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Cost-Reducing Traits for Agonistic Head Collisions: A Case for Neurophysiology

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Synopsis Many animal species have evolved extreme behaviors requiring them to engage in repeated high-impact collisions. These behaviors include mating displays like headbutting in sheep and drumming in woodpeckers. To our knowledge, these taxa do not experience any notable acute head trauma, even though the deceleration forces would cause traumatic brain injury in most animals. Previous research has focused on skeletomuscular morphology, biomechanics, and material properties in an attempt to explain how animals moderate these high-impact forces. However, many of these behaviors are understudied, and most morphological or computational studies make assumptions about the behavior without accounting for the physiology of an organism. Studying neurophysiological and immune adaptations that covary with these behaviors can highlight unique or synergistic solutions to seemingly deleterious behavioral displays. Here, we argue that selection for repeated, high-impact head collisions may rely on a suite of coadaptations in intracranial physiology as a cost-reducing mechanism. We propose that there are three physiological systems that could mitigate the effects of repeated head trauma: (1) the innate neuroimmune response; (2) the glymphatic system, and (3) the choroid plexus. These systems are interconnected yet can evolve in an independent manner. We then briefly describe the function of these systems, their role in head trauma, and research that has examined how these systems may evolve to help reduce the cost of repeated, forceful head impacts. Ultimately, we note that little is known about cost-reducing intracranial mechanisms making it a novel field of comparative study that is ripe for exploration.

Introduction

Repetitive headbutting, ramming, and other behaviors that require high-impact head collisions have evolved in many different animals. The most extreme form of agonistic behavior (ramming) may have even been present in dinosaurs (Geist 1966; Snively and Theodor 2011). Interestingly, most extant animals that engage in high-impact head collisions are principally represented by species in the superorder Cetartiodactyla (Bovidae, Giraffoidae, and Cetacea) and the Picidae family (woodpeckers) (Fig. 1) (May et al. 1979; Simmons and Scheepers 1996; Gol'din 2014; Panagiotopoulou et al. 2016; Schuppe et al. 2016; Liu et al. 2017). Male bighorn sheep (*Ovis canadensis*; Family: Bovidae), for example, have curved horns and charge each other and butt heads at speeds of up to 20 mph during periods of male–male competition (Schaffer 1968; Kitchener 1988; Cappelli et al. 2018), creating a force that is strong enough to break a human femur bone (Tyler et al. 1993). Likewise, nearly all 230 extant species of woodpecker routinely hammer and drum their bill against trees to forage, excavate nests, and also to produce acoustic displays that help individuals negotiate territorial interactions (Gorman 2014) Miles et al (2018) and Schuppe and Fuxjager (2018). During bouts of drumming, great spotted woodpeckers (*Dendrocopos major*) experience deceleration forces more than 10× higher than what humans can safely tolerate before suffering a concussion or severe brain injury (Liu et al. 2017; McAteer et al. 2017). Although these examples represent some of the more extreme forms of head collisions, it is important to remember that other examples of head

collisions can be found throughout the animal kingdom. Indeed, many taxa perform less intense versions of headbutting or similar variants of this behavior. For example, many animals wrestle as a form of male–male aggression and often collide heads with their opponents during competitive disputes (e.g., green anoles [*Anolis carolinensis*]; [Wilczynski et al. 2015]), while species like elk (*Cervus canadensis*) fence with their elaborate antlers (Linden and Dumont 2019).

Why does headbutting behavior evolve in the first place? It presumably emerges as part of a complex behavioral repertoire that functions to help individuals (often males) establish a mating hierarchy, maintain a territory, and/or act as a fitness signal to potential mates (Schaffer 1968; Rico-guevara and Hurme 2018; Tinghitella et al. 2018). Yet, the emergence of headbutting behavior also raises some interesting questions about its costs, given that it puts the brain at great risk of being injured or permanently damaged. Humans have a recent appreciation of this cost, considering the broad recognition that contact sport athletes experience repetitive head and/or body collisions, which can have profound negative effects on brain function (VanItallie 2019). For example, 99% of professional football players and 87% of all football players (including high schoolers) show evidence of post-mortem neuropathy or aberrant behavior likely due to repeated head trauma (Mez et al. 2017). Even players that experience head collisions at relatively low velocities and that occur over short acceleration distances are prone to these pathologies. Thus, all animals that incorporate head collisions into their display routines are likely at risk of head injury, including the species that perform the milder versions of head collisions described above. In this way, the repeated evolution of headbutting behavior in non-human animals is an astonishing phenomenon considering the dire fitness consequences that might arise from it.

