Unconventional animal models for traumatic brain injury and chronic traumatic encephalopathy

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Abstract
Traumatic brain injury (TBI) is one of the main causes of death worldwide. It is a complex injury that influences cellular physiology, causes neuronal cell death, and affects molecular pathways in the brain. This in turn can result in sensory, motor, and behavioral alterations that deeply impact the quality of life. Repetitive mild TBI can progress into chronic traumatic encephalopathy (CTE), a neurodegenerative condition linked to severe behavioral changes. While current animal models of TBI and CTE such as rodents, are useful to explore affected pathways, clinical findings therein have rarely translated into clinical applications, possibly because of the many morphofunctional differences between the model animals and humans. It is therefore important to complement these studies with alternative animal models that may better replicate the individuality of human TBI. Comparative studies in animals with naturally evolved brain protection such as bighorn sheep, woodpeckers, and whales, may provide preventive applications in humans. The advantages of an in-depth study of these unconventional animals are threefold. First, to increase knowledge of the often-understudied species in question; second, to improve common animal models based on the study of their extreme counterparts; and finally, to tap into a source of biological inspiration for comparative studies and translational applications in humans.
1 | INTRODUCTION

Increasing brain size has been a key factor in human evolution (Lieberman, 2011). Although human head morphology evolved to accommodate high encephalization, it may have led to particular vulnerabilities such as brain trauma and other neurodegenerative conditions (Ghika, 2008) (Figure 1). Traumatic brain injury (TBI) is one of the main causes of death in the world and is usually the result of a violent impact to the head, violent angular acceleration, or blast exposure (Unterharnscheidt & Higgins, 1969; Wojnarowicz et al., 2017). TBI encompasses a wide range of injuries, from mild concussions to severe brain damage, and includes closed head, penetrating, and blast-induced brain trauma (Elder & Cristian, 2009; Lee & Ng, 2019). Closed head TBI is caused by a variety of events, such as sports-related or automotive accidents, in which a strong impact paired with compression forces disrupt neuronal and vascular functions of the brain beneath the site of impact. Concussion is a relatively mild neurological syndrome caused by the violent brain movement and compression within the skull (McCrory et al., 2013). It makes up over 75% of TBI cases in the United States, and it is frequent in automotive accidents, youth, athletes, victims of domestic violence, and military personnel (Meaney et al., 2014). Penetrating TBI occurs when an object, such as a high-velocity projectile, penetrates the skull and meninges, damaging the brain parenchyma.

Repetitive brain trauma is particularly dangerous, as it has been linked to progressive neurological deterioration. This was first noted in the 1920s, as "dementia pugilistica" in association with boxing (Martland, 1928) and later named chronic traumatic encephalopathy (CTE) (Corsellis et al., 1973), a progressive neurodegenerative disease (Hof et al., 1992). CTE is known to occur in a variety of contact sports (McKee et al., 2009) and has been diagnosed in deceased football players (Omalu et al., 2005). In a recent study, 177 of 202 former players of US Football were diagnosed with CTE (mean years of football participation 15.1) (Mez et al., 2017). TBI and CTE have lately been recognized in many other high-impact or contact sports, causing a spike in medical concern and neuroscience research (Zetterberg et al., 2019). However, CTE remains difficult to diagnose, resulting in inconsistent knowledge about the extent and progression of the disease (Iverson et al., 2019). Repetitive head injuries, either mechanical or blast induced, are also prevalent in military environments around the world (Elder et al., 2010). Blast-induced TBI is a common form of military-related TBI that results from exposure to an explosion’s kinetic energy that is transferred through the head, damaging brain structures. Depending on the level of exposure, blast energy induces brain parenchymal and vascular disruptions, uncoupling of the blood–brain barrier, and induction of chronic cerebral vascular degeneration (Gama Sosa et al., 2014). TBI is indeed a major cause of combat-related disability and while CTE may explain some of the chronic symptoms experienced by veterans, its prevalence remains difficult to measure (Dickstein et al., 2020; Omalu et al., 2011). Public awareness of military-related TBI and CTE has recently increased due to the conflicts in Iraq and Afghanistan. Estimates are that 10%-20% of veterans returning from these conflicts suffered a TBI during deployment, with many suffering repetitive injuries (Warden, 2006). Concerns are rising over the potential adverse consequences of subclinical blast exposures, now being referred to as military occupational blast exposure (Engel et al., 2018). The long-term aspects of repeated mild TBI in military personnel and civilians alike include possible progression into chronic neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases, as well as CTE (Bryant & Harvey, 1999; Villapol, 2018). Common examples of chronic behavioral sequelae, include cognitive dysfunction, motor deficits, frontal lobe dysfunction, and stress-related outcomes such as post-traumatic stress disorder (PTSD) (Kennedy et al., 2007; Osier et al., 2015). Clearly, much more research is needed to understand the repercussions of mild TBI, how repetitive mild TBI may lead to CTE, and how these injuries and their consequences can be prevented.

