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Histopathological Analysis of the Role of Horn on the Memory/Intelligence Processing Areas of the Brain and the Blood Brain Barrier in Female Sheep

Análisis Histopatológico de la Función del Cuerno en las Áreas de Procesamiento de Memoria/Inteligencia del Cerebro y la Barrera Hematoencefálica en Ovejas

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SUMMARY: Because horned animals are prone to conflict, they may have a higher risk of developing a brain contusion as a result of a horn strike. Repeated brain trauma can affect the hippocampus, which is involved in memory and orientation. The aim of this study was to histopathologically examine the blood-brain barrier (BBB) of the hippocampus in horned and hornless female sheep. Skulls of 4 horned and 6 hornless female sheep aged 16±4 months were obtained from the abattoir. Brains were carefully removed and preserved in 10 % formalin for 5 days. Sections were taken from bilateral hippocampus. Specimens were stained with hematoxylin-eosin (H&E) and Tunel methods, and the histopathologic structures of the BBB were examined by light microscopy. BBB damage scores evaluated. The results were analyzed by Mann-Whitney U test. The BBB damage score was found to be higher in horned female sheep. Astrocytes was more branching and BBB was more intact in hornless female sheep. This is considered that more astrocyte branches may play an important role in the protective mechanism. The difference in intracerebral defense mechanism against brain trauma in female sheep may serve as a model for investigating molecular mechanisms. The impairment of the BBB in the hippocampal region of horned female sheep may result in their memory, intelligence, and navigational abilities being lower than those of hornless female sheep. This could open the horizon for better advances in human and animal brain health.

KEY WORDS: Blood brain barrier; Female sheep; Hippocampus; Horn; Trauma.

INTRODUCTION

Comparative histopathology of animal brains may reveal different findings. Considering the confrontational characteristics of horned and hornless animals, it would seem that horned animals are more combative. These conflicts suggest that horned animals may be more susceptible to brain trauma. Therefore, a comparative study of horned and hornless animal brains may provide important information about both the effects of brain trauma and the histomorphologic differences that protect against this trauma.

It has been reported in the literature that male bighorn sheep have been involved in prolonged bouts of collision but have not suffered any serious brain damage (Wheatley *et al*., 2023). Similarly, it was reported that although bighorn sheep routinely receive severe blows to the head and horns as part of intraspecific fighting, this does not result in obvious negative consequences to their brains or horns (Drake *et al*., 2016). Contrary to these studies, there are researchers who suggest that in bighorn sheep, the energy generated during a collision may create unwanted stresses in the frontal skull that can lead to brain damage (Maity & Tekalur, 2011). Johnson *et al*. (2021), emphasized that the brain injury measures in their ram study may be helpful in assessing the actual injury criteria for blows to the human head.

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Traumatic brain injury (TBI) is a life-threatening condition. One study reported that TBI accounts for the majority of injury-related deaths in the United States (Cash & Theus, 2020). One of the brain tissue structures that can be affected by TBI is the blood-brain barrier (BBB). The BBB is a selectively permeable membrane that regulates the passage of molecules into the environment of neurons. TBI can lead to a pathological disruption of the BBB resulting in a variety of neurological disorders. It has been reported that BBB dysfunction is associated with epilepsy, but the molecular mechanisms underlying this dysfunction need to be clarified (Greene *et al*., 2022). Subconcussion is a much milder form of head impact that does not cause noticeable clinical symptoms (Gysland *et al*., 2012). Repetitive subconcussive blows can disrupt the BBB and cause neurobehavioral dysfunction (Hiles-Murison *et al*., 2021). Hippocampal oxidative stress after subconcussion has been reported in mouse models (Mao *et al*., 2018). Repeated exposure to subconcussive head trauma in sheep can cause damage to the hippocampal structure.

The hippocampus has a particularly important role in memory and orientation. There are studies that show that sheep are good at tasks that require learning spatial navigation (Marino & Merskin, 2019). In a study testing memory and reverse learning using a modified Y-maze, sheep were reported to be able to remember the maze for at least 22 weeks (Hunter *et al*., 2015). The aim of this study was to investigate the histopathologic structure of the BBB of the hippocampus associated with memory and, in particular, orientation in horned and hornless female sheep.

