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Research Paper

Establishing the ferret as a gyrencephalic animal model of traumatic brain injury: Optimization of controlled cortical impact procedures

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HIGHLIGHTS

- Subject-specific MRI-guided coordinates overcame anatomical variability.
- The temporalis muscle should be detached from the midline and reflected.
- The craniotomy should be closed to avoid the temporalis muscle pushing on the brain.
- This optimized surgical procedure created scaled injury in a reproducible location.
- Behavior and astrocyte/microglial responses were scaled to injury severity.

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ABSTRACT

Background: Although rodent TBI studies provide valuable information regarding the effects of injury and recovery, an animal model with neuroanatomical characteristics closer to humans may provide a more meaningful basis for clinical translation. The ferret has a high white/gray matter ratio, gyrencephalic neocortex, and ventral hippocampal location. Furthermore, ferrets are amenable to behavioral training, have a body size compatible with pre-clinical MRI, and are cost-effective.

New methods: We optimized the surgical procedure for controlled cortical impact (CCI) using 9 adult male ferrets. We used subject-specific brain/skull morphometric data from anatomical MRIs to overcome across-subject variability for lesion placement. We also reflected the temporalis muscle, closed the craniotomy, and used antibiotics. We then gathered MRI, behavioral, and immunohistochemical data from 6 additional animals using the optimized surgical protocol: 1 control, 3 mild, and 1 severely injured animals (surviving one week) and 1 moderately injured animal surviving sixteen weeks.

Results: The optimized surgical protocol resulted in consistent injury placement. Astrocytic reactivity increased with injury severity showing progressively greater numbers of astrocytes within the white matter. The density and morphological changes of microglia amplified with injury severity or time after injury. Motor and cognitive impairments scaled with injury severity.

Comparison with existing method(s): The optimized surgical methods differ from those used in the rodent, and are integral to success using a ferret model.

Conclusions: We optimized ferret CCI surgery for consistent injury placement. The ferret is an excellent animal model to investigate pathophysiological and behavioral changes associated with TBI.

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Abbreviations: CCI, controlled cortical impact; DPI, days post injury; GFAP, glial fibrillary acidic protein; HPI, hours post injury; IBA-1, ionized calcium-binding adapter molecule 1; MRI, magnetic resonance imaging; NOR, novel object recognition; PBS, phosphate-buffered saline; ROI, region of interest; TBI, traumatic brain injury; WPI, weeks post injury.

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1. Introduction

Each year an estimated 1.7 million people sustain a traumatic brain injury (TBI) (Faul et al., 2010). Many of those affected are young and the resulting cognitive, physical, and behavioral impairments impede functioning in work and leisure activities for many years (Faul et al., 2010; Ponsford et al., 2014).

Mice and rats have been used for decades to study basic mechanisms of pathophysiology following TBI and to develop therapeutics. The translation of basic research findings into beneficial clinical outcomes in TBI patients however, is challenging (Morales et al., 2005; Statler et al., 2001; Stein 2015; Xiong et al., 2013). One obstacle may be the structural differences between the brains of rodents and humans (Defelipe, 2011; Duhaime, 2006; Gennarelli, 1994; Johnson et al., 2015; Mychasiuk et al., 2016). The human cortex contains extensive sulci and gyri and substantial white matter, whereas the rodent brain is lissencephalic with a low volume of white matter. Since TBI produces damage that reflects cranial and cerebral geometry, and often affects white matter in humans, this is a significant distinction (Johnson et al., 2013). The location of the rodent hippocampus differs as well, as it is situated in a superior position making it susceptible to specific types of experimental brain injury, whereas the ferret hippocampus is located primarily in the temporal lobe, as it is in the human.

A number of larger animals have brains with features similar to humans such sulci and gyri in the cerebral cortex and large amounts of white matter. Swine, sheep, and non-human primates are used to study TBI, but not as frequently as rodents because they present complications related to housing, cost, and/or ethical concerns. In addition, they require greater monitoring during the injury and related surgery. Conducting an MRI requires using human equipment due to their larger size; immunohistochemical markers established in rodents require further refinement to be effective, and behavioral tests have not been fully developed and standardized (Manley et al., 2006). Nevertheless, a translationally relevant animal model would be extremely valuable for studying the complex pathogenesis of TBI. In order to address this need, we propose that the ferret (*Mustela putorius furo*), as a small, gyrencephalic mammal, may be a more relevant and well-suited model to study TBI.

