Blast Injury Results in Widescale Purkinje Cell Damage in the Human Cerebellum

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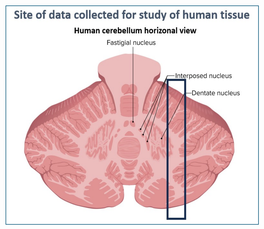
Abstract:

In recent years, the cerebellum has emerged as an important neural structure controlling many functions in addition to balance and coordination, including cognition, emotion, and sleep. Unfortunately, we know little about the precise effects of traumatic brain injury on the cerebellum. Occupational blast exposure from artillery, explosives, and other weapons is a key characteristic of military training that can lead to repeated microtraumas in the brain. To assess the impact of blast related injuries on the human cerebellum, we evaluated the histopathology of warfighters who were either exposed to multiple explosive blasts or control subjects not known to experience such injuries. The brain samples were obtained from the DoD/USU Brain Tissue Repository. In individuals exposed to blasts, we observed a dramatic loss of Purkinje cell immunoreactivity for calbindin throughout the cerebellum, especially evident in ventral cerebellar regions. The Purkinje cell dendritic morphology revealed by calbindin immunoreactivity was also stunted. In addition, Bergmann glia exhibited altered morphology showing a strong beaded pattern rather than relatively smooth processes normally present in cerebellar cortex. We also observed abnormal appearing astrocytes immunoreactive for aquaporin 4, primarily located at the interface of cerebellar cortex and white matter. The cerebelli of control brains showed relatively normal Purkinje cell numbers and distribution, although we observed variability in different subjects not known to receive brain injury. This suggests that blast traumatic brain injury can substantially alter Purkinje cell number, distribution and dendritic morphology, as well as glial cell morphology, which likely impacts multiple neurologic functions. The variability in Purkinje cell number of control subjects also indicates that multiple life experiences may influence the health and morphology of Purkinje cells.

Introduction:

- Purkinje cells are the primary output neuron of the cerebellar cortex. They are responsible for integrating sensory and motor information to fine-tune movement by sending inhibitory signals to other brain regions via GABA.
- To study TBI in the warfighter, we simulate occupational blast exposure by placing anesthetized ferrets in a blast chamber where they are subjected to high pressure waves.
- We surprisingly found strong neuropathologic findings in the cerebellum of blast-injured ferrets and decided to assess and compare potential damage in human cerebelli donated by Special Operations warfighters exposed to occupational blast throughout their careers.



Patient History: Human Subjects

ID #	Age	Branch	Occupation	Blast Exposure	Alcohol Abuse	Cause of Death	Psychological Diagnoses	Relevant Additional Past Medical History
1	54	Navy	SEAL	Yes	Yes	Suicide	PTSD	Frequent falls, neuropsychiatric issues, polysubstance abuse
2	47	Navy	SEAL	Yes	Unknown	Suicide	PTSD	Steven Johnson Syndrome
3	47	Navy	SEAL	Yes	Yes	Suicide	PTSD	Irritability, cognitive changes, chronic pain, CTE
4	58	Navy	Administrative	No	Unknown	Sudden cardiac death		Hypertension, hyperlipidemia, glucose intolerance
5	44	National Guard	Administrative	No	Unknown	Sudden cardiac death	Depression	Hyperlipidemia, COPD
6	48	Army	Administrative	No	Unknown	Unknown		Migraines, hyperlipidemia, blood clots, cardiac issues

Figure 1: On the left, this diagram shows the site of tissue used for study of human cerebellum. The table shows the past medical history of human subjects. Injured subjects were Special Operations warfighters.