In turn, we posit that high-impact head collision behaviors may require the coevolution of *both* conspicuous morphological and neurophysiological traits that offset the costs of head trauma (hereafter called "cost-reducing traits" [Møller 2008]). Putative morphological adaptations to high-impact head collisions likely evolved as a progression from thicker skin to head protrusions to horns as agonistic behaviors became more extreme (Geist 1966). Similarly, in woodpeckers, drumming likely evolved from a lower-impact foraging repertoire to become a repeated high-impact head collision (Garcia et al. 2020). If so, then neurophysiological traits may have been critical in ensuring the integrity of brain function during times when the tissue is vulnerable to damage.

However, most research has focused on comparative, biomechanical, or computational modeling of morphological traits as a major cost-reducing trait. For example, the spiraling horns of bighorn sheep, the compartmentalization of the enlarged forehead of sperm whales (*Physeter macrocephalus*), and the hyoid bone in woodpeckers have been proposed to provide protection by distributing the force over a large surface area (Panagiotopoulou et al. 2016), allowing for the absorption of force (Linden and Dumont 2019), or diverting the force of the impact away from the brain (Wu et al. 2015). However, the mechanics of these displays are often poorly understood, meaning that morphological evidence and computational simulations are largely speculative at best. For example, in a computational study, researchers have argued that the woodpecker hyoid bone, which wraps from the base of the skull and inserts into a nostril, acts to divert deceleration forces away from the brain (Wu et al. 2015). However, the hyoid bone may just be an innovative solution to store a long tongue, as homologous structures are present in other birds with long tongues that do not experience head collisions (e.g., hummingbirds [Weymouth et al. 1964]).

In contrast to morphological traits, neurophysiological cost-reducing traits are rarely considered in the context of head trauma in non-human species. Therefore, we consider three neurophysiological systems that could act as cost-reducing traits: (1) innate neuroimmune responses, (2) glymphatic system functioning, and/or (3) choroid plexus physiology and its production of cerebrospinal fluid (CSF). To contextualize the proposed hypotheses for each of the three neurophysiological systems, we first define head trauma and identify differences in neuropathies that can manifest due to acute and/or chronic head impacts. We then highlight the few studies that have found evidence of head injury in animals and discuss what this means for the hypothesis of neurophysiological cost-reducing traits. Finally, we explore the immune system, glymphatic system, and the choroid plexus/CSF, each in turn, to provide an overview of current knowledge (primarily from rodent models and human studies) and outlining how changes in specific neuroimmune function, brain structures, and neurophysiology can facilitate or evolve alongside behavioral adaptations that would otherwise cause significant trauma. Where possible, we provide specific hypotheses to be explored in these neurophysiological systems as a starting roadmap for new explorations in comparative biology. Overall, our

