Review article

# Challenges and demand for modeling disorders of consciousness following traumatic brain injury

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## ABSTRACT

Following severe traumatic brain injury (TBI), many patients experience coma — an unresponsive state lacking wakefulness or awareness. Coma rarely lasts more than two weeks, and emergence involves passing through a state of wakefulness without awareness of self or environment. Patients that linger in these Disorders of Consciousness (DoC) undergo clinical assessments of awareness for diagnosis into Unresponsive Wakefulness Syndrome (no awareness, also called vegetative state) or Minimally Conscious State (periodic increases in awareness). These diagnoses are notoriously inaccurate, offering little prognostic value. Recovery of awareness is unpredictable, returning within weeks, years, or never. This leaves patients' families with difficult decisions and little information on which to base them. Clinical studies have made significant advancements, but remain encumbered by high variability, limited data output, and a lack of necessary controls. Herein we discuss the clear and present need to establish a preclinical model of TBI-induced DoC, the significant challenges involved, and how such a model can be applied to support DoC research.

## 1. Introduction

An estimated 2.8 million people visit the emergency department due to traumatic brain injury (TBI) annually in the US, and ~11% are then hospitalized due to injury severity and coma — a temporary state of unconsciousness in which the patient is unawake and unaware (see Box 1 for terminology) (CDC Injury Center, 2016; Selassie et al., 2008; "TBI," 2017). Many patients that experience coma after TBI emerge into prolonged disorders of consciousness (DoC), in which they regain wakefulness (i.e. eye opening, arousal), with limited awareness of self or environment (Laureys et al., 2004). Within the DoC, absence of any behavioral signs of awareness is diagnosed as unresponsive wakefulness syndrome (UWS) — also referred to as vegetative state — and intermittent periods of increased awareness are diagnosed as minimally conscious state (MCS) (Laureys et al., 2010, 2004). The number of patients suffering from DoC is unclear, and inaccurate diagnoses are all too common (Monti et al., 2010; RCP London, 2015; Schnakers et al., 2009). Our current understanding of these disorders relies mainly on individual case studies or clinical trials in heterogeneous DoC patient populations, which are limited in their data output and ability to impose controlled experimental design (Schnakers and Monti, 2017). Experimental treatments including neuromodulatory approaches (deep brain stimulation in the thalamus (Schiff et al., 2007), transcranial direct current stimulation (Thibaut et al., 2017), and vagus nerve stimulation (Corazzol et al., 2017)) or pharmacological agents (GABA agonist zolpidem (Whyte et al., 2014) and dopaminergic agent amantadine (Giacino et al., 2012)) appear to increase awareness within subsets of patients in prolonged DoC; however, the clinical, heterogeneous nature of these studies limited the extent of controlled, mechanistic investigation. Spontaneous recovery from prolonged DoC lasting over a year occurs more regularly than previously believed, but controlling for spontaneous recovery in clinical trials poses significant logistical and ethical difficulty, and therefore the vast majority of studies do not include placebo or untreated controls (Giacino et al., 2016; Schnakers and Monti, 2017; Whyte et al., 2013a). There are no standards for patient selection for these therapies, which seems to be based

**Box 1**
Terminology discussed.

> **Wakefulness:** state of brain activation in which sensory information can be acquired; clinical signs include eye opening, sleep/wake EEG activity, reaction to noxious stimuli
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> **Awareness**: higher state of brain activation in which acquired sensory information can be processed; clinical signs include eye tracking, command-following, meaningful vocalization
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> **Disorders of Consciousness (DoC):** impaired states of wakefulness and/or awareness that often accompany moderate-to-severe traumatic brain injury (TBI) in humans
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> **Brain Death:** total absence of wakefulness, awareness, brainstem reflexes, and breathing drive; considered clinically and legally dead
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> **Coma:** absence of wakefulness and awareness with detectable brainstem reflexes and/or breathing drive; very rarely lasts more than two weeks
>
> **Unresponsive Wakefulness Syndrome (UWS):** also called "vegetative state"; DoC following emergence from coma in which wakefulness returns without awareness; can temporarily precede recovery of awareness by hours or decades, or remain permanently; current prognostic methods are notoriously inaccurate
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> **Minimally Conscious State (MCS):** a spectrum of DoC following emergence from coma in which wakefulness returns with intermittent signs of awareness; duration and prognostic ability similar to UWS.

instead on institutional preference or experience. Recruitment for clinical studies faces legal and ethical hurdles, further exacerbated by underreporting, often leading to limited sample sizes even in multi-center studies. A preclinical model would offer precise control, reduced variability, rich data output, and reduced ethical complexity. Validating a new model requires significant effort and modeling traumatic coma/DoC presents unique challenges. However, preclinical modeling is a vital tool for understanding and treating human maladies and DoC research stands to benefit greatly by expanding the field into preclinical investigation.

## 2. Nature of the problem

Due to their desperate prognoses, administering experimental systemic treatments to patients in DoC faces little resistance. However, denying treatment to impose necessary negative controls is often unacceptable to caregivers, which greatly restricts the conclusions that can be drawn from subsequent findings. Although there is some pathological overlap, various initial injuries can lead to DoC, and recovery rates also cover a broad range, leading to considerable within-group variability. Inaccurate diagnoses are unfortunately common when distinguishing between UWS and MCS, with misdiagnosis rates around 41% contributing additional variability and error (Monti et al., 2010; RCP London, 2015; Schnakers et al., 2009). Data output is limited, and post mortem analyses — when available — have high variability due not only to differences in patient population and injury, but also to medical complications after injury and the time of death relative to injury (Giacino et al., 2013; Whyte et al., 2013b). Underreporting and ethical/legal complications surrounding consent create additional difficulties by hindering the enrollment of enough patients to overcome high variability and achieve sufficient statistical power. For example, in Pennsylvania and a number of other states, once an incapacitated patient has a legally-appointed guardian that patient cannot be enrolled in research without a judge's permission, even if the guardian wants to enroll them (Wright et al., 2017). While high variability and limited experimental control over treatments and data collection are common problems that are broadly encountered in medical research, clinical DoC studies must contend with the combination of these issues with few options for addressing them.