The human brain is naturally shielded against mild trauma by the surrounding soft and hard tissues. The meningeal layers surrounded by cerebral ventricles and cerebrospinal fluid have a strain-relieving effect that reduces cortical displacement and buffers against vein rupture (Ivarsson et al., 2000). Despite these protections, the human brain can only sustain a limited amount of force, leaving it vulnerable to injury from severe impact (Greenwald et al., 2008). When forceful movement occurs and the brain comes into contact with the skull, it causes rapid deformation of the neural tissue (Bayly et al., 2005), eventually resulting in concussion. Even mild strain can cause harmful depolarization of cortical neurons (Shaw, 2002), while severe trauma can cause lesions in the white matter when axons are stretched.
beyond their physical injury threshold (Braun et al., 2020; Graham et al., 1995). Other causes of primary brain injury include contusions caused by coup and/or contrecoup impacts, blood vessel rupture between the brain and dura mater, and decreased blood flow due to brain edema. Secondary injury can then manifest over hours or days and contribute significantly to further neurological disability (McKee & Daneshvar, 2015). Secondary injury from TBI is less well characterized than the acute pathologies but can especially hinder patients'
survivability and quality of life. Examples of chronic histopathological outcomes include lesions, gross contusions, and changes to the cerebrovascular system. The brain can also show global volume reductions in response to TBI, most often affecting both the gray and white matter of the hippocampus, prefrontal, orbitofrontal, and temporal lobes. In CTE cases, atrophy occurs additionally in the thalamus, hypothalamus, and mamillary bodies (McKee et al., 2016; McKee & Daneshvar, 2015). This volume loss is thought to be caused primarily by axonal degeneration and is most commonly visualized by immunohistochemical detection of amyloid precursor protein and neurofilament proteins (McKee et al., 2016). Another diagnostic sign of CTE cases is the presence of neurofibrillary tangles that increase in number with case severity. Neurofibrillary tangles are accumulations of hyperphosphorylated tau protein, that in CTE cases are created by axonal injury principally in cortical layers II and III (Hof et al., 1992) and centered around small blood vessels or clustered in the depths of the cortical sulci (Blennow et al., 2016). Besides neurons, TBI also affects oligodendrocytes, microglia, and astrocytes, (Arneson et al., 2018) which are some of the cells responsible for an inflammatory response to brain trauma. Astrocytes release cytokines and chemokines that mediate the inflammatory response by recruiting peripheral cells, damaging neurons, and activating microglia (Arneson et al., 2018). The microglia activate within minutes and can remain so for years, suggesting a chronic inflammatory state in severe cases (Ramlackhansingh et al., 2011). Oligodendrocytes are mainly responsible for maintaining axon myelination in the central nervous system but can undergo apoptosis and necrosis following TBI events. However, brain trauma is also accompanied by an increase in oligodendrocyte progenitor cells, indicating the possibility for oligodendrocyte regeneration (Flygt et al., 2016). Depending on the specific type of shear injury, blast-related brain trauma presents a particular subset of related pathologies. These lesions often include vascular injuries that follow penetrating cortical vessels; high-energy blast exposure, for example, results in vascular tearing of the blood-brain barrier (Figure 2). In addition, blast exposure induces long-term uncoupling of the neurovascular unit with astrocytic and vascular degenerations. These observations suggest that blood vessels are very susceptible structures along which the damaging blast energy is transmitted (Gama Sosa et al., 2013, 2014).

The study of TBI has been vastly extended through the use of animal models to replicate injury on the molecular, cellular, and organismal level. Several models were initially developed in the 1980s, mainly in non-human primates, cats, dogs, and pigs (Morganti-Kossmann et al., 2010). Rodents then became the main models in the 1990s and remain the default today. However, the many anatomical differences between rodents and humans (Figure 1a,b) can cause difficulties when translating therapies for human application. Indeed, most neuroprotective therapeutic approaches for TBI developed in these animal models have failed advanced clinical trials (Xiong et al., 2013). In addition, TBI is not a single physiological event. It is instead qualified as a cascade of complex processes that run in parallel with each other, including focal and diffuse brain damage (McKee & Daneshvar, 2015). Depending on the source, intensity, and extent of the injury, TBI can be highly variable among patients (Langlois et al., 2006). Animal models are often limited to the study of a single trigger of TBI (e.g., impact, blast, rotational, unique, repetitive) (Morganti-Kossmann et al., 2010), and certainly some species are more amenable to modeling a specific aspect of TBI than others. Although not specific to TBI, dolphins, sea lions, polar bears, wolves, sheep, and goats are among the menagerie of species investigated as natural models for age-related neurodegeneration and tauopathies (the neurodegenerative disease involving the aggregation of tau protein into neurofibrillary tangles) (Youssef et al., 2016). Even Drosophila flies have been used to model the cellular and mechanical mechanisms of TBI (Shah et al., 2019), proof that a wide variety of models can be adapted for different research conditions and scientific approaches.