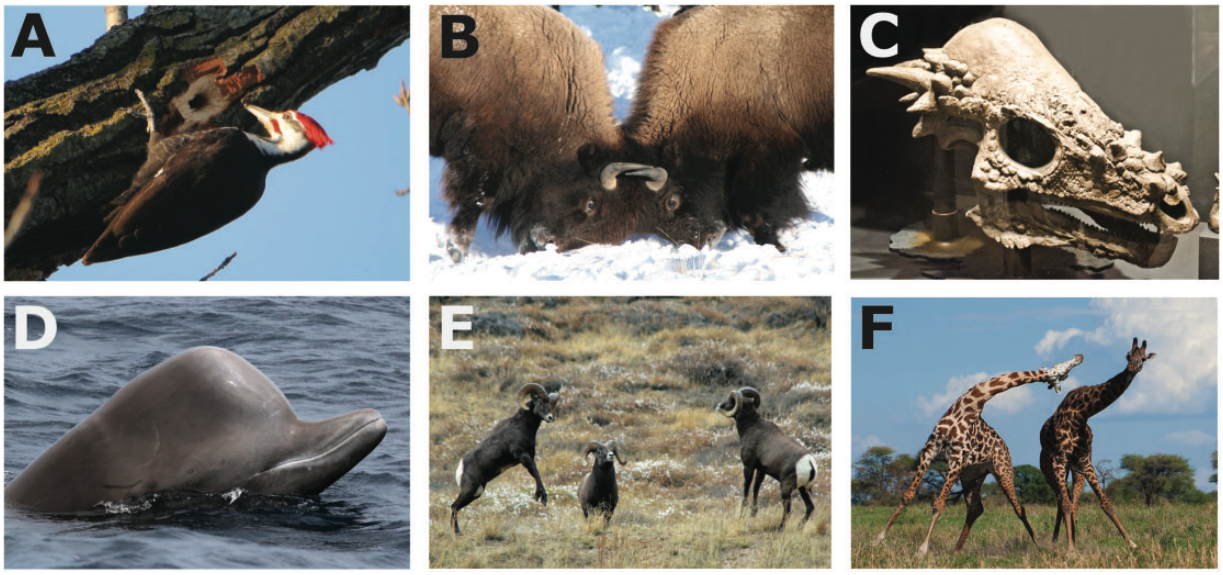

Fig. 1 Examples of extant and extinct animals that experience high-impact forces as a result of male–male competition or territoriality. (**A**) Pileated woodpecker (*Dryocopus pileatus*) drilling into a large tree branch (credit: Wildreturn licensed under CC BY 2.0). (**B**) Two male American bison (*Bison bison*) ramming in male–male combat. (credit: USFWS Mountain Prairie licensed under CC BY 2.0). (**C**) Fossilized skull of a male Pachycephalosaurus. Note the protruding dome on the dorsal skull suggestive of ramming behavior (credit: Tim Evanson licensed under CC BY-SA 2.0). (**D**) Northern bottlenose whale (*Hyperoodon ampullatus*), like sperm whales (not shown), have an enlarged head that is thought to be used during intrasexual combative behavior ([Gowans and Rendell 1999] credit: Cephas licensed under CC BY-SA 4.0). (**E**) Two male bighorn sheep (*Ovis canadensis*) rearing to get ready to ram each other (credit: webmink licensed under CC BY-NC-SA 2.0). (**F**) Male giraffes (*Giraffa camelopardalis*) engaging in necking behavior, wherein they use their strong neck muscles to swing their head in a pendular motion to strike their rival ([Simmons and Scheepers 1996] credit: Plastic Tiger licensed under CC BY-NC 2.0). See online version for color photographs

intent is to briefly review the literature of agonistic head trauma and propose these three neurophysiological avenues for future research. Headbutting and other repetitive high-impact behaviors such as territorial drumming in woodpeckers present a great opportunity for a comparative approach to examine the proximate costs and implications of such behaviors.

Types of head trauma

Before we can discuss coadaptations that potentially underlie the evolution of high-impact head collisions in animals, we must first distinguish between the different forms of head injuries and brain trauma. The severity of the trauma depends on the magnitude of the force, duration of contact, and the velocity of acceleration–deceleration inertia. High-impact forces for long durations can cause extreme trauma, whereas moderate or high forces for short durations can cause milder traumatic brain injuries (TBIs). Many (non-human) species engage in behaviors that might incur head trauma that can be classified as two distinct, but related, phenomena. The first, and the most acutely deleterious type of head trauma, is known as TBI, which can be put into two major classifications—focal injury and diffuse injury (Werner and Engelhard 2007). Focal injuries are typically caused by localized penetrating or blunt force impacts such as head collisions during a car crash, fall, or a blow to the head during an assault. These impacts typically lead to very specific neurological deficits based on the location of the trauma (Shaw 2002; Xiong et al. 2013). In contrast, diffuse injuries are often a function of acceleration–deceleration inertial forces that cause widespread trauma as a result of the brain bouncing back and forth inside the skull (also called coup-contrecoup injury), excessive heat and energy caused by cavitation (Pan et al. 2017), or rotational injury (shearing and tearing) to the neural tissue (Meaney and Smith 2011; Hong et al. 2016; Mead et al. 2017). Diffuse injuries are the most common form of trauma that stem from head-to-head collisions, at least in humans (Mez et al. 2017). Diffuse TBIs can span a spectrum of severity from mild to lethal and can lead to moderate temporary psychological issues or major permanent physical disabilities. Either together or independently, these diffuse and/or focal traumatic phenomena cause acute and chronic disruption to neural signaling. Moreover, there can be a delayed secondary injury from an overactive response from the immune system causing unintended necrosis, apoptosis, cytotoxicity, or unregulated inflammation of

non-neural structures vital in maintaining brain homeostasis.

The second phenomenon is called chronic traumatic encephalopathy (CTE), or "punch drunk syndrome" (named as such because it is often seen in retired boxers that develop motor deficits [Meaney and Smith 2011]). CTE is a chronic condition that stems from an accumulation of the deleterious effects of repeated subconcussive or mild, diffuse TBI events throughout an organism's life. In humans and other non-human laboratory mammals, CTE can result in neuropsychological disorders including depression, memory loss, and impulsivity (Tellier et al. 2009). In many cases, there are notable gross neuromorphological changes, such as enlargement of the ventricles, cerebral atrophy, and loss of pigmentation in the substantia nigra (a dopamine-producing region of the brain important for movement [McKee et al. 2015]). On a molecular scale, postmortem analysis of CTE in humans and rodents primarily includes the presence of neurofibrillary tangles (aggregates of hyperphosphorylated tau proteins) and amyloid plaques (accumulation of necrotic tissue around amyloid-beta (Aβ) aggregates [McKee et al. 2015]). Both neuropathies disrupt normal neural communication. Even mild repeated head trauma can have surprisingly deleterious effects on neural function, as seen in gridiron football players (McKee et al. 2009). Athletes help to highlight how deleterious repeated head trauma can be, even though many non-human species exhibit many similar cost-reducing traits such as horns or thicker skulls (like football helmets), low mass and low acceleration differentials between the competitors, and behavioral preparations for collision such as muscle contractions and vertebrae alignment. For a full dive into the ontogeny of the cellular pathologies that contribute to CTE or TBI, see the following reviews: Shaw (2002); McAteer et al. (2017); Vink (2018); Zanier et al. (2018). In humans, aberrant behaviors associated with CTE (only confirmed by a post-mortem diagnosis) can present itself in adulthood, which includes the prime breeding years, especially in males (Gardner and Yaffe 2015; McKee et al. 2015). Therefore, animals would significantly increase their fitness if they can mitigate the acute effects of TBI while delaying the presentation of CTE-like pathologies until after the prime breeding years.