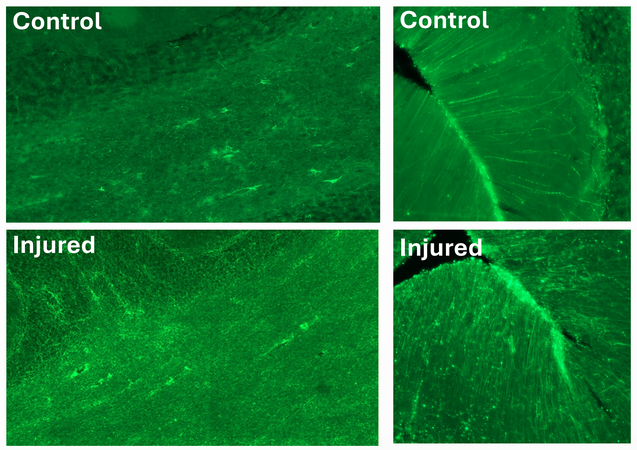


Figure 2: GFAP immunoreactivity (marker for Bergmann glia) is increased in the white matter of the cerebellum (left) in injured brain. The cerebellar cortex (right) shows an abnormal beading pattern along Bergmann glia in injured brains, while Bergmann glia in control brains are predominantly straight and not beaded.

The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of War.

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Thank you to the servicemembers & their families for donating their brains to the DoW/USU Brain Tissue Repository.

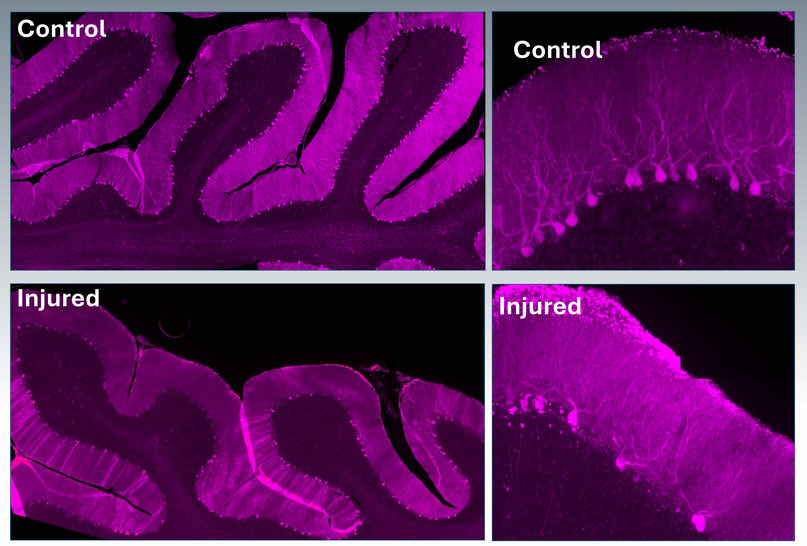

Figure 3: Examples of control and injured cerebellum showing Purkinje cells labeled with calbindin. Injured subjects consistently showed decreases in Purkinje cell number and dendritic density.

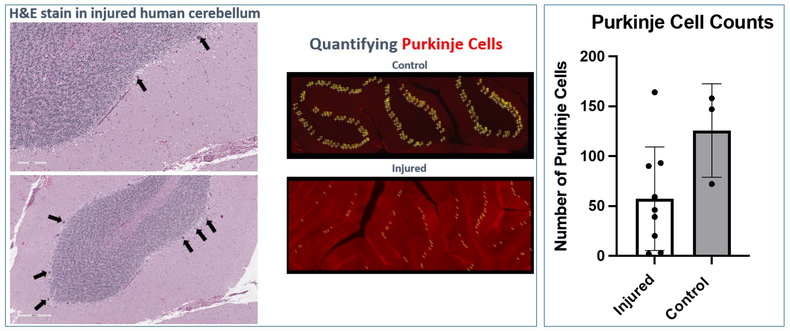

Figure 4: On the left, hematoxylin and eosin (H&E) staining in the cerebellum of an injured human subject. Note sparse Purkinje cells throughout the cortex (designated by arrows). On the right, immunohistochemistry for calbindin labeling Purkinje cells and subsequent quantification using Image J multi-point tool. Purkinje cell counts are decreased in injured human cerebelli.