An additional problem for most clinically oriented animal experiments of TBI, is the failure to account for the natural anatomical and physiological properties of their model species. This is especially the case for large animal models, whose brains are more representative of the human brains in size and gyriﬁcation (Figure 1). Comparative adaptations for injury prevention in animal models, as well as recovery time from brain injury in different species, are both avenues lacking research that could inform human injury in terms of prevention, survival, and recovery. Besides humans and common laboratory models, other species are rarely investigated as potential sources for solutions to TBI. Although arguably more challenging to implement, they could represent novel approaches to the TBI epidemic. Animals adapted to either extreme environments or extreme behaviors have in some cases evolved specific protections to avoid or reduce brain trauma. Popularized examples include the headbutting of bighorn sheep and other bovids, the woodpecker’s pecking, and the whale’s high-pressure deep dives as well as their exposure to high-amplitude underwater sound. Studying these adaptations and whether they indeed result in a successful avoidance of brain trauma, can inspire the design of similar protective mechanisms for humans. In addition, exploratory studies of these real-world species can only serve to inform gaps in the current anatomical knowledge of their more common laboratory counterparts.

Several publications have reviewed the extensive literature on animal models of TBI and CTE. Most recently, Gold et al. (2013), Vink (2018), and Hoogenboom et al. (2019) have covered the subject thoroughly. Here, alongside a short review of the current animal models of TBI and CTE, we aim to bring focus to other potential species that can be used for observational studies of head trauma and the possibilities they may provide.

2 OVERVIEW OF CURRENT PRECLINICAL MODELS OF TBI AND CTE

Animal models have been established in many species to study the various aspects that together make up the complex process of TBI. This process often develops into a secondary phase with elongated and evolving secondary injuries, at which point models aim
to investigate the potential for therapeutic intervention. Whereas early models investigated the biophysical aspects of TBI, more recent studies have focused on understanding the molecular cascades affected by these injuries (for review see Xiong et al., 2013) and how they are influenced by injury type, be it focal, diffuse, unique, or repetitive.

### 2.1 Current TBI simulation techniques

Static force is only represented by crushing nervous tissue (David & Aguayo, 1985), however, most TBI models aim to measure dynamic mechanical force, taking into account acceleration (linear or angular) and velocity. This enables modeling of coup–contre injuries,
stretches of blood vessels, nerves, and other tissues, as well as brain inflammation (Phipps, 2016). Blast injury models simulate indirect force, through controlled standardized explosions or by delivering compressed air through a metal tube, generating blast shockwaves that may induce brain damage (Risling & Davidsson, 2012). These models are mainly applied to rodents and have recently been adapted to pigs (Sus scrofa domesticus) (Bauman et al., 2009; Kim et al., 2020). Shock tube models are, however, not standardized across research groups and it is difficult to simulate non-ideal blast waves that occur in real-life settings (Elder et al., 2014). However, recent work has improved injury simulations and shock-wave accuracy (Vu et al., 2018). Direct force models can further be separated into non-impact and impact head acceleration models (for an overview, see fig. 1 in Cernak, 2005). Non-impact head acceleration is performed mostly on larger animals because of the relationship of inertial force to brain size. It involves rapid head acceleration, either linearly or rotationally and was developed in non-human primates (Gennarelli et al., 1982) and pigs (Ross et al., 1994).

