Alzheimer’s disease. Brain. Arch Immunol Ther Exp (Warsz) 60:251–266. https://doi.org/10.1007/s00005-012-0181-2

144. Veillard-Baron A, Frisell B, Raffestin B, Baker AH, Edditahibi S, Adnot S, D’Ortho M-P (2003) Inhibition of matrix metalloproteinases by lung TIMP-1 gene transfer limits monocrotaline-induced pulmonary vascular remodeling in rats. Hum Gene Ther 14:861–869. https://doi.org/10.1089/1089849034303765701150

145. Zagorevich L, Osse P, Zhuang ZW, Moodie K, Mulligan-Kehoe MJ, Simons M, Couffignal T (2010) Micro computed tomography for vascular exploration. J Angiogenes Res 2:7. https://doi.org/10.1186/2043-2984-2-7

146. Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G (2003) Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nat Neurosci 6:43–50. https://doi.org/10.1038/nne980

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more: biomedcentral.com/submissions