Preclinical study offers many advantages and potential for advancement. Modeling DoC can provide properly controlled experiments and greater experimental output that are necessary for drawing precise, detailed conclusions. Reproducible injury criteria and a homogenous animal population can minimize within-group variability. By breaking down the larger pathology into smaller components, preclinical studies can lead to a deeper understanding of DoC and provide more informed inclusion criteria that can reduce within-group variability for future clinical trials. In addition, pre-clinical models afford the opportunity to test various interventions not only for their effects on behavior and non-invasive monitoring, but also on systems-level circuits and individual cells. However, while establishing an animal model would provide greater homogeneity and practicality, it would also require a considerable amount of effort to establish the limits of model validity. Model validation typically begins with mechanism and manifestation. The model would first need to accurately reproduce the mechanisms of severe TBI in humans, requiring similar brain mass, neuroanatomy, and injury physics. The model would also need to accurately reproduce the manifestations of severe TBI in humans, most importantly prolonged loss of consciousness, which does not occur in the common animal models utilizing contusion injury. Beyond the basics of mechanism and manifestation, model validation should demonstrate the ability to distinguish between wakefulness and awareness, the potential for spontaneous recovery, and response to treatment that parallels what is observed in humans. Technical and conceptual challenges have thus far stymied preclinical investigation of DoC and the desperately needed clarity and power it can provide, but these challenges are not insurmountable.

## 3. Validation of mechanism and manifestation

Most TBI cases are closed-head diffuse injuries, and loss of consciousness is associated with damage from rotational acceleration in closed head TBI, not with linear acceleration or impact contusions (Denny-Brown and Russell, 1941; Langlois et al., 2006; Ommaya and Gennarelli, 1974). Rapid acceleration/deceleration of the head relative to the body produces rotational loading in the brain, resulting in mass-dependent generation of forces throughout the brain that can locally create large deformations damaging at the cell and tissue levels (Cullen et al., 2016; Denny-Brown and Russell, 1941; Holbourn, 1943; Povlishock, 2016). In TBI, rotational loading is associated with loss of consciousness, while the compressive forces of impact are not (Cullen et al., 2016; Povlishock, 2016). DoC can also arise from non-TBI etiologies such as stroke, but we will focus our discussion on modeling TBI-induced DoC, as the heterogeneity of non-TBI DoC would require different approaches with their own unique challenges (Cruse et al., 2012; Newcombe et al., 2010). Rotational acceleration injury was previously found to produce coma followed by several days of prolonged "intermediate coma" requiring supportive care and lacking spontaneous motor activity in a subset of non-human primates (Gennarelli et al., 1982). That study did not draw parallels between this "intermediate coma" state and human DoC, but their observations are nevertheless encouraging for modeling wakefulness without awareness

in a gyrencephalic animal model. In addition to establishing the connection between rotational acceleration and loss of consciousness after TBI, research in non-human primates spanning the 1940s through 1980s found that pontine damage and diffuse axonal injury were strongly correlated with coma severity (Denny-Brown and Russell, 1941; Gennarelli et al., 1982; Ommaya and Gennarelli, 1974). Axons comprising the white matter are viscoelastic, like Silly Putty, meaning they can accommodate slow shearing forces by stretching, but intra-axonal components may "snap" with rapid acceleration (Cullen et al., 2011; Galbraith et al., 1993). These early, seminal studies in non-human primates established the connection between loss of consciousness and the diffuse axonal injury resulting from rotational acceleration and shearing forces during TBI. Due to ethical concerns, the field has moved away from non-human primate research, and the only contemporary model for rotational acceleration TBI utilizes swine (Cullen et al., 2016).

While we still have much to learn from the rodent models of TBI, modeling trauma-induced coma or DoC in rodents presents significant limitations. Precise pharmacological lesions in rats at several discrete locations in the ascending reticular activating system (ARAS) have produced prolonged coma and revealed circuits and cell types that are necessary for maintaining consciousness (Fuller et al., 2011). Rodents have also been studied extensively in the context of sleep and anesthesia, informing our understanding of the neural correlates of consciousness, changes in global and local connectivity, the asymmetric processes of induction and emergence, and the pharmacological mechanisms of anesthetics (Hudson et al., 2014; Joiner et al., 2013; McCarren et al., 2014; Olcese et al., 2016; Pal et al., 2017). The focal contusion-based methods commonly used to model TBI in rodents have also been vital in identifying pathological mechanisms, particularly having to do with cellular and molecular sequelae of secondary injury following mechanical perturbation. However, it appears that it is not possible to model loss of consciousness caused by diffuse TBI using rodents. Indeed, evidence suggests that the biomechanical forces and recovery patterns associated with loss of consciousness after TBI cannot be replicated in the rodent brain. While inclusion of head acceleration has been attempted in rodent models of TBI, the maximum "loss of righting reflex" observed after severe injury was 25 min, with most values in the range of 8 to 15 min (Sauerbeck et al., 2018). That study went on to report that rotational acceleration "did not reach scaled thresholds" and pathological analyses indicated that "impact may be the biggest driver of injury" in their model. Unfortunately these results are unsurprising, given that rodent brains are simply too small to produce the diffuse rotational acceleration-induced forces present in a human brain during TBI. Shearing force is a product of brain mass and rotational acceleration, and the relatively large cortical mass in swine allows for scaling to human shearing forces through increased rotational loading (Holbourn, 1943; Margulies et al., 1990; Ommaya et al., 1967), while rotational accelerations required to scale forces to rodent brains are not feasible. Swine and humans also have similarly high white:gray matter ratios (both ~60:40), compared to the low ratios in rats and mice (14:86 and 10:90, respectively) (Bailey et al., 2009; Howells et al., 2010; Zhang and Sejnowski, 2000). In addition, the architecture of the cortex and sub-cortical structures determine the pattern of pathology after closed-head TBI, making the gyrencephalic porcine brain much more relevant to human neuropathology than the smooth lissencephalic brains of smaller mammals (McKee et al., 2009; Meaney et al., 1995). Finally, even at injury levels described as "severe" in rodents, the animals are performing normal behaviors (e.g., eating, grooming, etc.) within an hour after injury, and the administration of higher injury levels does not produce prolonged unconsciousness but rather induces immediate herniation and death without the possibility of reanimation (Dixon et al., 1987; Hsieh et al., 2017; Johnson et al., 2015; Marklund, 2016). As such, it appears that from a practical standpoint physical trauma levels cannot be calibrated such that rodents experience a period of prolonged (i.e. several hours to days)

