An open-head traumatic brain injury model in adult zebrafish (*Danio rerio*)

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Traumatic brain injury (TBI) is the leading cause of death in children ages (≤ 19 years old), yet very little is known of how the brains recovers from such an event. We created a new model of open-head TBI in zebrafish (*Danio rerio*) to investigate genetic changes that may occur following brain injury. Following TBI, recovering zebrafish immediately displayed an altered swimming pattern, swimming in circles or a spiral torpedo pattern. TBI zebrafish had a 70% survival rate and began to swim normally within 3 to 7 days post brain injury, suggesting recovery from the injury. Future use of the TBI model in zebrafish will allow us to investigate how specific genes and gene regulators may help the brain recover from a brain injury.

Traumatic Brain Injury (TBI) is the number one cause of death and disability in children and is defined as a head injury with symptoms ranging from a mild concussion to a complete lack of responsiveness, as detailed by the Glascow coma scale[1]. Currently, there is a paucity of knowledge regarding how the brain responds to cell damage and repair after a traumatic injury, due to a limited number of model systems available to study TBI. Our goal is to establish a new model of TBI in a genetically tractable vertebrate model system that will provide insights toward comprehending the genetic regulatory circuits that promote brain repair and recovery.

Zebrafish is an excellent vertebrate model system for understanding cellular and genetic processes of organ repair and regeneration[2]. The most critical aspects of zebrafish as a model system are the numerous available genetic resources for gene manipulation and remarkable capacity for regeneration of multiple organ systems, including the CNS[3,4]. Zebrafish also have a short life cycle, simple husbandry and a highly conserved genome with mammals. Given the high degree of genome conservation between zebrafish and mammals, fundamental knowledge of TBI in zebrafish can provide valuable insight in shaping future therapeutic treatment in humans. With these advantages, establishing a TBI model in zebrafish would allow for unprecedented insight into the biology of brain injury and recovery, and the genetic circuits that define a CNS regenerative response.

The adult zebrafish brain structure and specific locations of neurons responsible for swimming behaviors are well characterized[5]. We used this information to create a TBI model in the adult zebrafish that produced a distinct and reproducible behavioral change with injury.

To induce an acute injury to the brain, anesthetized (2-phenoxyethanol, 1:1000) adult zebrafish were secured in an agarose mold. A deep puncture injury was produced in the left brainstem and midbrain by inserting a blunted sterilized syringe needle (26 3/8 gauge, 0.2 mm) through the foramen magnum at the base of the skull, as illustrated by the gray shaded area in Figure 1A (modified from Rupp *et al.*[5]). Fish were allowed to recover in water and resuscitated via mechanical water dispersal over their gills. This injury disrupts brain tissue on the left side of the brain, as