The ferret is used in numerous fields of anatomic and physiologic research because it possesses many features that are similar to humans. Examples include immune and respiratory systems, auditory systems, cerebrovascular research, endocrine system, and gastric anatomy (Atkinson et al., 1989; Bakthavatchalu et al., 2016; Cabot and Fox, 1990; Gold et al., 2015; Oh and Hurt, 2016). Ferrets are advantageous for many practical reasons such as a comparatively low cost, sociability to allow group housing thereby minimizing space needs, and body dimensions with a slender torso that allows use of specialized pre-clinical MRI scanners for *in vivo* imaging. Ferrets are also amenable to many types of behavioral testing (e.g., Christensson and Garwicz, 2005; Gold et al., 2015; Haddad et al., 1976; Rabe et al., 1985; Zhou et al., 2016). For a full review of various issues to consider when choosing the ferret as a laboratory animal see Ball (2006). The recent publication of the ferret genome increases the usefulness of this animal and demonstrates that less genetic divergence occurs between humans and ferrets than between humans and mice (Peng et al., 2014).

The ferret was one of the first animals used to develop the controlled cortical impact (CCI) technique for TBI research (Lighthall, 1988; Lighthall et al., 1990; see Osier and Dixon, 2016 for a review). The CCI technique uses a pneumatically or electromagnetically driven metal rod to directly strike the surgically exposed dura, causing brain injury. The benefit of this model is the level of control of the impact – velocity, impact depth, angle, and duration. In the ferret, the impactor strikes a gyrus, and the biomechanical forces would likely be transmitted throughout the brain in a similar way as in the human brain, which also has sulci and gyri. The increased amount of white matter in the ferret compared to the rodent increases the likelihood of diffuse axonal injury. While the CCI technique was used initially with the ferret, the resulting pathology and functional impairments were not fully characterized at that time and the ferret has not been used for CCI study since. Several current brain injury studies using ferrets investigate the effect of blast, primarily to examine the threshold for intracranial hemorrhage, cardiorespiratory instability and overall survival risk assessment but did not study the specific effects on the brain (Rafaels et al., 2012; Rafaels et al., 2016). The ferret is also being used in brain imaging studies (Feng et al., 2013; Sawada et al., 2013). The ferret is becoming recognized as a model to investigate multiple aspects of brain function and the effects of injury. Recently, the ferret was proposed to study perinatal brain injury (Empie et al., 2015). The information presented here provides evidence that ferrets are important and highly useful to characterize neuropathological changes following brain injury.

Our goal was to optimize surgical procedures to perform controlled cortical impact in the ferret. We also provide preliminary evidence that the injury characteristics and consequent functional impairment exhibit elements similar to the human condition through imaging, histology, and behavioral testing in animals at short and long term time-points.

2. Materials and methods

2.1. Animals and housing

All animal procedures were conducted with the approval of the Uniformed Services University of Health Sciences Institutional Animal Care and Use Committee and in accordance with the animal care guidelines issued by the National Institutes of Health. Adult male ferrets (*Mustela putorius furo*), 5–9 months of age, weighing 1.3–2.1 kg, were purchased from Marshall Bioresources (North Rose, NY, USA) and housed 2 per cage prior to surgery (modified rabbit cage by Lenderking Caging Products, Millersville, MD) and 1 ferret per cage after surgery. Ferrets had *ad libitum* access to food and water before and during experimental procedures and were on a 12-h light/12-h dark cycle with the room temperature range of 61–72 °F (16–22 °C) and humidity of 30–70%. All animals survived for 1 week after the injury except 1 animal that survived 16 weeks. A total of 15 ferrets were used: nine to optimize the surgery, five additional animals using the optimized surgical protocol with varying severities of injury, and one control animal. The naïve control did not receive anesthesia and was employed for comparison with MRI, immunohistochemistry and behavioral results (see Table 1). Also note that the ferrets used for optimizing the surgery had a range of injuries from mild to severe.

2.2. Navigation to the target brain site

The target brain site was the primary somatosensory cortex of the left hemisphere (McLaughlin et al., 1998). This region can be identified as the dimple in the posterior sigmoid gyrus, located between the coronal and cruciate sulci and anterior to the ansinate sulcus (see Fig. 1A). We used a skull landmark previously identified by Lawes and Andrews (1987) to navigate to the target brain site. This landmark is the junction of the supraorbital crests, which we will refer to as the anterior landmark (Fig. 1B). In the animals studied earlier in this set of experiments, the anterior landmark was identified visually during surgery and pre-determined coordinates measured on the skull to determine the craniotomy site. In the later studied animals, a baseline structural MRI identified the anterior