Evidence of brain injury in animals from head collisions

It does appear that the brains of animals with these behavioral displays (muskox and woodpeckers) have microscopic evidence of neuropathies that are similar to CTE neuropathies in humans ([Farah et al. 2018; Ackermans 2020; Ackermans et al. 2021]; personal communication [DJT]: Nicole Ackermans). For example, in fixed museum specimens, researchers found that male downy woodpeckers (*Dryobates pubescens*) show histological evidence of neurofibrillary tangles and hyperphosphorylated tau, whereas there was no evidence of such neuropathologies in similarly preserved non-drumming species of birds (Farah et al. 2018). More extensive sampling, controlled experiments, and other potential explanations (e.g., phosphorylation rates of tau in woodpeckers) must be ruled out, but preliminarily results suggest that these animals do accumulate some sort of brain injury in their lifetimes. Moreover, there have been anecdotal reports of muskox bleeding through the nose or being dazed after a rut (a bout of headbutting), but there have not been substantive reports of chronic abnormal behavior in breeding adults in either muskox or woodpeckers, suggesting that these animals are able to better endure the cost of these neuropathies and perhaps come up with cost-reducing mechanisms to delay the buildup of these neuropathies. In other words, perhaps these animals have cost-reducing neurophysiological and morphological traits that help stave off the inevitable and allow them the ability to live long enough to produce more offspring than any rivals.

Neurophysiological cost-reducing traits

In turn, these species may have intracranial coadaptations that help to mitigate moderate or severe TBI and delay accumulation of CTE-like neuropathies. We suggest three potential physiological and/or neuromolecular systems might be specialized to help attenuate the effects of repeated high-impact head trauma: the innate immune system, the glymphatic system, and the choroid plexus. We briefly describe the general function of these systems, how they respond to brain trauma in traditional animal models and humans that have not evolved to withstand high-impact forces, and how they can be reshaped to potentially assuage, alleviate, or avoid acute and/or chronic trauma-associated damage to the brain.

Innate immune response

The local and systemic innate immune system response to a high-impact or traumatic head collision can have a profound effect on the prognosis and extent of the injury (Riera Romo et al. 2016). Mechanisms underlying the inflammatory process may have therefore evolved to help buffer the brain from its own immune overresponse when individuals engage in headbutting. This idea is rooted in an

emerging literature that explores the evolution of the innate immune system in animals. The innate immune system is an organism's immediate defense to invading pathogens and trauma-induced injury. Due to its roles in both providing protection from foreign pathogens and initiating repair of trauma-induced injury, the innate immune system must properly distinguish between not just self from non-self, but also healthy self from injured self. In the brain, innate immune activation is critical in repairing damage caused by head injury. Counterintuitively, however, an overactive immune response can also lead to greater damage and secondary injury. In rats, experimentally induced TBI causes neuronal damage and necrosis, which results in the activation of the innate immune system to clear necrotic and apoptotic cells, along with the neurotoxic proteins and molecules that can no longer be properly contained by these cells (Nguyen et al. 2002; Popovich and Longbrake 2008). The activation of the resident innate immune cells (astrocytes, microglia, and central nervous system-associated macrophages) leads to the production of proinflammatory cytokines. Overproduction of proinflammatory cytokines then initiate the secondary injury cascade, resulting in the recruitment of peripheral immune cells, additional inflammation, and increased neuronal damage (Ottens et al. 2007; de Rivero Vaccari et al. 2015), ultimately exacerbating the damage after the initial TBI.