MATERIAL AND METHOD

Animals: The skulls of 4 horned and 6 hornless female sheep aged 16±4 months living in rural areas were obtained from the abattoir. The brains were removed by careful dissection.

Magnetic Resonance Imaging (MRI): All brains were imaged in sagittal, coronal, and axial planes using 1.5T magnetic resonance imaging (MRI).

Histopathologic Examination: The right and left temporal lobes were carefully separated and sections were taken from both hippocampi (Fig. 1).

Tissue Monitoring Procedure: Brains were immersed in 10 % formalin and washed in running water for 4 hours. Tissues were dehydrated by passing through increasing alcohol series (50 %-60 %-70 %-70 %-70 %-80 %-80 %-80 %-80 %-96 %-100 %), transparency by passing through xylene series and infiltration by passing through paraffin series and paraffin and tissue blocks were prepared. The

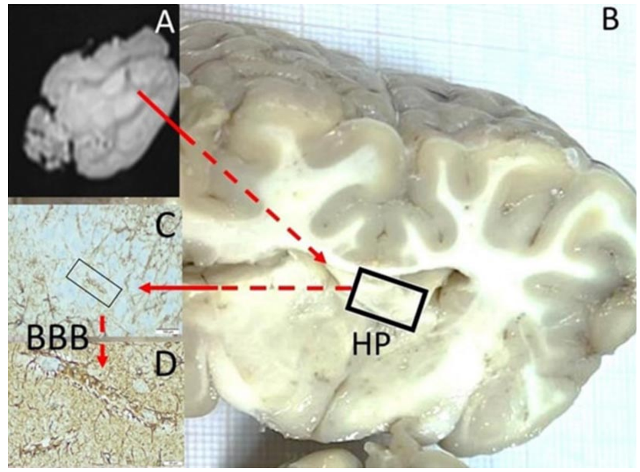

Fig. 1. Magnetic Resonance Image – MRI (A) and anatomical appearances of the hippocampus (B); and histological appearances of the blood brain barrier (BBB) of the hippocampus (C and D), C- microstructure of the BBB X 4, D- magnified structure of the BBB X 40.

blocked tissues were cooled at +4° C and five sections of 5 µm thickness were taken from each block with a Leica RM 2145 microtome device.

Hematoxylin and eosin (H&E) staining: The samples were placed on polylysine slides and kept in an oven at 65 °C for 15 min and then passed through a xylene series to remove the paraffin completely. Tissue samples were washed with distilled water and stained with hematoxylin. After washing to remove excess stain, eosin staining was performed for counterstaining. Excess eosin was removed with 96 % alcohol, and sections passed through alcohol and xylene series were coverslipped with entellan for microscopic examination.

Immuno-histochemical (IHC) Staining: Staining of 5 µm samples on polylysine slides with GFAP (Glial Fibril Acidic Protein) was performed automatically on a Ventana BenchMark® GX automated immunohistochemistry instrument. Samples were covered with Entellan and coverslips for microscopic examination.

The preparations were evaluated and photographed by computerized light microscopy. The BBB damage score was calculated using the scoring system of Aydin *et al*. (2006), Endothelial cell shrinkage (1 point), cellular angulation (2 points), pericytoplasmic halo formation (3 points), nuclear condensation (4 points), and less than 5 periarteriolar astrocytes per arteriole section [n/section (5) points] were scored for a total of 15 points. A total score <6 was considered intact/mild impairment, 6 - 10 was considered moderate impairment, and >10 was considered severe impairment.

Statistical Analysis. The data of our study were statistically analyzed using the IBM 20.00 SPSS program. Significance between two independent groups was analyzed by Mann-Whitney U test. P<0.05 was considered significant.

RESULTS

Hornless female sheep showed anatomically more intact brain tissue, a better-looking MRI and histopathologically more intact arterioles and capillary endothelial structure, more numerous and more branched astrocytes (Fig. 2). However, a horned female sheep showed anatomically less intact brain tissue and a poor appearance on brain MRI, and histopathologically, more degenerated arterioles and capillary endothelium and fewer branching astrocytes were observed (Fig. 3). Post-traumatic gliotic areas, enlarged subarachnoid spaces, and arachnoid-pia adhesions were observed on the brain surfaces of horned female sheep. BBB damage scores are shown in Table I.