unconsciousness without inducing sudden death. In contrast, rotational acceleration TBI in swine produces loss of consciousness even at mild (concussion) levels, and through to severe levels of injury with a duration-to-severity relationship mirroring that which is observed in humans (Browne et al., 2011; Cullen et al., 2016; Smith et al., 2000). In summary, while the low cost and ease of genetic manipulations in rodent models makes them attractive, the brains of swine and humans share several properties — not found in smaller mammals — that are necessary for producing the damage from rotational acceleration injury central to human TBI and loss of consciousness. The porcine model therefore presents unique potential for mechanistic validity by reproducing the forces of human TBI, as well as manifestation validity by producing traumatic unconsciousness.

## 4. Rotational acceleration TBI and prolonged unconsciousness in swine

The porcine model of closed-head rotational acceleration injury has led to considerable advances in the treatment and understanding of TBI over the last few decades, and is currently the only model of diffuse TBI capable of reproducing the type of pathology associated with coma and DoC in humans (Cullen et al., 2016; Smith et al., 2000). Significantly delayed return to consciousness (after anesthesia removal) is often observed in this model following rotational acceleration in planes causing axonal shearing in brainstem (axial or sagittal), but not the coronal plane (Eucker et al., 2011; Smith et al., 2000). Brainstem deformation from rotational acceleration in the axial or sagittal plane is apparent in the schematics of Fig. 1. It was reported that axial rotation in this swine model of closed head TBI induced deep coma that lasted for up to the full duration of the 8 h study period (Smith et al., 2000). In that study, axonal pathology was quantified throughout the brain, and white matter damage in the brainstem — specifically the pons — correlated strongly with coma duration (no other regional correlations were observed). Severe axonal lesions in brainstem were also unique to coma in non-human primates after lateral rotational acceleration (Gennarelli et al., 1982). Orexin-saporin lesion of glutamatergic neurons in the parabrachial/precoeruleus area of the pons results in coma in rats (Fuller et al., 2011), and lesions in the parabrachial area of the pontine tegmentum have also been associated with coma in humans (Fischer et al., 2016).

Although the large gyrencephalic porcine brain more closely models the human brain compared with smaller mammals, they are still quadrupeds, which makes for differences in the musculoskeletal architecture of the head and neck. These differences can affect centers of mass and rotation. However, of the possible angles, rotation of the porcine head in the sagittal plane aligns most closely with the centers of mass and rotation corresponding to sagittal rotation of the human head (Cullen et al., 2016). Sagittal rotation is also ideal for modeling frontal impact in humans, common in explosive scenarios, sports impacts, or vehicular collisions. A study comparing coronal, sagittal, and axial (horizontal) acceleration at slower speeds in neonatal swine found that sagittal rotation was associated with the longest periods of unconsciousness and apnea even when compared to higher velocity axial acceleration, and brainstem pathology was only observed in the sagittal and high-speed axial groups that experienced significant periods of unconsciousness (Eucker et al., 2011). We drew upon archived tissue to look for evidence of axonal pathology in the pons after sagittal rotation injury. We observed buildup of amyloid precursor protein (APP) in swollen axons and retraction bulbs, indicating that there is significant axonal pathology in the pons of adult swine after sagittal acceleration injury (Fig. 2).