Direct impact models can be classified into two more categories: penetrating injury with direct brain deformation, and non-penetrating injury with impact acceleration, each using constrained and unconstrained head models. Constrained head models of penetrating injury include the fluid percussion (FPI) and controlled cortical impact (CCI) injury models. The FPI model combines both diffuse and focal injury either by applying force along the medial skull sutures, creating a diffuse, medial FPI (MFPI), or by applying a focal, lateral injury to the cerebral cortex, creating a lateral FPI (LFI). LFI targets a single hemisphere of the rodent brain to replicate progressive gray matter damage, intracranial hemorrhage, and brain swelling (Graham et al., 2000). Because FPI offers the possibility of studying long-term effects and the ability to model focal as well as diffuse injuries, it has become the most used, and therefore best characterized TBI model (Thompson et al., 2005). The CCI model only creates a focal injury and although it is advantageous in its reproducibility and high precision, its inability to create diffuse injury limits its application, as not all TBI cases show signs of contusion (internal hemorrhage due to ruptured brain capillaries) (Osier & Dixon, 2016). CCI was first described in ferrets (Mustela putorius furo) (Lighthall, 1988), and later applied to rats (Rattus norvegicus) (Dixon et al., 1991), mice (Mus musculus) (Smith et al., 1995), pigs (Hannay et al., 1999), and rhesus macaques (Macaca mulatta) (King et al., 2010). High-velocity missile injury models are rarely used unless specifically intended to simulate firearm- and shrapnel-related injuries on both constrained and unconstrained head models. High-velocity missile injury and similar penetrating ballistic brain injury models have also been described in rhesus macaques (Allen et al., 1982), as well as in cats (Felis catus) (Carey et al., 1989) and rats (Williams et al., 2005). The weight-drop model creates form of non-penetrative injury that causes widespread axonal damage without damaging the cranium itself, by dropping a weight onto a metal disk affixed to the skull. This model’s main advantages are low cost and minimal invasiveness, and it is mainly applied to rodents. Unconstrained head models of non-penetrating injury, on the other hand, are modeled using bolt guns, mostly on sheep (Ovis aries) (Lewis et al., 1996).

It should be noted that the extent of damage created in models using experimentally inflicted injury can be problematic. In some cases, models require non-clinically relevant craniotomy, as is mandatory for FPI, CCI, and weight-drop models (Hoogenboom et al., 2019), and the creation of additional damage independent of TBI can impede further cellular and molecular investigation (Finnie, 1993).

### 2.2 Small animal models

Rats and mice are the species most commonly used as experimental animal models for TBI (Morganti-Kossmann et al., 2010). Rodents are ideal models because of their relative low cost, short generation times, and availability of genome manipulation. In addition, there are aspects of TBI and CTE pathology that are well replicated in rodents, establishing a precedent for experimental studies. For example, motor, sensory, and cognitive behavior assessment techniques are well characterized and widely applied to record TBI effects on behavior in these animals (Gold et al., 2013). In the case of CTE, only one rodent model has reproduced a chronic tauopathy in a model of repetitive mild blast injury (Dickstein et al., 2020). In this model young adult rats were subjected to three repetitive blast injuries designed to model mild TBI or subclinical blast exposures. These rats then developed a chronic cognitive and behavioral phenotype, including PTSD-related traits that were present for over a year (Perez-Garcia et al., 2019).

However, rodents do have significant limitations as research models to study the neuropsychiatric consequences of TBI, as they do not always show replication of human trauma. Secondary axonal injury, for example, occurs more rapidly in rodents than in humans and other animal models, and immature rodent models for pediatric TBI tend to create focal rather than diffuse injury (Finnie, 2001). While rodents can show similar motor dysfunction and tissue degradation to humans in response to TBI, they do not lapse into the comatose state most common in humans (Morganti-Kossmann et al., 2010). Even within rodents, disparities exist between mice and rats in terms of cognitive capacities, physiology, and behavior that can be analyzed in each species as a model. Unfortunately, very few effective therapies have resulted from experimental animal models (Sorby-Adams et al., 2018). This lack of translation is most often attributed to neuroanatomical differences between rodent and human brains. Specifically, the smaller brain, lissencephalic cerebrum, weak tentorium cerebelli, low ratio of white to gray matter (Sorby-Adams et al., 2018), rectangular skull shape, thin skull, and different brain orientation relative to the spinal cord in rodents, do not faithfully replicate human anatomy (Figure 1a,b). In addition to the lack of clinical translation, few rodent model studies focus on the chronic progression of neurovascular changes related to TBI or CTE (Gama Sosa et al., 2013, 2014, 2019; Jamerian et al., 2019), as seen in Figure 2 with the appearance of tortuous and pinched vessels in blast-exposed rats. As CTE can originate from repetitive
mild instances of TBI, the severe TBI models most often applied to rodents may be maladapted to study CTE (Goldstein et al., 2012; McKee et al., 2012). Finally, CTE can only be diagnosed in human postmortem, making the development of models replicating this injury difficult regardless of species.

Apart from rodents, the ferret is a relatively small animal model with a gyrencephalic brain that has showed promise as a model for TBI research. Ferrets also have a high white to gray matter ratio, a ventral hippocampus, and are large enough for brain MRI, unlike rodents. A CCI experiment on ferrets resulted in increased astrogliosis with injury severity, alongside motor and cognitive impairments (Schwerin et al., 2017). Blast tube experiments also altered astroglia densities and phosphorylated tau expression after injury (Schwerin et al., 2021), suggesting that ferrets represent an interesting translational model for some features of TBI.