Because of its importance in organismal homeostasis and health, the innate immune system is highly evolutionarily conserved (Salzet 2001; Riera Romo et al. 2016). Nonetheless, there is evidence of evolutionary divergence in the mechanisms of innate immune functioning within vertebrates resulting in lineage-specific changes to the system itself. For example, in birds and mammals, interferons (signaling proteins involved in viral defense and the inflammatory response) share a common core of regulatory genes, but each mammalian lineage can increase or decrease gene expression and may have additional regulatory genes (Shaw et al. 2017). The evolution of a unique set of regulatory genes in each mammalian lineage has led to each possessing a unique interferome (the entirety of interferon-regulated genes). Moreover, divergent evolution has also resulted in multiple allelic variants of key innate immune receptors (e.g., toll-like receptors) both between and within mammalian species (Smirnova et al. 2000; Gardiner et al. 2001). The presence of heterogeneity within the innate immune receptors raises the possibility that innate immune function could be altered across species, but the extent to which allelic variants lead to altered function needs further study (Magor and Magor 2001). Together, the evolution of a variety of allelic variants in key innate immune processes across vertebrates and the lineage-specific interferomes raise the possibility that natural selection can act on the resident innate immune system. However, to our knowledge, no study has demonstrated divergent evolution in neuroimmune cellular processes that regulate a response to head trauma. This presents a unique opportunity for the comparative community to examine whether animals have a blunted immune response to repeated head collisions or experience increased negative feedback on the immune system to prevent a robust response.

Experimental evidence suggests that a blunted inflammatory response after TBI is neuroprotective. A recent study in *Drosophila melanogaster* used gene editing to create flies that were heterozygous for the proinflammatory innate immune response transcription factor, nuclear factor-κB (NF-κB), homolog *Relish*. The results of their study showed that heterozygous individuals had altered protein expression in the brain and had increased survival following an experimentally induced TBI (Swanson et al. 2020). These results suggest that a minor change in the inflammatory response to TBI can result in decreased inflammation. Thus, this change may be neuroprotective by improving recovery from trauma. What is most exciting about these data is that they illustrate how slight genetic alterations could have co-evolved in species that repeatedly experience head collisions and potentially ameliorate their negative effect on the organism and its viability.

Another way to modulate innate immune cell responsivity is through steroid hormone signaling. Indeed, steroid hormones may be an important evolutionary target to help regulate headbutting because they directly modulate aggression *and* the innate immune system (Wingfield et al. 2001). For example, glucocorticoids (e.g., cortisol) have an immune-enhancing effect when local levels are increased slightly for a short duration and immunosuppressive effects when local levels are increased more for longer durations (Sorrells and Sapolsky 2007). Glucocorticoid levels increase in response to a myriad of stressors such as environmental challenges, infection, or injury (including TBI). Neuroimmune cells such as microglia also locally regulate glucocorticoid levels within discrete brain regions (Moisan et al. 1990; Mellon and Deschepper 1993; Holmes and Seckl 2006; Gottfried-Blackmore et al. 2010; Taves et al. 2015; Tobiansky et al. 2018, 2020; Hamden et al. 2021). In the nervous system, glucocorticoids decrease inflammation by providing

negative feedback on proinflammatory pathways and decreasing cytokine gene expression in microglia (Blais et al. 2002; Nadeau and Rivest 2002). In fact, due to the severely deleterious effects of an overly robust immune response to TBI, medical practitioners currently give glucocorticoids to suppress the immune response in humans after a TBI (Bergold 2016). Thus, an evolutionary push for increased local glucocorticoid regulation or enhanced negative feedback on immune cells in the brain may promote long-term brain health by decreasing damage due to secondary injury from neuroinflammation. We would like to note that local and systemic levels of other sex steroids such as progestogens, androgens, and estrogens also have immunoregulatory effects *and* are important for the modulation of sexually selected behaviors (Rolff 2002; Owen-Ashley et al. 2004), but a review of the literature of these sex steroid hormones is beyond the scope of this paper.

We should also recognize that a more general over-suppression of the innate immune response may similarly be disadvantageous. For example, if the immune system is overly suppressed systemically, then contusions and scrapes become susceptible to serious infection (this would not likely be beneficial for individuals in constant battle for resources and mates). Such an effect, however, has the potential at least to create a trade-off that sits on the crux of reproductive aggression and its function. Namely, immunosuppression needed to headbutt might make individuals more susceptible to injury and in doing so create a handicap that can provide honest information about an individual's quality. Low-quality individuals may not be as good at withstanding the costs associated with reduced innate immune function that occurs as part of the physiological preparation for headbutting. Organisms can navigate this trade-off if the changes selectively modify the responsiveness of resident neural immune cells rather than cells in both the brain and in the periphery. Indeed, macrophages have been shown to have different variants in the brain than in the periphery, providing evidence that changes to immune function can be specific to the brain (Gonzalez-Perez et al. 2012). Furthermore, discrete brain regions have been shown to modulate local glucocorticoid levels (likely via local production), thereby allowing for a fine-tuned regulation of immune function and neural processes (Taves et al. 2016; Tobiansky et al. 2018; Hamden et al. 2021). The idea of trade-offs between immune function, steroid levels, and reproductive success is not new but warrants further investigation in this context (Bonneaud et al. 2003; McEwen and Wingfield 2003; Martin et al. 2008).