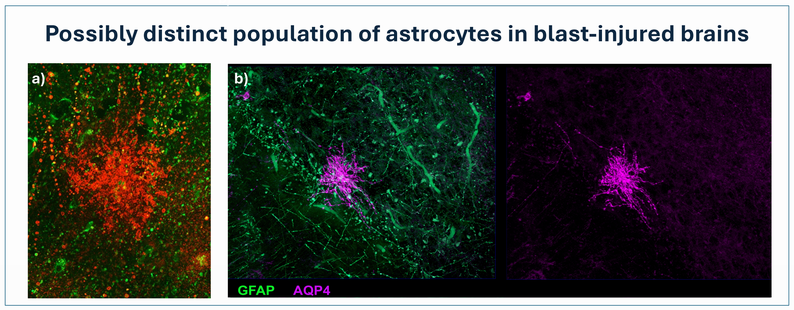

Figure 5: Previously, we observed a distinct population of AQP4+ astrocytes (red) in human blast-injured prefrontal cortex (left) that did not co-localize with GFAP (green); this abnormal population was not observed in controls. We found a similar abnormal population of AQP4+ (purple) astrocytes in the cerebellar cortex at the interface between grey and white matter in injured brains (right) that did not co-localize with GFAP (green).

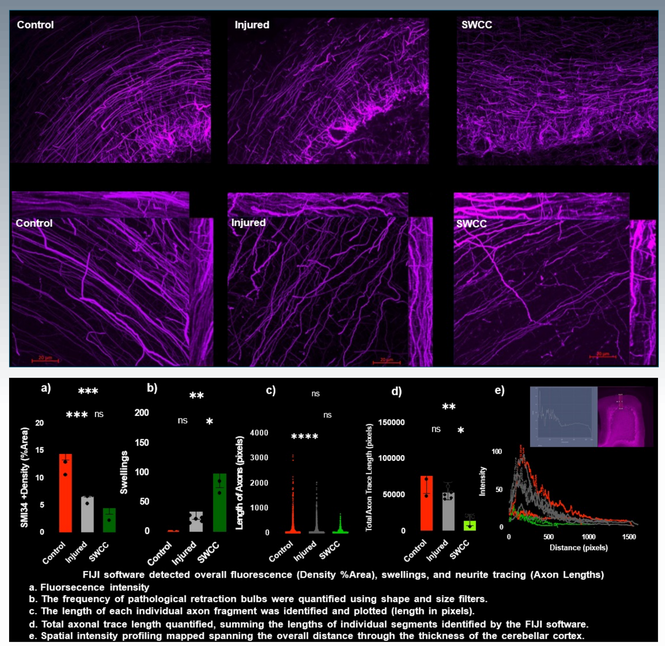

Figure 6: Above, images from cerebellar folia immunoreacted for SMI34, a marker of axonal integrity. Across all subjects, axons are observed throughout the molecular layer of the cerebellar cortex. Blast injured and SWCC operators show reduced density and organization of axons, in addition to pathologic retraction bulbs at axon terminals.

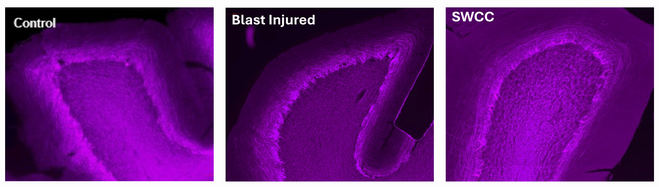

Figure 7: Side-by-side comparison of SMI34 labeling of axons in the molecular layer of the cerebellar cortex. SWCC brains show the greatest reduction in axonal density.

Conclusions:

- Blast-injured human cerebelli show increased immunoreactivity and abnormal, pathological beading in Bergmann glia.
- Blast-injured human cerebelli show severely decreased Purkinje cell numbers and dendritic density compared to controls.
- A distinct population of astrocytes may become activated in the prefrontal and cerebellar cortices at grey-white matter interface regions in response to blast injury.
- Axonal integrity in the cerebellar cortex is disrupted in blast-injured subjects with the most significant disruption noted in Navy SWCCs.
- Cerebellar pathology may be associated with disrupted cognition and other behavioral changes observed in Special Operations warfighters exposed to occupational blast.