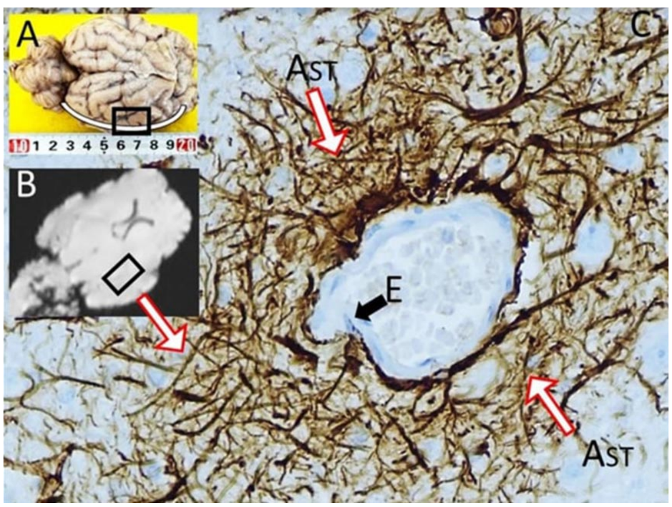

Fig. 2. In a hornless sheep, anatomically more intact brain tissue (A), a better-looking MRI (B) and more numerous and more branched astrocytes (AST) with more robust arteriol and arteriolar endothelium (E) are monitored (LM, GFAP, x40/C).

BBB damage score in bilateral hippocampus (hornless: 12, horned: 8; Table I):

- hornless female sheep: <6 (intact or mild damage) in 6 samples, 6-10 (moderate damage) in 4 samples, >10 (severe damage) in 2 samples,
- horned female sheep: <6 (intact or slight damage) in 2 samples, 6-10 (moderate damage) in 3 samples, >10 (severe damage) in 3 samples

The difference between hornless and horned sheep in these scores was statistically significant ($p<0.05$).

Table I. Blood-brain barrier damage scores of bilateral hippocampus in horned and hornless female sheep brains.

Score	Horned (n: 4) Total: 8 (right + left)	Hornless (n: 6) Total: 12 (right + left)	p
< 6	2	6	<0.005
6 - 10	3	4	<0.001
>10	3	2	<0.0001

DISCUSSION

Because horns are used as a means of conflict in animals, they are subjected to head trauma and therefore brain trauma. It was emphasized that horn ramming of male bighorn sheep did not cause significant brain damage and that keratin-rich horn and bony horn nuclei may play a role in mitigating brain damage by reducing brain acceleration through elastic mechanisms that dissipate energy (Wheatley *et al*., 2023). It has been reported that conical spiral horns are important in absorbing shock and that the trabecular horn core structure absorbs shock 3 times more (Drake *et al*., 2016). The trabecular bone structure distributes the stresses over a larger volume and absorbs more of the impact energy (Fuller & Donahue, 2021). Situations where the energy threshold is exceeded lead to acceleration in the brain (Johnson *et al*., 2021). It has been reported that the absorption of impact energy is greatly reduced and acceleration is increased by 49 % when the front half of the long horns are cut in Bighorn male sheep (Drake *et al*., 2016).

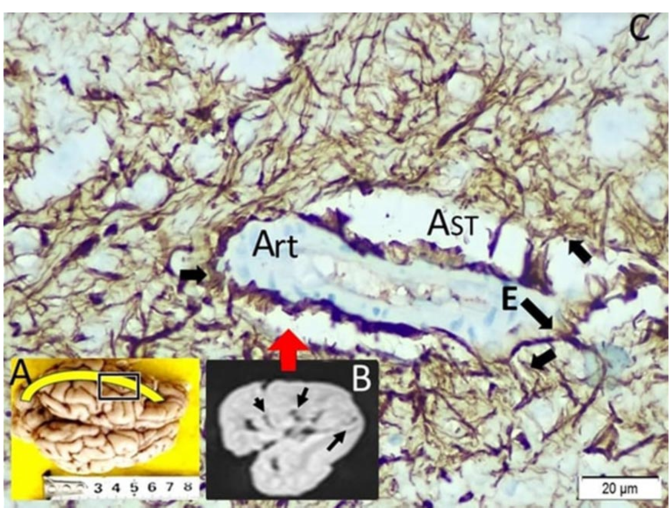

Fig. 3. In a horned sheep, anatomically the less intact brain tissue (A), a bad-looking MRI appearances of brain (B), black arrows indicate ischemic, traumatic and gliotic areas (B) and less number and less branched astrocytes (AST) with more deformed arterioles (Art) and degenerated arteriolar endothelium (E) are monitored (LM, GFAP, x40/C).