The glymphatic system

Another way to possibly offset negative effects of repeated head trauma is by altering the function of the glymphatic system. This newly described system provides an important mechanism for regulating and disposing of waste produced by normal brain function, as well as cytotoxins produced by mild trauma (Jessen et al. 2015). The glymphatic system is composed of a network of perivascular spaces formed by astroglial cells, which help form the brain–blood barrier, maintain neurons, and regulate synapses in the brain (Rouach et al. 2008; Ezan et al. 2012; Jessen et al. 2015). These perivascular spaces permeate the brain parenchyma (neuronal and glial cells) and facilitate the exchange of CSF and the interstitial fluid within the space between the endothelial cells and the endfeet of the astroglial cells (Iliff et al. 2012; Jessen et al. 2015). Subsequently, the glymphatic system may be the "missing link" for understanding brain edema or brain swelling after a TBI because this system regulates CSF and interstitial fluid exchange in the brain (Thrane et al. 2014). Suppression of glymphatic system functioning is thought to trigger neuron degeneration, brain trauma, and stroke, suggesting that this system plays an integral part in maintaining homeostasis and is an ideal candidate for increasing efficiency as a cost-reducing trait (Zemlan et al. 2002; Gaberel et al. 2014).

The glymphatic system's ability to clear wastes from the CSF and interstitial fluid may play an integral role in mitigating the effects of TBI. Often after a TBI, there is a release of proteolytically cleaved tau (C-tau) protein (an intercellular microtubule-associated protein) in the interstitial fluid of the brain (Gabbita et al. 2005). The amount of C-tau accumulated in the brain correlates with the severity of brain trauma (Zemlan et al. 2002). After a TBI, glymphatic system functions are reduced by ~60%, and this suppression persists for at least 1 month after the injury occurred (Iliff et al. 2014), resulting in higher levels of C-tau in the brain for a longer period. This is important because high levels of C-tau in the brain lead to the uptake of C-tau by cells, initiating the formation of fibrous tangles, scarring, and prolonged inflammation in the brain (Guo and Lee 2011; Iliff et al. 2014). The glymphatic system helps to clear cytotoxic proteins like C-tau from the brain and could be crucial for preventing further brain injury after a TBI has taken place (Jessen et al. 2015).

While the glymphatic system has been studied in mice and humans in order to understand TBI, CTE,

and other neurodegenerative disorders (Jucker and Walker 2011), the role of the glymphatic system has yet to be evaluated in taxa that experience high-impact forces on the head. This begs the question: How might the glymphatic system have evolved differently in animals that engage in ramming or drumming and endure repeated head trauma? Do bovids have glymphatic systems that are quicker at clearing out cytotoxins and maintaining functions after a bout of ramming to avoid any long-term effects on the brain? If the glymphatic system relies on diffusion and bulk flow (Kaur et al. 2020) to move solutes through the perivascular spaces, then the size and shape of the perivascular tunnels could influence the speed that waste can be cleared. Since larger perivascular spaces are associated with age and decreased cognitive function in humans (Heier et al. 1989; MacLullich et al. 2004; Doubal et al. 2010), we might expect animals that have headbutting behaviors to have smaller perivascular spaces when compared with non-headbutting animals. Smaller perivascular space cross-sectional area could help increase the speed of the fluid within these spaces, but this has yet to be comparatively explored in headbutting animals. Additionally, the ability of these perivascular spaces (especially smaller, tighter spaces) to remain open and operational after swelling could also play a role in ameliorating head trauma recovery after ramming. For example, mice with a deletion of the gene encoding the water-regulating aquaporin 4 protein (AQP4) demonstrate a failure to clear injected solutes from their brains (Iliff et al. 2012), meaning that alterations to AQP4 to increase active water transport at the endfeet of the astroglial cells lining the perivascular spaces could increase the efficiency of bulk flow, despite small or swelling spaces, to clear cytotoxic molecules after a high-impact collision and could evolve in animals that engage in ramming. Moreover, it is not entirely understood how AQP4 mechanistically regulates the fluid exchange in the glymphatic system, meaning that this remains to be explored in both a biomedical and a comparative context (Benveniste et al. 2020; Kaur et al. 2020).

Another potential cost-reducing mechanism could reside in the functioning of the meningeal lymphatic system, as the glymphatic system passes off waste to the meningeal lymphatics (Louveau et al. 2017; Bolte et al. 2020). In a recent study, mice that were injected with vascular endothelial growth factor C (VEGF-C) after head trauma showed great improvement in the clearing of proteins, necrotic cells, and cellular debris, thus lessening gliosis, ultimately leading to reduced inflammation (Bolte et al. 2020). The amount of VEGF-C released in the brains of ramming animals could also have evolved to increase waste clearing after TBI as another possible adaptation. VEGF-C dilates lymphatic tubes, which could lead to an increase in drainage in response to compression from swelling (Louveau et al. 2015), and thus leads to improvements to the side effects of head trauma. We could then expect that animals that engage in head collisions may release higher levels of VEGF-C than closely related species that do not display these behaviors. Because the glymphatic system is not fully understood in traditional animal models and has yet to be examined in non-model systems, there are exciting new research opportunities to determine how animals can compensate for repeated head collisions.