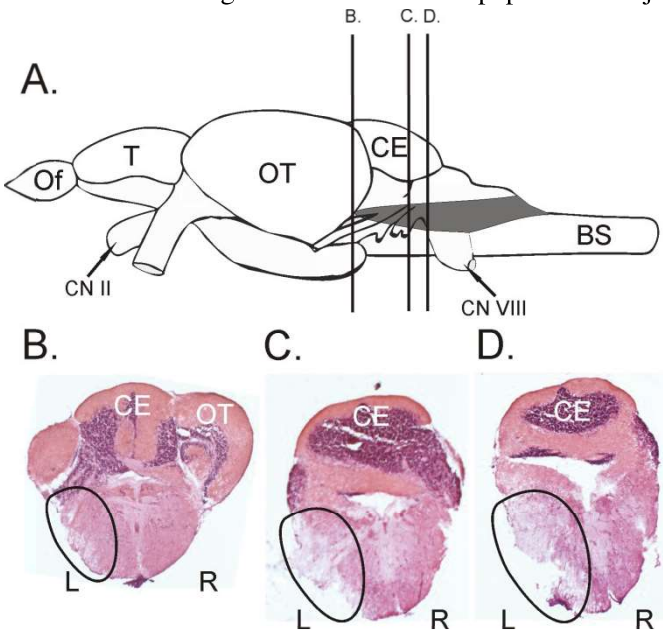

Figure 1. A new TBI model in zebrafish. (**A**) A schematic of the adult zebrafish brain, depicting the injury site (gray-shaded area) and planes of cryosections (b'-d'). (rostral=left; dorsal=top). (**B-D**) Hematoxylin and eosin stained brain tissue sections highlighting the injured site (circle). At the level of the cerebellum and midbrain structures the puncture wound disrupts brain tissue only on the left side; where the blunted syringe entered the tissue (C, D). (BS=brain stem, CE= cerebellum, CN II=cranial nerve II (optic nerve), CN VIII=cranial nerve VIII (vestibulocochlear nerve), Of=olfactory bulb, OT=optic tectum, T=telencephalon).

highlighted by a circle in the hematoxylin and eosin stained brain tissue sections (Fig 1B-1D). Our preliminary analysis indicates the medulla and midbrain, cranial nerve nuclei ten, eight, and seven and lateral lemniscus were all impacted by this wounding protocol. Although this acute model of TBI is an aggressive wounding regimen, there was ~70% survival rate. Specifically, TBI was performed on 44 adult zebrafish; of these, 13 fish did not survive past 24 hours. In order to control for length of anesthetic and fish manipulation stress, 25 zebrafish were anesthetized for the same duration as TBI zebrafish, and a small incision was made perpendicular to the midline at the base of the posterior cranial plate (sham control). The survival rate for sham zebrafish was 84% at 24 hours.

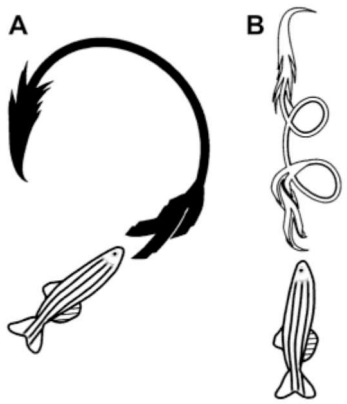

Figure 2. Swimming patterns following TBI. (A) After acute injury, ~70% of recovering TBI zebrafish swam in a counterclockwise circle. (B) Conversely, 30% of injured zebrafish swam in a corkscrew like fashion.

The lateral lemniscus is a collection of axons in the brainstem that is thought to contribute to swimming behavior. Thus, we predicted that injured fish would exhibit an altered swimming pattern. We found that all surviving injured fish exhibited varying degrees of defects in swimming coordination. Approximately 70% (n = 31) of TBI fish swam in a circular, counter-clockwise pattern, turning toward the side of the injury while 30% (n = 13) of the fish swam in a spinning, corkscrew, clockwise fashion turning away from the side of the injury (Fig 2). Upon recovery, control zebrafish all swam normally. Remarkably, surviving TBI zebrafish showed improved or achieved normal swimming behavior at 3 to 7 days post-injury, suggesting rapid recovery from an acute injury.

In summation, our new TBI model in zebrafish establishes the foundation for future studies to elucidate the cellular and genetic bases of brain recovery and regeneration from an acute injury. One avenue of future investigation is to examine gene expression following acute adult zebrafish brain injury. In humans gene expression is altered by severe and mild TBI and can be detected in plasma[6]. Using both open and closed head injury models in adult zebrafish we will explore gene expression changes that are associated with specific brain tissues, *i.e.,* neurons, astrocytes and blood vessels) and specific brain regions (such as hippocampus and the cerebellum). There are several candidate microRNAs described from other TBI models that we will investigate in our adult zebrafish model. We will explore microRNA gene expression changes over time following TBI. Furthermore, we will seek to correlate behavioral changes with neuronal regeneration and gene expression.

This research was supported by Maine IDeA Network of Biomedical Research Excellence (P20-RR016463).

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