### 2.3 Large animal models

Large animal models for TBI can be considered more relevant for translational research in certain cases because of their size, high white to gray matter ratio, and gyrencephalic brain when compared to rodents (Figure 1a–e) (Vink, 2018). Non-human primates especially have been used as TBI models using multiple techniques (Vander Vorst et al., 2007), although smaller primate species used in research, such as marmoset monkeys (Callithrix jacchus) have less gyrencephalic brains than larger species, principally represented by macaque monkeys. Primate hominid species with a brain morphology more directly comparable to that of humans can no longer be used in laboratory research.

In comparison to rodents, sheep and pigs have a more similar white and gray matter distribution to that of humans (Vink, 2018), but with species-specific differences in biomechanical brain properties (Pervin & Chen, 2011). Domestic pigs continue to be used as models for TBI due to their resemblance with human anatomy in terms of brain size (Figure 1a,e), composition, organization, vasculature, development, and inflammatory response to injury (Kinder et al., 2019). When simulating diffuse axonal injury, pig models sustain several cellular pathologies seen in humans, including axon clubbing, swelling, and accumulation of neurofilament, beta amyloid, and tau proteins (Kinder et al., 2019). In addition, CCI pig models showed an increase in glial fibrillary acidic protein (GFAP) expression in astrocytes corresponding to injury severity (Baker et al., 2019), as is the case in humans. One disadvantage of the pig model is the lack of behavioral studies, resulting in limited methods for the assessment of motor impairments of TBI, with no behavioral or cognitive impairment testing adopted as standards (Kinder et al., 2019). The tau gene structure was originally determined from cattle brains (Himmler, 1989); although cattle (Bos taurus) are not used to model TBI today (Pervin & Chen, 2009). Domestic sheep have thus emerged as the preferred large animal model for tauopathies and have been used for a variety of techniques. For example, aging sheep and goats are being considered as potential models for Alzheimer’s disease (Braak et al., 1994; Reid et al., 2017) as they show increasing neurofibrillary tangles and tau deposition with age (Härtig et al., 2000) that are similar in appearance to those in humans, with a highly conserved sequence of key proteins involved in Alzheimer’s disease pathogenesis. In the future, these and other large mammalian species may allow for long-term post-injury measurements, including the development of cognitive impairments over time with increased study of behavioral cues and the development of specific behavioral tests for these species.

### 3 Potential species for comparative studies of brain trauma

Aside from common laboratory species such as mice, rats, zebrafish, Drosophila, and C. elegans, other species are uncommon as models in neuroscience. Multiple species across taxa have naturally evolved adaptations for brain protection in relation to their various behaviors and environments. The different evolutionary path followed by each species toward the avoidance of brain trauma may provide insight into solving our own brain protection problems. These species’ adaptations hold value at the anatomical and physiological scales as sources for observational comparative studies focusing on TBI. Accruing specimens from non-laboratory animals can be more logistically difficult and time-consuming with regard to the scarcity of the materials. However, partnerships with zoos, farms, and wildlife organizations make it possible to collect brain samples from naturally deceased or humanely euthanized animals, and increased interest in real-world models will only serve to increase characterization. The lack of appropriate reagents, established protocols, and techniques could also hamper studies using these models. Pilot studies with available samples would enable quality control for different preservation methods and provide validation for reagent interactions. Even if these real-world models cannot be used for preclinical treatments in the absence of standardized behavioral tests, they can be useful to reveal biological protection or susceptibility to trauma. In-depth anatomical investigations of animals specifically adapted to extreme behaviors or environments could reveal structures specifically evolved to protect the brain from high forces. These can take on the form of macroscopic structures, including a thickened skull, large sinuses, cushioning structures such as face pads or a specialized tongue, as well as additional appendages such as large horns or antlers; or protections on a physiological level. This will not only establish a baseline for model species, but also develop a better understanding of the capacities of the mammalian brain as a whole and serve to orient us toward solutions applicable to humans.