The choroid plexus

The choroid plexus is the final intracranial structure that we will consider that may have evolved to help with cost mitigation from high-impact head collisions. We should first note that the choroid plexus and the glymphatic system are intrinsically linked because the choroid plexus produces most of the CSF necessary for glymphatic system function. However, the choroid plexus should also be considered independently because it does perform many other independent homeostatic functions. The choroid plexus is non-neuronal tissue that floats in the ventricles of the brain and is composed of a network of tightly packed choroid plexus epithelial cells that surround fenestrated capillaries (Damkier et al. 2013). The fenestrations of the capillary walls in the choroid plexus allow it to make CSF, which is a clear, serum-like fluid that helps maintain biochemical and cellular homeostasis, provides neutral buoyancy that suspends the brain in the cranium, and acts as a cushion during a traumatic event (Lun et al. 2015). The viscosity and pressure of CSF are critical to this latter function. For example, higher viscosity and/or pressure prevent the brain from hitting the skull after an impact (Lun et al. 2015). The choroid plexus regulates viscosity and pressure via water-regulating proteins (e.g., aquaporins), glucose transporters, and tightly controlling protein content in the CSF (Damkier et al. 2013; Marques et al. 2017; Praetorius and Damkier 2017).

The choroid plexus also plays an active role in waste clearance through waste-removing proteins on the surface of choroid plexus epithelial cells, resident sentinel immune cells, and flushing waste via the glymphatic system. If selection for repeated head collisions was strong, we would hypothesize that

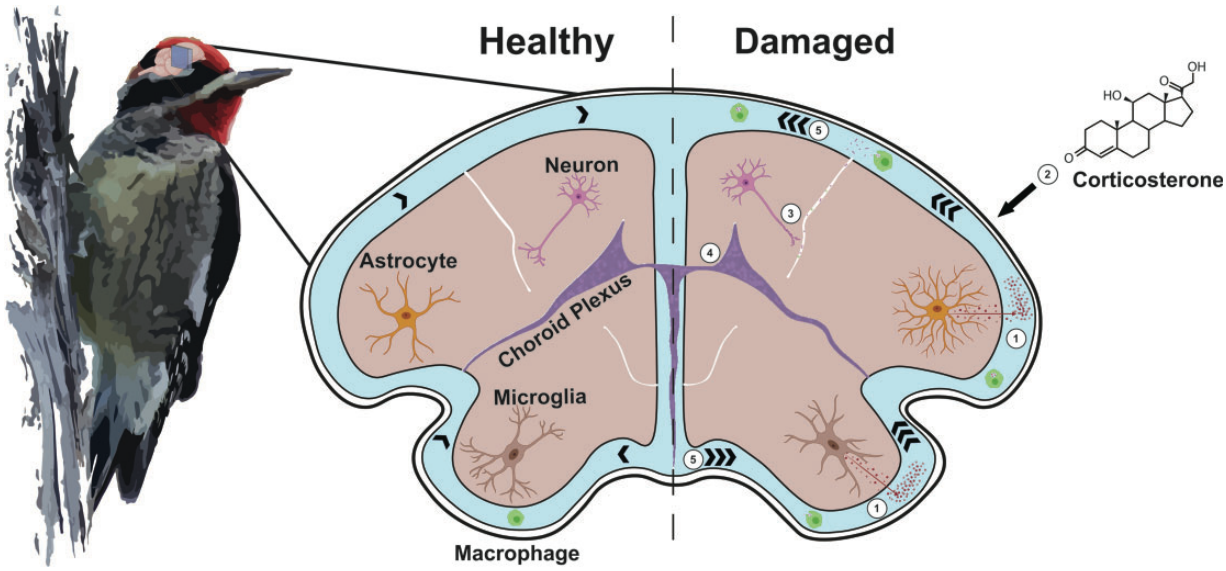

Fig. 2 Possible physiological compensatory adaptations to mitigate the effects of brain injury represented in transverse section of a yellow-bellied sapsucker (*Sphyrapicus varius;* a woodpecker) brain. Possible alterations to these systems may include the following: ① decreased release of proinflammatory cytokines by microglia (neural immune cells), astrocytes, and central nervous system-associated macrophages; ② increased active regulation of anti-inflammatory steroid levels in the brain; ③ increased efficiency by the glymphatic system in clearing damaged cells from the brain via the perivascular spaces (represented by the white line); ④ and ⑤ increased relative choroid plexus tissue (in purple), which would increase cerebrospinal fluid production, waste-clearance membrane proteins, and sentinel macrophage cells. Figure created, in part, using BioRender. Image of bird is modified from a photo credited to puuikibeach licensed under CC BY 4.0. See online version for color images