In our study, female sheep were selected, not rams (male sheep). Female sheep also come in horned and hornless varieties. Horned female sheep have smaller horn lengths (~ 8 cm) than horned male sheep. Considering the small horns and biomechanical structure of female sheep horns, it can be assumed that these horns are not sufficient to absorb high impact energy. In the present study, the fact that horned female sheep showed significant brain damage supports that horn structures may be less effective at absorbing impact energy than bighorn sheep. In this study, hornless female sheep were found to have less brain damage (Fig. 2; Table I). The less confrontational nature of hornless female sheep may result in less trauma exposure and therefore less brain damage in these sheep. However, differences related to the structure of the BBB and other protective mechanisms are discussed below. Acceleration in head trauma also affects the BBB. Studies suggest that BBB dysfunction often follows head trauma and may persist for several days, weeks, or even years after the acute event (Tomkins *et al*., 2008). The BBB is a selective semipermeable membrane that regulates the movement of molecules and ions between the blood and the brain. This structure is a highly selective barrier that prevents the passage of pathogens and toxic components from the blood into the brain and ensures the delivery of nutrients to the brain tissue.

The BBB consists of endothelial cells (ECs), pericytes (PCs), capillary basement membrane and astrocyte end feet (Dotiwala *et al*., 2023). Tight junctions between capillary endothelial cells form a highly selective diffusion barrier that prevents most blood-borne substances from entering the brain (Luissint *et al*., 2012). Disruption of the tight junction can lead to a variety of neurological disorders, including stroke, neuroinflammatory disorders, and intraventricular hemorrhage (Ballabh *et al*., 2004). Astrocytes involved in the formation of the BBB are found in both gray matter (protoplasmic astrocytes - long, unbranched extensions) and white matter (fibrous astrocytes - short, highly branched extensions) (Chaboub & Deneen, 2012). Pedicels of both protoplasmic and fibrous astrocytes also surround nerve fibers, neuronal somas, and the abluminal surface of vessels. Astrocyte endfeet coat the vascular wall and play a critical role in the induction and maintenance of the tight junction barrier (Dotiwala *et al*., 2023).

In the present study, astrocyte branching around the endothelium was more numerous, and the blood-brain barrier was more intact in hornless female sheep (Fig. 3). Although horned female sheep are more prone to fighting, hornless female sheep can also suffer head trauma from both fighting and play. The horn structure absorbs impacts to the skull and protects against brain damage. Although this may seem like an advantage for horned female sheep, it can be said that the more astrocyte branches around the BBB in hornless sheep, the more advantages there are in terms of making the barrier stronger and more protected. Because hornless female sheep have no horn structure to absorb impacts to the skull, the brain tissue is exposed to more trauma. This could lead to the further development of protective mechanisms in the tissue of the brain. Therefore, it can be assumed that astrocytes, which play an important role in the protection and integrity of the BBB, are more numerous and branched in hornless female sheep.

TBI can lead to pathological disruption of the BBB and, consequently, to a number of neurological disorders (Zhou *et al*., 2020). It has been reported that the permeability of the BBB is increased in epilepsy (Greene *et al*., 2022). However, less attention has been paid to its role in brain microvascular endothelial cells and BBB damage, which is the pathological process in post-traumatic brain injury (Fang *et al*., 2022). The underlying mechanisms associated with the BBB dysfunction are not fully understood (Greene *et al*., 2022). Perivascular inflammation following cerebrovascular disruption in moderate to severe TBI results in hemorrhage, coagulation, thrombotic necrosis, and acute immune cell infiltration and neurodegeneration (Ma *et al*., 2020). One of the effects of TBI is secondary injury, which occurs when components in the bloodstream come into contact with the brain parenchyma due to disruption of the BBB (Cash & Theus, 2020). In addition, proinflammatory cytokines such as TNF-a and IL-1b released after trauma have been found to increase endothelial permeability in the BBB (Chodobski *et al*., 2011).