It is reasonable to hypothesize that high velocity rotational acceleration in the sagittal plane in adult swine can induce prolonged coma and emergence into a state of wakefulness without awareness. However, the survival of the unconscious swine for longer than 8 h after severe rotational injury has yet to be attempted; therefore, the needs for
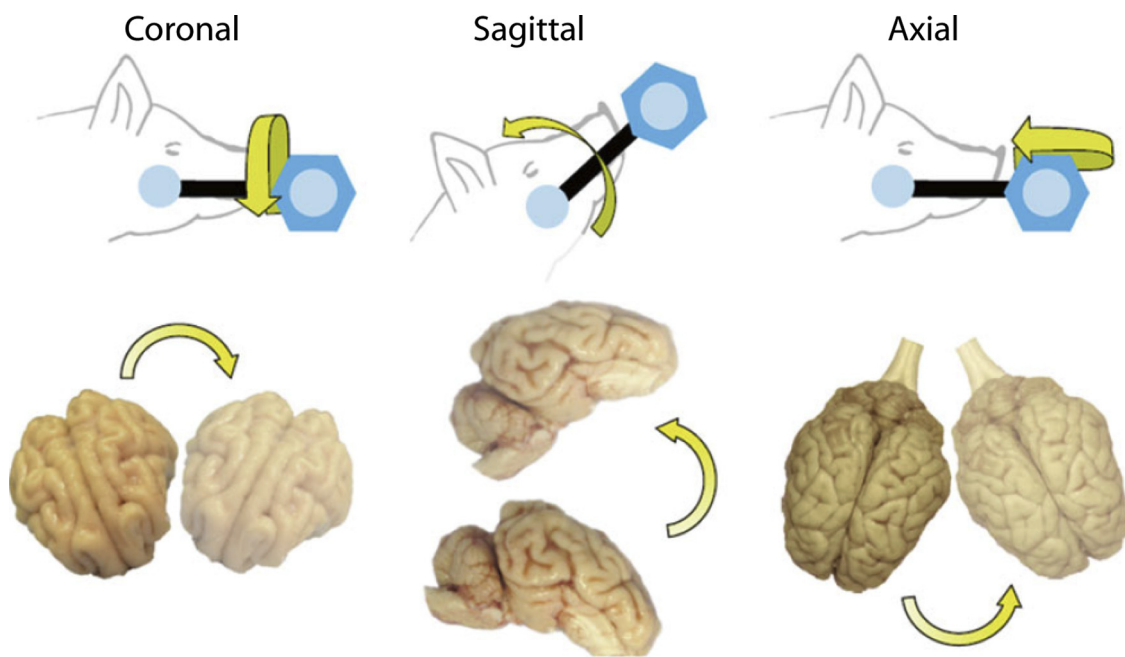
**Fig. 1.** Rotational acceleration injury in swine. Head rotational acceleration in different anatomical planes is facilitated by customized bite plates. Rotation in the sagittal or axial plane preferentially deforms the brainstem, while rotation in the coronal plane produces minimal brainstem deformation in swine. Figure adapted with permission from Cullen et al., 2016.
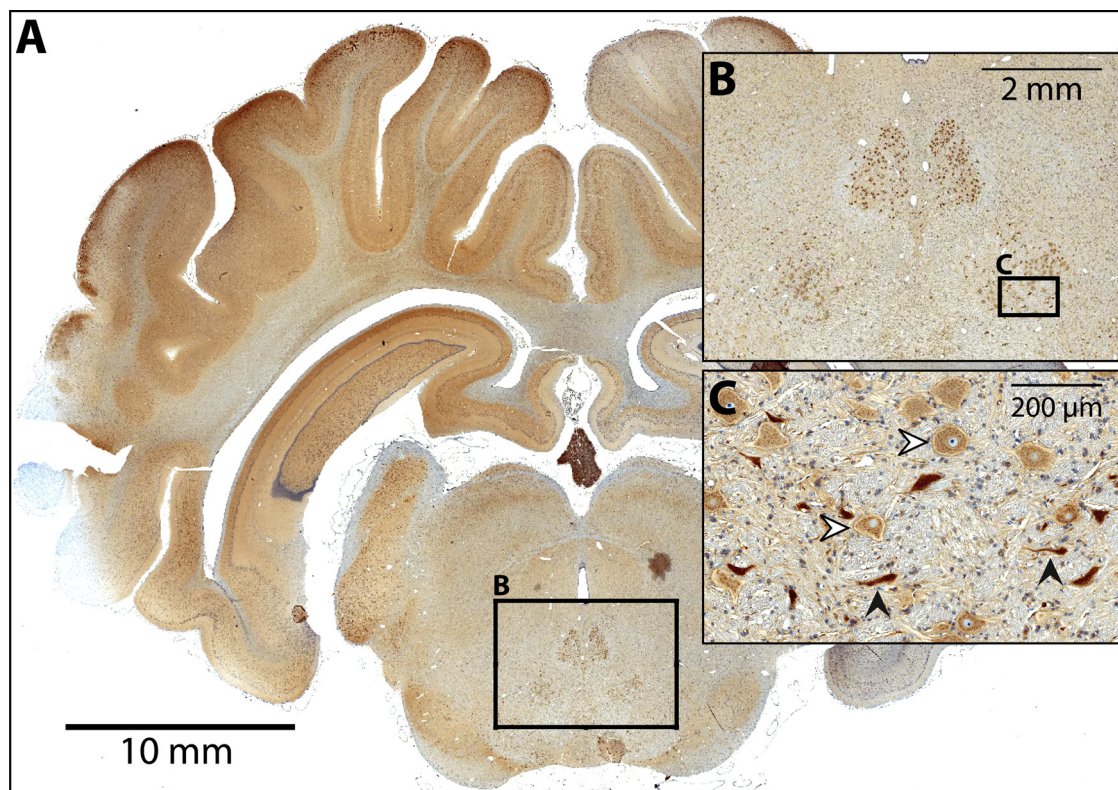


**Fig. 2.** Neuropathology in the porcine brain following rapid head rotational acceleration in the sagittal plane. Brain sections cut in the coronal plane were stained with a primary antibody for amyloid precursor protein (APP) and secondary antibody conjugated to 3,3′-Diaminobenzidine (DAB), with a hematoxylin counterstain. (A) APP accumulation – the hallmark pathological feature of diffuse axonal injury (DAI) – was evident throughout the brain in damaged axons as well as in neuronal cell bodies, generally an indication of stressed neurons. (B) Pontine lesions highlighted via APP accumulation. (C) High magnification in the pons providing examples of APP buildup in the somata of stressed cells (white arrows) and in axonal retraction bulbs (black arrows) indicative of axonal pathology.

critical clinical care are currently unknown. Moving beyond this acute time period will require the involvement of neurointensivists and veterinarians to establish protocols that meet the clinical needs of the animals and ensure that they are not exposed to any unnecessary discomfort. Here, the human neurointensive care unit should be recreated for swine, complete with continuous multimodal neuromonitoring to include cerebral blood flow, intracranial pressure (ICP), mean arterial pressure, continuous electroencephalography (EEG), cerebral microdialysis, and brain tissue oxygen content (PbO2), as well as IV fluids and respiratory support. There must also be an adaptation of sedation and analgesia protocols, as well as care algorithms for managing multiple parameters including temperature, ICP, and cerebral perfusion pressure. The investment of resources and expertise to define clinical care protocols and validate such a model is worthwhile given the potential to improve our understanding of the mechanisms and neural substrates underlying traumatic DoC, and allow for truly translational, controlled, in-depth evaluation of potential treatments to reduce damage after severe TBI and restore aspects of consciousness.

## 5. Validating pathological manifestations: circuits of interest

Correlation between coma and brainstem pathology following rotational acceleration injury in swine (Smith et al., 2000) is consistent with the hypothesis that DoC are dependent on damage to the ascending reticular activating system (ARAS). As part of the ARAS, projections from the pons (particularly the parabrachial nucleus) activate various neuronal subtypes in the basal forebrain, lateral hypothalamus, and thalamus (Fig. 3). These regions then send out their own projections propagating arousal up into the cortex, and cortical projections

provide feedback to the lower areas, including the pons (Fig. 3A). Building on this understanding, deep brain stimulation (DBS) in the central thalamus improved awareness in a chronic MCS patient (Schiff et al., 2007), leading to the development of the mesocircuit hypothesis. In short, the mesocircuit model describes a mechanism for wakefulness without awareness after injury in which loss of GABAergic tone from striatum results in increased activation of inhibitory neurons projecting from globus pallidus to central thalamus, thereby suppressing thalamic activity (Fig. 3B) (Brown and McKenna, 2015; Fridman and Schiff, 2014; Schiff, 2016).