these animals could increase the surface area or number of choroid plexus epithelial cells, they can then increase the CSF–epithelia interface, allowing them to more effectively remove proteins associated with brain damage such as Aβ (a cytotoxic protein) by increasing CSF production and proteins involved in removing toxic molecules from the CSF. Moreover, the choroid plexus helps to regulate and mediate immune responses in the brain (Lun et al. 2015). Moving along the CSF-facing choroid plexus epithelial membranes, sentinel macrophages constantly survey the CSF and recruit a variety of immune defenses once trauma is sensed (Dani et al. 2019). This is important because many issues that arise after brain trauma are not from the injury itself, but from the immune response to the injury (see discussion of secondary injury cascade above) (Morganti-Kossmann et al. 2002; Maas et al. 2008). For example, proinflammatory cytokines released after a mechanically induced injury can disrupt neuronal signaling by altering ion channel function (Chen et al. 2017). Therefore, the choroid plexus can mitigate any unnecessary damage caused by an overactive or restrained immune response by altering infiltration of immune cells and/or cytokine release (Needham et al. 2019).

Finally, the choroid plexus can evolve to mitigate the effects of brain trauma and secondary pathology by (1) increasing the size of the choroid plexus or choroid epithelial cells to increase the surface area of CSF-regulating proteins, which will help to increase CSF production and regulate CSF viscosity and density or (2) increasing the amount of CSF- or cytotoxin-regulating proteins, and/or cytokine release in a given cell. Indeed, the only comparative study that exists (to our knowledge) shows that the choroid plexus of headbutting domestic sheep is relatively more voluminous compared to other mammals, including humans, cats, and pigs (Cserr et al. 1980). An example of a how the molecular biology of the choroid plexus can change to decrease cost associated with trauma is a study that examined the protein klotho (a catalytic transmembrane protein), which is produced in distinctly high amounts by the choroid plexus and directly regulates immune function in the brain. A decrease in the expression of this protein, either via natural aging or via genetic manipulation, in mice leads to an increase in immune-mediated neuropathogenesis (Zhu et al. 2018). It stands to reason that a consistent upregulation of this protein may retard age-related or trauma-related neuropathology, thereby increasing years in peak breeding state. Overall, little is known about the comparative function of the choroid plexus, how it differs structurally and functionally between taxa, and how it may have evolved to

ameliorate the costs of high-impact head collisions. We are currently in the process of examining differences in the choroid plexus size and morphology in woodpeckers and closely related non-woodpecker, non-drumming species to determine if such a difference does exist, and if so, when it came online (Fig. 2). This lack of knowledge suggests that a comparative approach to understanding the role of the choroid plexus could lead to exciting findings in basic and translational research.

Conclusion and future directions

The major goal of this article is to suggest that neurophysiological and immunological coadaptations must be considered alongside skeletomuscular adaptations in mitigating head trauma from high-impact head collisions in a taxa's behavioral repertoire. From a biological and evolutionary perspective, there is no reason to think that musculoskeletal morphology should be the only cost-reducing traits for head collisions—selection for a behavior should logically act on both morphology and physiology to increase fitness. Moreover, others have hypothesized that extreme headbutting behavior has progressed in a gradual manner, suggesting that cost-reducing morphology and physiology evolved in tandem with one another (Geist 1966). As such, we proposed changes to three intracranial systems that may have been coadapted to mitigate head trauma and neuropathies. First, the innate immune system, which is universal and essential in vertebrates and invertebrates, can vary significantly in a species-specific manner to adapt to local trauma. Second, the glymphatic system is a prime candidate for evolutionary manipulation due to its importance in clearing cytotoxic waste from the brain that can accumulate after repeated head collisions. Finally, the choroid plexus is important in protecting the brain by producing CSF to cushion the brain, plays a major role in the glymphatic system, directly removes cytotoxic molecules from the ventricles, and acts as an interface between the brain and peripheral immune system. Above, we highlight how these systems can be coadapted to mitigate neuropathies from repeated head collisions and give examples of proteins or pathways that can be altered to provide greater protection to the brain. By co-opting cost-reducing mechanisms in the brain to delay the effects of cumulative damage, these animals can increase potential fitness by extending their prime breeding years. Ultimately, the research and hypotheses laid out above raise significantly more questions than they answer, and we hope they stimulate discussion and further research on this important topic.

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Conflict of Interest

The authors declare no conflicts of interest.

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Authors' Contributions (CRediT taxonomy)

D.J.T. and M.J.F. conceptualized the study; D.J.T., K.M.L., and J.E.H. visualized the study; D.J.T., K.M.L., and J.E.H. contributed to writing the original draft; D.J.T., K.M.L., J.E.H., J.D.B., and M.J.F contributed to review and editing.

Data availability statement

Data availability was not used, presented, or analyzed in this paper. Thus, this would not be applicable for our manuscript.

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