In our study, the BBB damage score was calculated using the scoring method in the study by Aydin *et al*. (2006), to evaluate BBB damage. In this study, endothelial cell shrinkage, cellular angulation, pericytoplasmic halo formation, nuclear condensation, and pericapillary astrocyte number in the BBB were evaluated, and the BBB damage score was determined by scoring according to the mentioned study. A BBB damage score of <6 indicated more intact or mild damage, and most of the tissue samples in this category belonged to hornless female sheep (Table I). The difference between the horned and hornless groups was statistically significant in all scoring categories (Table I; $p<0.05$).

A previous study found that BBB permeability increased rapidly in the early phase after trauma (Shlosberg *et al*., 2010). There are also studies reporting that BBB permeability returns to normal within days and weeks after TBI (Tomkins *et al*., 2008). However, little quantitative data are available on the relationship between the extent of BBB damage and the mechanism and severity of TBI (Shlosberg *et al*., 2010). TBI is known to induce astrocyte activation, which has a role in remodeling processes such as neurogenesis, synaptogenesis, BBB repair, and extracellular matrix formation (Zhou *et al*., 2020). In hornless female sheep, the high number and branching of astrocytes is important for protection and may also speed up post-traumatic remodeling and repair events. In our study, the stronger blood-brain barrier in hornless sheep can be explained by this information. Mild head impacts that do not cause obvious clinical symptoms are defined as subconcussion (Gysland *et al*., 2012). Repeated subconcussive impacts are thought to cause milder brain damage and neurobehavioral dysfunction (Hiles-Murison *et al*., 2021). Studies have shown that subconcussive impacts cause disruption of the BBB and the CSF-blood barrier and may play a role in the development of ventriculomegaly (Ott *et al*., 2010). Subconcussive impacts have been reported to induce oxidative stress in the hippocampus in mouse and rat models, suggesting a possible causal condition for cognitive decline associated with BBB dysfunction (Mao *et al*., 2018). BBB disruption in the hippocampus has been shown in clinical studies to be a critical feature of individuals with early cognitive dysfunction (Ni *et al*., 2022).

In this study, the blood-brain barrier of the hippocampus, which is associated with memory and navigation functions, was examined histopathologically. In hornless female sheep, more robust arterioles and capillary endothelium and numerous branched astrocytes were observed in the BBB (Fig. 2). In horned female sheep, more deformed arterioles, more degenerated capillary endothelium and fewer and less branched astrocytes were observed (Fig. 3). Looking at the BBB damage score of bilateral hippocampal structures (hornless: 12, horned: 8); 6 samples were <6 (intact or mild damage), 4 samples were 6-10 (moderate damage), 2 samples were >10 (severe damage) in hornless female sheep, and, 2 samples were <6 (intact or mild damage), 3 samples were 6-10 (moderate damage) and 3 samples were >10 (severe damage) in horned female sheep (Table I). There was a statistically significant difference in BBB damage scores between horned and hornless female sheep ($p<0.05$), and the BBB in the hippocampus of hornless female sheep was more intact or less damaged. Based on our findings, it can be said that the hippocampus, and therefore memory and navigational skills, are better preserved in hornless female sheep than in horned female sheep. The literature review did not identify any study on the differences in the hippocampal BBB between horned and hornless female sheep.

CONCLUSION

As a result, the absence of a horn structure to absorb the blows to the skull during trauma leads to more concussion and more brain tissue damage is expected to occur in this case. However, in our findings, more intact or less damaged brain tissue and BBB were detected in hornless female sheep. This clues that protective mechanisms are better developed in the brain tissue of hornless female sheep. In this study, it was found that astrocytes showed more branching in the brain tissue of female sheep. This suggests that more astrocyte branches may play an important role in this protective mechanism. Structural differences in the astrocytes of horned and hornless female sheep may serve as a model for future investigations of molecular mechanisms. This means that a better understanding of histopathological mechanisms in the animal brain may contribute to a better understanding of similar mechanisms in the human brain.

Ethical Approval. The Local Animal Experiments Ethics Committee of our university decided that ethical approval was not required because the experimental animal study involved dead animals (decision date: 29.09.2022/ 2200298220). The study was conducted in accordance with the National and Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines.