To reiterate, wakefulness is a base state of activation/arousal in which sensory information can be gathered, and awareness is a state in which that information can be processed. The presence of detectable bottom-up EEG signals in response to sensory stimulation in the absence of top-down cortico-thalamic processing of that input is strongly indicative of wakefulness without awareness (Boly et al., 2011). In general terms, the ARAS is associated with wakefulness, and awareness is associated with connections between the ARAS and cortex as well as connections within cortex. Coma is a state without wakefulness or awareness, and emergence from coma begins with the return of wakefulness preceding the return of awareness. The spectrum of DoC encompasses patients that linger in this awake/unaware state for varying periods of time, with varying levels of awareness.

Direct damage to connections within the cortex or between cortex and ARAS (particularly thalamus) are associated with impaired awareness, but techniques required to directly test for necessary or sufficient connections cannot be ethically applied in humans. The mesocircuit hypothesis also posits the contribution of subcortical damage to circuits that normally provide disinhibition (inhibiting inhibitory
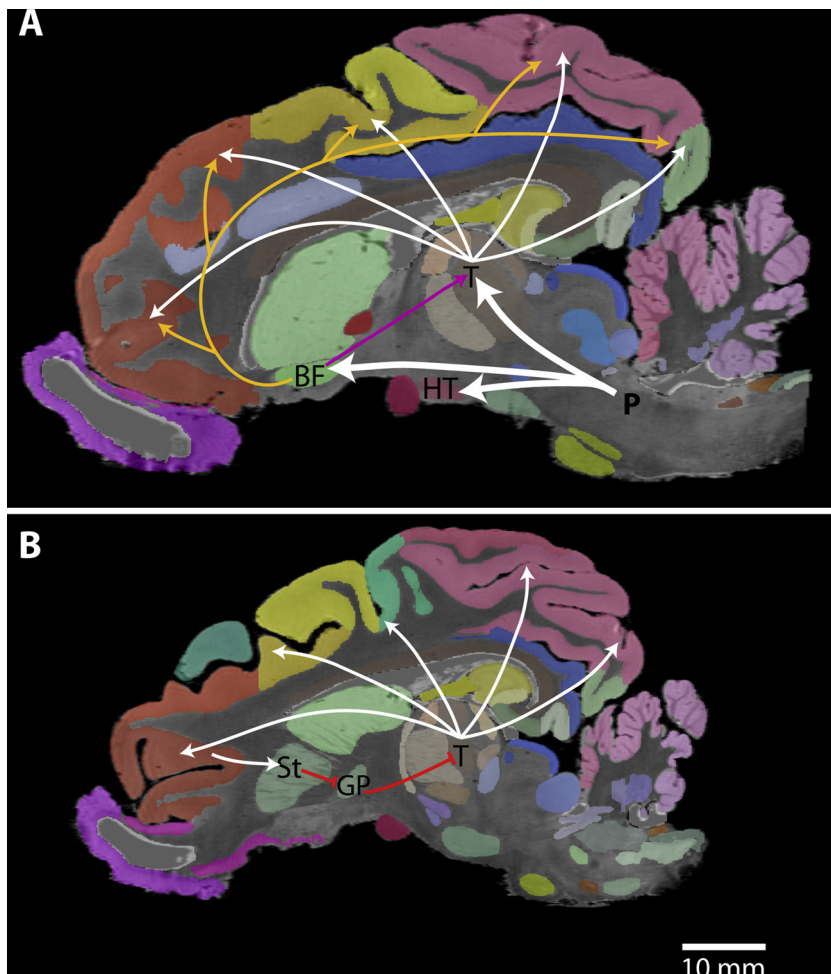


**Fig. 3.** Sagittal cross-sections of a porcine brain with simplified depictions of circuits associated with wakefulness and awareness. (A) Aspects of the Ascending Reticular Activating System (ARAS) and its cortical connections. The parabrachial nucleus of the pons (P) sends projections (thick white arrows) to the thalamus (T), basal forebrain (BF), and hypothalamus (HT). BF synchronizes activity in T via high frequency GABAergic signaling (pink arrow); BF projects mixed excitatory and modulatory afferents throughout cortex (yellow arrows). T projects excitatory afferents throughout cortex (slim white arrows). (B) Aspects of the mesocircuit model. Striatum (St) projects inhibitory afferents (flat red arrows) to Globus Pallidus (GP), which projects inhibitory afferents to Thalamus (T). Excitatory afferents (white arrows) project from T throughout cortex, and also from cortex back to St. Excitation of St inhibits GP, thereby disinhibiting T. Frames A and B are of the same scale, with a smaller brain section in B due to greater distance from midline. Mapped magnetic resonance images of swine brain are courtesy of 3D Slicer (http://www.slicer.org) (Fedorov et al., 2012; Saikali et al., 2010) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

signaling) of thalamus, resulting in over-inhibition of thalamus after injury (Brown and McKenna, 2015; Fridman and Schiff, 2014; Schiff, 2016). The GABA agonist zolpidem can improve awareness in a subset of patients suffering from DoC (Whyte et al., 2014), and it has been suggested that this seemingly paradoxical effect may arise from replacing lost inhibition of globus pallidus by striatum in the mesocircuit, thereby disinhibiting thalamus (Brown and McKenna, 2015; Fridman and Schiff, 2014; Schiff, 2016). Improved recovery from DoC in response to treatment with the dopaminergic agent amantadine has also been attributed to thalamic disinhibition, but the mechanism has not been tested directly (Schnakers and Monti, 2017).