#### 3.1 Wild hogs

Wild counterparts of the domesticated pig model may hold some promise for future TBI studies. The giant forest hog (Hylochoerus...
meinertzthageni) and warthog (Phacochoerus sp.) are to domestic pigs, what bighorn rams are to domestic sheep, in that they exhibit extreme anatomy and behavior. Giant forest hogs and warthogs have been observed butting heads as a show of dominance between males (Frädrich, 1971; Geist, 1966) and indeed, d’Huart and Kingdon (2013) describe mature male forest hogs charging at each other, hitting foreheads, and producing “a very loud cracking noise” (for an example of this behavior, see https://www.youtube.com/watch?v=ld0b8cv9QDs. ortnermi, 2017). Thickened bony ridges on the forehead (Ewer, 1970), “a double-layered cranium,” and thick facial tissue pads are claimed to absorb the shock from these aggressive clashes (d’Huart & Kingdon, 2013), although these hypotheses have never been tested experimentally. On rare occasions, these head clashes can result in skull fractures and even death, raising the question of the cost these fights take on the hog’s neuroanatomy. Anatomical and histological investigations would be essential to begin to determine the hog’s main source of protection, or whether the suspected factors provide any additional brain protection at all. Perhaps, similarly to the thick fat pad found between a sheep’s horns, the giant forest hog’s facial pads serve both as a means of protection and as a sexual signal. Further investigation could provide more insight into the skull and brain anatomy of the domestic pig TBI model, and would be an interesting avenue to consider for the betterment of athletic and military helmets.

3.2 | Combative bovids

Apart from some domesticates, all bovid species have horns, sometimes present in both males and females. These adornments most often serve as a signal for sexual selection and a defense against predators (Emlen, 2008). Of all representatives of the Bovidae family, only the Caprinae subfamily (sheep-, goat-, and muskoxen-like animals) use their horns for headbutting, while all other subfamilies practice more of a sparring ritual when two males engage in the rut, similar to deer. Muskoxen bulls (Ovibos moschatus) are among the largest animals to practice headbutting, with heads formed by helmet-shaped horns and a body mass averaging 300 kg (De Magalhães et al., 2005). Because of their remote habitat, these animals remain understudied and there have been no reports in the literature relating muskoxen death to observable brain injury, although anecdotal observations of animals “acting dazed” after the rut have been noted (Smith, 1976). Bighorn sheep (Ovis canadensis) have some of the most prominent headgear in the natural world, and thus are the extreme representative of their taxonomic family (Figure 1c,d). The bighorn sheep’s horns and skull have been investigated for their biomechanical properties (Drake et al., 2016; Eck, 2014; Huang et al., 2017; Johnson et al., 2017; Kitchener, 1988; Trim et al., 2011; Zhang et al., 2018) and have even inspired biomaterial design for football helmets (Johnson, 2016). All studies investigating headbutting in goats and sheep imply that these animals successfully absorb all shock through the horns, however, a recent study found the biomechanical properties of the bighorn ram’s horncores to be no different than those of other mammalian cortical bone (Fuller & Donahue, 2021). Furthermore, all biomechanical studies were either performed only on horn cores and horn sheaths (Fuller & Donahue, 2021; Huang et al., 2017; Johnson, 2016; Johnson et al., 2017; Kitchener, 1988; Trim et al., 2011; Zhang et al., 2018), skeletonized skulls, on finite element analysis on models thereof (Drake et al., 2016; Maity & Tekalur, 2011). Only one study used a skinned goat head with raw muscles intact as a model for impact loading (Jaslows & Biewener, 1995) and no studies accounted for the material properties of the brain and its surrounding meninges and cerebrospinal fluid, the interaction of different anatomical structures (vessels, muscles, fat, meninges, fluid, skin, bone), or the physiological properties in live animals. In fact, whether these animals sustain brain damage at all under natural conditions despite their substantial headgear has yet to be investigated. In addition, all studies failed to account for female skull anatomy and behavior in headbutting species. Indeed, wild ewes have been observed butting heads even more frequently than males in the mouflon (Ovis orientalis) (McClelland, 1991), stone sheep (Ovis dalli), and bighorn sheep (Geist, 1971) without sustaining any apparent ill effects. Quite often, when striking their opponents, each animal has its head tilted to the side so as to strike between the horns on the forehead of their rival, rather than horn to horn (Geist, 1971). Given that ewes have smaller horns, or even lack horns, as is the case in domestic breeds, these appendages cannot be the only line of defense against trauma. Thus, the namesake appendages of the bighorn sheep may be used mainly as a marker for sexual fitness and selection, rather than solely a protective or combative mechanism. Another hypothesis (Jaslows & Biewener, 1995), is that the thick skull and expansive frontal sinuses of these animals are their main defense against impact (Figure 1d). However, Farke (2008) used phylogenetic and finite element analysis to model domestic goat skulls (Capra aegagrus hircus) with and without frontal sinuses and concluded that larger sinuses had no apparent effect in shock reduction. Instead, the sinuses most likely serve as a scaffolding during maturation to support the rapid growth of large horns and reduce their weight.