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RESUMEN: Debido a que los animales con cuernos son propensos a los conflictos, pueden tener un mayor riesgo de desarrollar una contusión cerebral como resultado de un golpe con un cuerno. Los traumatismos cerebrales reiterados pueden afectar el hipocampo relacionado con la memoria y la orientación. El objetivo de este estudio fue examinar histopatológicamente la barrera hematoencefálica (BHE) del hipocampo en ovejas con y sin cuernos. Se obtuvieron de un matadero los cráneos de 4 ovejas con cuernos y 6 sin cuernos de 16 ± 4 meses de edad. Los cerebros se extrajeron cuidadosamente y se conservaron en formalina al 10 % durante 5 días. Se tomaron secciones del hipocampo bilateral. Las muestras se tiñeron con hematoxilina-eosina (H&E) y métodos Tunel, y las estructuras histopatológicas de la BHE se examinaron mediante microscopía óptica. Se evaluaron las puntuaciones de daño de la BHE. Los resultados fueron analizados mediante la prueba U de Mann-Whitney. Se descubrió que la puntuación de daño de la BHE era mayor en las ovejas con cuernos. Los astrocitos estaban más ramificados y la BHE estaba más intacta en las ovejas sin cuernos. Se considera que más ramas de astrocitos pueden desempeñar un papel importante en el mecanismo de protección. La diferencia en el mecanismo de defensa intracerebral contra el trauma cerebral en las ovejas puede servir como modelo para investigar los mecanismos moleculares. El deterioro de la BHE en la región del hipocampo de las ovejas con cuernos puede provocar que su memoria, inteligencia y capacidades de orientación sean inferiores a las de las ovejas sin cuernos. Esto podría abrir el horizonte para mejores avances en la salud del cerebro humano y animal.

PALABRAS CLAVE: Barrera hematoencefálica; Oveja hembra; Hipocampo; Cuerno; Trauma.

REFERENCES

Ballabh, P.; Braun, A. & Nedergaard, M. The blood–brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol. Dis., 16(1)*:1-13, 2004.

Cash, A. & Theus, M. H. Mechanisms of blood–brain barrier dysfunction in traumatic brain injury. *Int. J. Mol. Sci., 21(9)*:3344, 2020.

Chaboub, L. S. & Deneen, B. Developmental origins of astrocyte heterogeneity: the final frontier of CNS development. *Dev. Neurosci., 34(5)*:379-88, 2012.

Chodobski, A.; Zink, B. J. & Szmydynger-Chodobska, J. Blood–brain barrier pathophysiology in traumatic brain injury. *Transl. Stroke Res., 2(4)*:492-516, 2011.

Dotiwala, A. K.; McCausland, C. & Samra, N. S. *Anatomy, Head and Neck: Blood Brain Barrier.* Treasure Island (FL), StatPearls Publishing, 2023.

Drake, A.; Donahue, T. L. H.; Stansloski, M.; Fox, K.; Wheatley, B. B. & Donahue, S. W. Horn and horn core trabecular bone of bighorn sheep rams absorbs impact energy and reduces brain cavity accelerations during high impact ramming of the skull. *Acta Biomater., 44*:41-50, 2016.

Fang, J.; Yuan, Q.; Du, Z.; Fei, M.; Zhang, Q.; Yang, L.; Wang, M.; Yang, W.; Yu, J.; Wu, G.; *et al*. Ferroptosis in brain microvascular endothelial cells mediates blood-brain barrier disruption after traumatic brain injury. *Biochem. Biophys. Res. Commun., 619*:34-41, 2022.

Fuller, L. H. & Donahue, S. W. Material properties of bighorn sheep (Ovis canadensis) horncore bone with implications for energy absorption during impacts. *J. Mech. Behav. Biomed. Mater., 114*:104224, 2021.

Greene, C.; Hanley, N.; Reschke, C. R.; Reddy, A.; Mäe, M. A.; Connolly, R.; Behan, C.; O'Keeffe, E.; Bolger, I.; Hudson, N.; *et al*. Microvascular stabilization via blood-brain barrier regulation prevents seizure activity. *Nat. Commun., 13(1)*:2003, 2022.

Gysland, S. M.; Mihalik, J. P.; Register-Mihalik, J. K.; Trulock, S. C.; Shields, E. W. & Guskiewicz, K. M. The relationship between subconcussive impacts and concussion history on clinical measures of neurologic function in collegiate football players. *Ann. Biomed. Eng., 40(1)*:14-22, 2012.