Attempting to distinguish between circuits necessary for wakefulness or awareness can be problematic, as they are intertwined emergent properties. The lines are especially blurred when discussing the ARAS areas of basal forebrain and thalamus, due to their positions at the "highest" point of the ARAS and extensive connections with cortex and other brain regions. There is a threshold level of activation in ARAS (wakefulness) required for awareness of environment. Therefore, partial damage or incomplete recovery in the ARAS that does not reach the threshold for activating awareness could be sufficient to produce a wakeful/unaware state. This in no way detracts from the likelihood that damage to cortico-cortico or thalamo-cortico connections could be sufficient to produce a wakeful/unaware state independent of ARAS damage. However, TBI damage to these higher circuits does not typically occur without damage to ARAS, which prevents asking questions of sufficiency. Indeed, pathological analyses utilizing high angular resolution diffusion imaging suggest that damage to connections in the ARAS are more central to loss of consciousness than diffuse cortical damage, and that emergence into a wakeful/unaware state could be associated with sparing connections between brainstem and hypothalamus, which coordinates sleep/wake cycles (Edlow et al., 2013). The individual contributions of basal forebrain or thalamus and their synergistic contributions to consciousness and DoC are a continued source of debate.

Fast-firing GABAergic projections from basal forebrain to cortex and thalamus supply disinhibition and synchronization that are necessary for wakefulness and cortical activation (Brown and McKenna, 2015). In mice, specific chemogenetic activation of GABAergic neurons in basal forebrain led to extended periods of wakefulness and high frequency rhythms in cortex, while inhibiting them induced sleep (Anaclet et al., 2015). Pharmacological lesions in rat basal forebrain resulted in coma, while thalamic lesions had no detectable effect on wakefulness or cortical activity (Fuller et al., 2011). These rodent studies did not produce any instances of wakefulness without awareness (when they describe wakefulness, environmental awareness is also implied), but the circuits they investigated are associated with human DoC. While these studies suggest that a subset of neurons with somata located in thalamus are not necessary for consciousness in rats, they do not (and could not) test whether thalamic activation is sufficient to increase awareness in a wakeful/unaware state, as has been demonstrated with clinical DBS (Brown and McKenna, 2015; Fridman and Schiff, 2014; Schiff, 2016; Schiff et al., 2007). Additionally, evidence that activation of basal forebrain promotes wakefulness (and cortical activity) in rodents does not necessarily indicate that damage to that circuit is involved in eliciting DoC in humans, or that such stimulation could improve awareness in DoC patients. However, loss of fast-firing GABAergic projections from basal forebrain would desynchronize thalamic activity, thereby reducing thalamo-cortical signaling, which would generally align with the mesocircuit hypothesis that has thus far aligned with most clinical data. Furthermore, a large MRI study in DoC patients found that loss of awareness and responsiveness to sensory stimuli were associated with atrophy in thalamus and basal forebrain, respectively (Lutkenhoff et al., 2015). Neuropathology in the swine model of rotational acceleration TBI is present in the ARAS and thalamo-cortical regions associated with DoC in humans, providing additional validity for modeling manifestations of the injury. The interconnectedness of the substrates of

wakefulness and awareness presents a challenge in both clinical and preclinical studies investigating the pathological correlates of DoC. Thus far, precise lesion investigations have been performed in models that cannot recreate TBI-induced coma or DoC for comparative study. In an animal model of TBI-induced DoC, neuromodulation and pharmacological lesions could be applied in a controlled manner along with extensive post-mortem analyses to directly test the contributions of these brain regions to coma and wakefulness-without-awareness after injury.

## 6. Assessing awareness

While significant advancements have moved the study of consciousness from the realm of philosophy into more grounded scientific inquiry of neural correlates, considerable difficulties remain when modeling human aspects of consciousness in non-human animals (Boly et al., 2017; Crick and Koch, 1990; Koch et al., 2016; Odegaard et al., 2017; Storm et al., 2017). Aspects of higher consciousness such as metacognition, experience, and self-awareness are particularly challenging, since studying them in humans typically requires advanced command-following and self-reporting. However, patients in DoC cannot self-report, so when seeking to model these disorders the elusive aspects of higher consciousness are not the focus. Instead, it is necessary to evaluate awareness of environment and basic top-down processing, which can be assessed in animal models. However, the evaluation of awareness in an animal model must be based on proxies of awareness and it remains to be seen if these proxies will have meaningful correlations to the human condition.

Behavioral examination remains the gold standard for assessing awareness in humans, with the Glasgow Coma Scale (GCS) commonly applied during the acute injury period (Teasdale et al., 2014; Teasdale and Jennett, 1974), and the Coma Recovery Scale – Revised (CRS-R) applied thereafter to provide greater resolution in assessing levels of awareness (Giacino et al., 2004). In a recent talk at the 2018 Neurosciences in Intensive Care International Symposium on Coma and Consciousness, Dr. Joseph Giacino summarized the factors necessary for accurate assessment of awareness in patients with DoC (Giacino, 2018; Giacino et al., 2018, 2002), stressing the importance of six main principles: (1) identify and treat confounding factors (Whyte et al., 2013b), (2) use a validated, standardized exam like the CRS-R (American Congress of Rehabilitation Medicine, Brain Injury-Interdisciplinary Special Interest Group, Disorders of Consciousness Task Force et al., 2010), (3) maximize arousal prior to and during examination (Cortese et al., 2015), (4) perform serial examinations (Wannez et al., 2017), (5) assess multiple response systems, and (6) attempt to operationalize qualitative observations to be quantified for individualized behavioral assessment (Whyte et al., 1999; Whyte and DiPasquale, 1995). These basic principles are compatible with behavioral assessment in an animal model and must be applied. The arousal, visual, and motor scales of the CRS-R include techniques such as eye tracking and object recognition that are frequently applied in animal models in the context of TBI (Fujimoto et al., 2004; Jacotte-Simancas et al., 2014; Leigh and Zee, 2015; Sönmez et al., 2007). However, some aspects of human neurological exams rely on verbalization and language-mediated command-following, which represents perhaps the greatest challenge to adapting an exam for use in an animal model. One promising strategy for modeling language-based command-following would be to employ a classical (trace) conditioning paradigm with pre-injury training paired to autonomic responses (Bekinschtein et al., 2011; Powell et al., 2001). For example, subjects could be trained to associate a distinct auditory cue with a delayed noxious stimulus that elicits an autonomic response, and higher processing of that cue (in the absence of the noxious stimulus) could then be detected via autonomic responses such as changes in skin conductance, salivary pH, or eye blink. These autonomic responses in humans and other animals are often described as "unconscious" reactions because they do not require direct attention to