Observational records show headbutting is one of the most common behavioral patterns of bighorn and stone sheep (Geist, 1971), the frequency of which may cast these sheep as a possible model for athletes suffering from repetitive sports-related concussions and CTE. It is important, however, that experiments first establish if these animals suffer from TBI at all, as even solely from a physics aspect, it is evident that there is much more to learn about how sheep brains may be affected from head clashing (Courtney & Courtney, 2007). If combative bovids have an innate resistance to TBI, this could put into question the accuracy of the current head-impact models developed on domestic sheep, as Millen et al. (1985) noted early on in establishing sheep TBI models, and would demand a different approach to bovid models, as well as the potential of their translation to humans. Although anatomical differences such as horns need to be considered, the potential internal protective mechanisms surrounding the brain and/or a fast neural recovery process in such extreme animal models may also provide avenues for prevention or clinical therapies for TBI in humans.
3.3 | Cetaceans

Long life spans, large relative brain masses (Ridgway et al., 2016), and greater gyration of the cetacean neocortex relative to other mammals (Figure 1g) (Hof et al., 2005; Marino, 1998; Ridgway & Brownson, 1984) make cetaceans (whales, dolphins, and porpoises) unique models for investigating the relationship between behavior and trauma in the human brain. Although adapted for high-pressure habitats when deep diving (Reidenberg & Laitman, 2008), numerous studies have demonstrated marked impacts on cetacean physiology, behavior, and brain integrity caused by underwater pressure waves from explosions associated with construction or seismic activities. These impacts include irregular behaviors (DeRuiter et al., 2013; Todd et al., 1996) and elevated physiological costs (Williams et al., 2008, 2017, 2020), as well as potential brainstem and cranial trauma often associated with auditory trauma (Ketten et al., 2004). As in humans, large cetaceans can experience a thoracic effect, in which damage to the central nervous system may occur indirectly from the transfer of kinetic energy from a blast wave traveling across the body and into the head. This was evident in a study investigating minke whales (Balaenoptera acutorostrata) subjected to blast pressure waves when being hunted with explosive harpoons (Knudsen & Øen, 2003). The animals later revealed signs of TBI, in which the severity of damage to the skull and brain depended on the distance of the harpoon strike from the head, with closer explosions causing severe neurotrauma and intracerebral hemorrhage. Although these are extreme and unnatural situations, akin to the experimental techniques applied to rodents in terms of a large direct blast to the head, they demonstrate the susceptibility of these comparatively long-lived, large-brained animals to blast-induced TBI. The potential benefits of diving adaptations reported for cetaceans, including hypoxia protection (Pongonis, 2015) and neuro-protection (Williams et al., 2008) for shielding the brain from blast trauma remain to be investigated and show promise as an interesting avenue for further exploratory TBI research on recovered specimens. In an Alzheimer’s disease study, evidence of tau pathology was found in dolphins (Gunn-Moore et al., 2018), demonstrating that tau might also be detectable in immunohistochemical TBI studies on other cetaceans. One additional aspect of cetaceans’ relationship to TBI is the controversial take that sperm whales (Physeter macrocephalus) may use their large, sexually dimorphic melon (the organ on a whale’s forehead involved in echolocation) for male-to-male aggression, and occasionally defense against boats (Carrier et al., 2002; Panagiotopoulou et al., 2016). The counter-argument is that the male’s large head is used in a form of acoustic sexual selection (Cranford, 1999). Still, when modeling sperm whale head impacts, a study (Panagiotopoulou et al., 2016) demonstrated that the modeled junk organ, the enlarged melon of the male sperm whale, may reduce impact stress due to the arrangement of its connective tissue, and Carrier et al. (2002) suggested that sperm whale ramming could seriously injure an opponent. Ramming behavior is not limited to sperm whales and could be conserved basal behavior to Cetartiodactyls (a superorder including cetaceans and artiodactyls, even-toed ungulates) (Lusseau, 2003) as it has been recorded the four major cetacean lineages, including narwhals (Graham et al., 2020), humpback whales (Baker & Herman, 1984), bottlenose whales (Gowans & Rendell, 1999), bottlenose dolphins (Lusseau, 2007) (Figure 3), and killer whales (Goley & Straley, 1994). In pilot whales, unusual skull structures may even act as a form of “antlers inside” the head (Gol’din, 2014). This collection of extreme behaviors, in addition to adaptations for living in an aquatic habitat makes cetaceans an extremely interesting avenue to explore in terms of TBI reduction.