Hiles-Murison, B.; Lavender, A. P.; Hackett, M. J.; Armstrong, J. J.; Nesbit, M.; Rawlings, S.; Mcgonigle, T.; Warnock, A.; Lam, V.; Mamo, J. C.; *et al*. Blood–brain barrier disruption and ventricular enlargement are the earliest neuropathological changes in rats with repeated sub-concussive impacts over 2 weeks. *Sci. Rep., 11(1)*:9261, 2021.

Hunter, D. S.; Hazel, S. J.; Kind, K. L.; Liu, H.; Marini, D.; Owens, J. A.; Pitcher, J. B. & Gatford, K. L. Do I turn left or right? Effects of sex, age, experience and exit route on maze test performance in sheep. *Physiol. Behav., 139*:244-53, 2015.

Johnson, K. L.; Trim, M. W.; Mao, Y.; Rhee, H.; Williams, L. N.; Liao, J.; Griggs, J.; Horstemeyer, M. F. & Duan, Y. Finite element analysis of a ram brain during impact under wet and dry horn conditions. *J. Mech. Behav. Biomed. Mater., 119*:104400, 2021.

Luissint, A. C.; Artus, C.; Glacial, F.; Ganeshamoorthy, K. & Couraud, P. O. Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. *Fluids Barriers CNS, 9(1)*:23, 2012.

Ma, X.; Cheng, Y.; Garcia, R. & Haorah, J. Hemorrhage associated mechanisms of neuroinflammation in experimental traumatic brain injury. *J. Neuroimmune Pharmacol., 15(2)*:181-95, 2020.

Maity, P. & Tekalur, S. A. Finite element analysis of ramming in Ovis canadensis. *J. Biomech. Eng., 133(2)*:021009, 2011.

Mao, Y.; Black, A. M.; Milbourn, H. R.; Krakonja, S.; Nesbit, M.; Bartlett, C. A.; Fehily, B.; Takechi, R.; Yates, N. J. & Fitzgerald, M. The effects of a combination of ion channel inhibitors in female rats following repeated mild traumatic brain injury. *Int. J. Mol. Sci., 19(11)*:3408, 2018.

Marino, L. & Merskin, D. Intelligence, complexity, and individuality in sheep. *Anim. Sentience, 4*:1, 2019.

Ni, K.; Zhu, J.; Xu, X.; Liu, Y.; Yang, S.; Huang, Y.; Xu, R.; Jiang, L.; Zhang, J.; Zhang, W.; *et al*. Hippocampal activated microglia may contribute to blood–brain barrier impairment and cognitive dysfunction in post-traumatic stress disorder-like rats. *J. Mol. Neurosci., 72(5)*:975-82, 2022.

Ott, B. R.; Cohen, R. A.; Gongvatana, A.; Okonkwo, O. C.; Johanson, C. E.; Stopa, E. G.; Donahue, J. E.; Silverberg, G. D. & Alzheimer's Disease Neuroimaging Initiative. Brain ventricular volume and cerebrospinal fluid biomarkers of Alzheimer's disease. *J. Alzheimers Dis., 20(2)*:647-57, 2010.

Shlosberg, D.; Benifla, M.; Kaufer, D. & Friedman, A. Blood–brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat. Rev. Neurol., 6(7)*:393-403, 2010.

Tomkins, O.; Shelef, I.; Kaizerman, I.; Eliushin, A.; Afawi, Z.; Misk, A.; Gidon, M.; Cohen, A.; Zumsteg, D. & Friedman, A. Blood–brain barrier disruption in post-traumatic epilepsy. *J. Neurol. Neurosurg. Psychiatry, 79(7)*:774-7, 2008.

Wheatley, B. B.; Gilmore, E. C.; Fuller, L. H.; Drake, A. M. & Donahue, S. W. How the geometry and mechanics of bighorn sheep horns mitigate the effects of impact and reduce the head injury criterion. *Bioinspir. Biomim., 18(2)*:026005, 2023.

Zhou, Y.; Shao, A.; Yao, Y.; Tu, S.; Deng, Y. & Zhang, J. Dual roles of astrocytes in plasticity and reconstruction after traumatic brain injury. *Cell Commun. Signal., 18(1)*:62, 2020.

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