occur. However, when entrained to a separate sensory cue like a distinct tone or odor, triggering the autonomic response in the absence of the original stimulus first requires that the brain process that sensory cue for association with the original stimulus. Therefore, with pre-injury conditioning, autonomic responses to conditioned stimuli could be measured to assess the presence of top-down processing without relying on language processing or speech, and may provide a powerful surrogate for verbal command response. While human DoC patients are beyond pre-injury conditioning, there is evidence that trace conditioning can be acquired in some patients previously diagnosed into UWS (Bekinschtein et al., 2009). This phenomenon has been attributed to partially-preserved conscious processing without verbal or motor responsiveness to command — commonly referred to as covert cognition (Monti et al., 2010; Schnakers et al., 2015) — but this sort of passive processing would require further validation in a preclinical model to establish that it is truly a surrogate of awareness, and not simply a component of wakefulness. Multiple strategies to model command-following should be applied as part of the adapted neurological exam, and optimized in naïve animals prior to validation in the injury model. In terms of a validated, standardized neurological exam, a modified GCS has been in use for several decades to assess brain injury in dogs (Platt et al., 2001; Shores, 1983), and should also be applicable for swine during the acute injury period. When the CRS-R was introduced, it was validated via psychometric Rasch analysis that accounts for variability from the examiner and the examinee (Bodien et al., 2016; Giacino et al., 2004; La Porta et al., 2013; Wright and Masters, 1982), and similar techniques should be applied while validating behavioral exams to measure awareness in swine. Following psychometric validation, the adapted neurological exam can aid in validation of additional modalities for monitoring changes in awareness.

## 7. Assessing awareness: beyond behavioral examination

Despite recent advances, objective techniques for assessing surrogates of awareness such as EEG (Boly et al., 2011; Hauger et al., 2017; Schiff et al., 2014) and neuroimaging (Gosseries et al., 2016) — e.g. positron emission tomography (PET) (Heiss, 2012; Stender et al., 2015) and functional magnetic resonance imaging (fMRI) (Monti et al., 2010; Vanhaudenhuyse et al., 2010) — have not gained wide acceptance as clinical diagnostic tools in DoC patients, though the American Academy of Neurology has recently recommended that they may be employed in situations of ambiguous behavioral diagnoses (Bayne et al., 2017; Giacino et al., 2018). After adjusting the number and position of surface electrodes to accommodate the size and shape of the swine cranium, surface EEG recorded in a model of blast-induced TBI was largely in agreement with clinical observations, demonstrating feasibility for applying scalp EEG techniques in an adult swine model (Chen et al., 2017). Scalp EEG has also been applied in adult swine to develop prognostic markers for cerebral arterial gas embolism (Weenink et al., 2012), and auditory event-related potentials measured via scalp EEG in piglets informed development of a diagnostic algorithm for mild TBI (Atlan et al., 2018). EEG analysis could provide an objective surrogate of awareness in a swine model of DoC, independent of the ability to respond to commands (Estraneo et al., 2016; Schiff et al., 2014). Top-down (cortico-thalamic) signaling is associated with the explicit processing — or awareness — of stimuli after lower level (feed-forward) processing, and selective loss of top-down EEG signals is strongly associated with DoC (Boly et al., 2011). Global power analysis has also been used to differentiate between coma, UWS, and MCS, with distinctive patterns emerging across different frequency bands (Claassen et al., 2016; Schiff et al., 2014; van den Brink et al., 2018). Perhaps the most promising method for objectively assessing consciousness surrogates after brain injury relies on direct assessment of the integration and complexity of the cortex, without relying on the subject's ability to sense or interact with the environment. This is achieved by stimulating the brain with transcranial magnetic stimulation (TMS) and analyzing EEG for spatiotemporal complexity of the resultant patterns to calculate the perturbational complexity index (PCI). This technique has demonstrated efficacy in distinguishing between awake, asleep, and anesthetized human volunteers, as well as between patients at different levels of consciousness after emerging from coma (Casali et al., 2013; Casarotto et al., 2016).

Adapting and validating EEG methods to monitor awareness in swine will require adjusting for possible differences in signal characteristics in addition to electrode position and number, requiring comparison with naïve and anesthetized animals, and relying on prior psychometric validation of an adapted neurological exam. The combination of neurological examination, advanced neuroimaging, real-time EEG monitoring of global power spectra, and evaluation of top-down processing by EEG with auditory-evoked responses is compatible with large, gyrencephalic animal models, and immediately translatable to human DoC. Studying these techniques in a large, gyrencephalic animal model with low variability and direct correlation with detailed pathological output could contribute to reaching a consensus on their clinical utilization in concert with the more subjective, but widely accepted, neurological exams.

## 8. Potential role of an animal model of DoC in treatment research

As detailed in the recent state-of-the-art review from Schnakers and Monti, interventions for promoting recovery from DoC have demonstrated some efficacy in clinical trials, but there remains a need for additional investigation and more rigorous controls (Schnakers and Monti, 2017). Experimental therapies for DoC fall into two main categories of neuromodulation: electromagnetic and pharmacological. The electromagnetic approaches are intended to amplify activity in discrete brain regions to accommodate for diminished pathways, and techniques include DBS in the central thalamus (Chudy et al., 2017; Magrassi et al., 2016; Schiff et al., 2007; Yamamoto et al., 2010), transcranial direct current stimulation (tDCS) (Angelakis et al., 2014; Estraneo et al., 2017; Thibaut et al., 2017, 2015, 2014), transcranial magnetic stimulation (TMS) (Casali et al., 2013; Formaggio et al., 2016; Gosseries et al., 2015; Piccione et al., 2011; Xia et al., 2017), and vagus nerve stimulation (Corazzol et al., 2017). Low intensity focused ultrasound (LIFUP) has emerged as a promising noninvasive alternative to DBS, relying on sonic mechanical perturbation in discrete areas to modulate neuronal activity (Monti et al., 2016). Pharmacological candidates include the GABA agonist zolpidem (Sutton and Clauss, 2017; Whyte et al., 2014) and the dopaminergic agent amantadine (Giacino et al., 2012; Horiguchi et al., 1990; Lehnerer et al., 2017; Meythaler et al., 2002), both of which have been speculated to act via disinhibition of thalamus. Studying the effects of these drugs in an animal model of DoC would allow for a detailed mechanistic investigation that could improve treatment strategies and identify novel therapeutic targets. While some studies of tDCS, zolpidem, and amantadine have attempted to control for spontaneous recovery, most clinical trials do not (Schnakers and Monti, 2017). Significant spontaneous recovery can occur years into prolonged traumatic DoC, and functional recovery has recently been found to take place between 5 to 10 years after injury (Giacino et al., 2016; Hammond et al., 2018; Whyte et al., 2013a). Furthermore, differences in genetics, age, pre-existing medical conditions, injury severity, anatomical injury location(s), comorbidities, acute clinical care, and chronic clinical care (among other differences in the clinical population) contribute to the variability of spontaneous recovery and response to treatment. A large animal model of traumatic DoC would provide the negative controls necessary to account for spontaneous recovery rates, and would also control for the factors contributing to the variability of spontaneous recovery and response to treatment. Controlled design, reduced variability, increased data output via maximized and standardized multimodal neuromonitoring, and availability of extensive, in-depth post-mortem analyses in models similar to human pathology will significantly expand our understanding of existing