3.4 | Birds

Woodpeckers (Picidae) are known for their ability to pound their heads powerfully against trees, seemingly without experiencing observable brain injury (Bock, 1999). Because of this extremely specialized behavior, their physiology has been investigated for its potential to inspire solutions to TBI (Figure 1f) (Farah et al., 2018; May et al., 1979; Wang et al., 2011). Some studies suggest that the shape of their skull allows for distribution of force that avoids harming their brains (Bock, 1999; Wang et al., 2011), while others imply that the linear as opposed to rotational (angular) trajectory of their pecking motion, in conjunction with their small brain size, reduces brain injury (May et al., 1979). Yet other hypotheses include the specialized anatomy of their hyoid apparatus, which wraps around the skull, posited as a cushioning mechanism that helps protect the brain (Yoon & Park, 2011). None of these hypotheses, however, have been proven conclusively outside of theoretical models and although these adaptations may contribute to brain protection, there has been no conclusive neuropathological evidence that supports woodpeckers avoiding TBI altogether. Nonetheless, as with bighorn sheep, the woodpecker’s unique anatomy has also inspired football helmet prototypes aimed at reducing TBI (Mao et al., 2014). A recent immunohistological study (Farah et al., 2018) noted accumulations of phosphorylated tau throughout the entire brain of woodpeckers, as compared to non-pecking bird species, with accumulations specifically concentrated around astrocytes. Due to the small sample size of the study and the general lack of knowledge surrounding woodpecker neurophysiology, it is unclear whether the tau accumulations were related to aging, pecking, or other factors. However, this study is a prime example of why conclusions about animal models should not be reached hastily. It is important to note that the woodpecker’s brain lack the gyri and sulci that, in humans, act as the key sites of tau accumulation due to the mechanical forces of a brain injury (Vink, 2018). Another avian species known for its head impacts is the helmeted hornbill (Rhinoplax vigil), a large bird sporting solid “ivory” casques on its bill. These birds have been observed headbutting casque-to-casque mid-air in interactions lasting up to two hours, creating loud sounds and such forces that the birds were thrown backwards (Kinnaird et al., 2003). Further anatomical investigations have not been made, however, on this critically endangered species. Although less extreme than woodpeckers and hornbills, one bird has been used as a laboratory model for TBI. Specifically, zebra finches (Taeniopygia guttata) have become part of...
an increasing body of work on neuroinflammation and steroid hormone production in relation to TBIs (Pedersen et al., 2018). In a zebra finch experiment by Pedersen et al. (2016), a penetrating brain injury was performed through craniotomy and insertion of a needle 3 mm into the brain. Estradiol synthesis in response to the resulting brain damage showed anti-inflammatory effects by decreasing cytokine activity, an interesting finding that will benefit from comparisons in other non-avian species.

4 | CONCLUSION

Decades of work in the field of neuroscience on conventional animal models have led to the discovery of specific mechanisms and pathways that have vastly improved our understanding of TBI. Integrating this knowledge with findings in more unconventional species could further our understanding of the diverse factors involved in TBI and their importance in the development of preventive measures against what has become one of the leading causes of death in the world. Conventional animal models continue to offer valuable insight into certain aspects of TBI, such as the progression of diffuse versus focal injury, or the development of cognitive and behavioral changes. However, the diversity and individuality of traumatic brain injuries contribute to the difficulties of translation from animal models to clinical settings and demand a variety of approaches in return. Being that CTE can only be diagnosed postmortem, the development of secondary injury leading to CTE remains difficult to model and is thus understudied. More in-depth work is needed to understand the molecular, physiological, and cellular pathways affected by CTE, as well as its long-term outcomes and progression into neurodegenerative diseases. Avenues of research offered by less explored models include systems specifically evolved for neural protection, often in gyrencephalic brains of similar size to those of humans. Seeking inspiration from unconventional animal models that routinely survive repetitive impacts can provide new perspectives addressing the numerous questions that still surround acute and chronic brain injury. Inspiration from unconventional models may stem from externally evolved brain protections (horns, thick skull, padding etc.) or the capacity to resist, survive, and recover from TBI more efficiently than humans on a physiological and molecular level.

Research is currently underway to establish the presence or absence of pathological markers of naturally occurring brain injury in various extremely adapted mammals that offer themselves as potential species for the study of TBI and CTE (Ackermans et al., 2021). Antibody-based detection of glia- and microglia-specific markers in brain tissue is being used in bovids to highlight astrocytic and glial morphology and density, as glia can change shape as a function of activation following central nervous system injury (Tischer et al., 2016). Another technique being applied for TBI detection is highlighting the presence of abnormally phosphorylated tau proteins linked to neurofibrillary degeneration and tauopathy. Exploratory histology, alongside in-depth anatomical descriptions are the baseline for investigating TBI in these uncommon species, and although studies are underway, the animal kingdom is a vast reservoir of untapped potential that remains to be explored for this purpose. Including a diverse array of brains into the study of TBI can only promote further expansion of the field by increasing our knowledge of the development as well as the avoidance of TBI in a vast array of species.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the Journal of Neuroscience Research, this manuscript
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presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

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CONFLICT OF INTEREST

No competing financial interests exist.

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