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J.C. O'Donnell et al.                                                                                                    Neuroscience and Biobehavioral Reviews xxx (xxxx) xxx–xxx

| Major Challenges | Possible Solutions |
|---|---|
| **1. Reproducing Human TBI Mechanisms** | **1. Rotational Acceleration TBI in Swine** |
| a. (Large brain mass) x (Rotational acceleration) = Damaging forces | a. Sufficient brain mass to achieve forces via scaled-up acceleration |
| b. Neuroanatomy dictates patterns of neuropathology | b. Gyrencephalic cortex; high white-to-gray matter ratio |
| **2. Reproducing Human TBI Manifestations** | **2. A Neuro ICU for Swine** |
| a. Prolonged loss of consciousness is not associated with the ubiquitous rodent contusion models of TBI | a. Prolonged loss of consciousness is observed in the swine model with rotational acceleration |
| b. Severe brain injuries are survivable in humans due to advanced neuromonitoring and clinical care | b. Invest expertise and resources to apply protocols from the human neuro ICU to the swine model to extend survival |
| **3. Distinguishing between Wakefulness and Awareness** | **3. Replicate, Modify, or Create Surrogate Measures** |
| a. Gold-standard exams are the GCS and CRS-R | a. Utilize the veterinary Modified-GCS and adapt/validate the CRS-R |
| b. Response to verbal commands is a component of human exams | b. Utilize pre-injury trace conditioning with autonomic responses as surrogate for response to verbal commands |
| c. No concensus for clinical use of objective measures like EEG | c. Take advantage of the power of the preclinical model to evaluate objective techniques, beginning with EEG approaches |

**Fig. 4.** Summary of Major Challenges and Possible Solutions.

treatments for promoting increased awareness.

Preclinical studies can improve our ability to detect the effects and determine the mechanisms of experimental treatments that have been attempted clinically, and can also provide a platform to develop new technologies to facilitate repair and recovery after TBI. While there is still work to be done to identify necessary or sufficient circuits, DoC are widely believed to exist as a result of a loss of connections between brain areas. As the mammalian brain develops and grows, the mechanical forces of the brain's growth gradually stretch small young neurons to form the long axon tracts in the developed brain (Bray, 1984; Franze, 2013; O'Toole et al., 2008; Zheng et al., 1991). Regrowing lost axons between disconnected brain regions after rotational acceleration injury is stymied by the post-injury environment (e.g. inflammation, glial scarring) and the fact that the brain is already full size with established distances between nuclei, and therefore the mechanical stretch forces from the surrounding tissue that contributed to creating long axonal tracts no longer exist. While they are years away from clinical implementation, there are technologies under development to replace and repair these lost connections. For instance, efforts are under way to create tissue-engineered brain pathways that consist of aligned bundles of axons projecting from phenotypically-constrained neuronal populations (Cullen et al., 2012; Harris et al., 2016; Struzyna et al., 2014, 2015b, 2015a, 2017, 2018). These anatomically-inspired constructs may then be stereotaxically implanted to replace discrete pathways in the brain, and may also potentially promote regeneration via axon-mediated axonal growth. Additionally, recently developed "living electrodes" consisting of microtissue engineered aligned axonal bundles can be electrically or optogenetically activated to excite or inhibit discrete brain structures based on the neuronal cell type used for fabrication, offering significant advantages over current methods for DBS (Adewole et al., 2016; Serruya et al., 2017). With the goal of restoring lost connections between brain areas, these early-stage technologies represent an entirely new therapeutic approach to complement the pharmacological and electromagnetic approaches currently under investigation for treating DoC. A large animal model of traumatic DoC will provide the necessary platform to develop these nascent technologies, and could eventually facilitate translation into clinical use.

## 9. Conclusions and a path forward

Given that the swine model of rotational acceleration TBI can effectively produce extended periods of unconsciousness, the challenges to modeling TBI-induced DoC are not insurmountable (as summarized in Fig. 4). Efforts to expand this swine model of severe TBI beyond the acute phase should utilize interventions similar to those following severe trauma in humans, including prophylactic pain management, mechanical respiration, continuous neuromonitoring, and standards of intervention to manage factors such as intracranial hypertension. In addition to neurological examination and EEG (continuous and evoked), such a model could provide extensive pre and post injury data including clinical monitoring measures (e.g. intracranial pressure, brain tissue oxygenation, cerebral perfusion pressure and flow, cerebral autoregulation, heart rate, $SpO_2$, end-tidal $CO_2$), regular microdialysis and blood sampling for biomarker discovery and pharmacodynamic analysis of drugs, high-resolution diffusion tensor imaging, gross pathology, immunostaining, and biochemical analyses. This model would flourish with cross-pollination from the clinical DoC research community to adapt their techniques, ask relevant questions, and interpret results. Establishing and characterizing a preclinical model for TBI-induced DoC — while challenging — will open these disorders to basic science investigation, provide inclusion criteria for clinical trials, and create a pathway for developing new diagnostics and therapeutics.

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