Development of an experimental model of traumatic brain injury using organotypic hippocampal slice cultures

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INTRODUCTION

Traumatic brain injury (TBI) is currently one of the leading causes of global cognitive disorder and is mainly caused by falls, traffic accidents and sport contacts (*Victoria E. Johnson, 2013*). Therefore, the comprehension of the pathophysiological mechanisms is a major challenge. Development of robust *in vitro* models is important for the understanding and apprehension of the molecular and cellular mechanisms of this pathology.

OBJECTIVES

The aim of this work is the development of a relevant model of an indirect traumatic brain injury using organotypic hippocampal slice cultures. The goal was to characterize the injury in terms of cell death, either for necrosis and apoptosis. Then, some characteristics of mild TBI hallmarks were explored to validate this model. Three groups were created: control, sham (handled through the stages of shock protocol but not shocked) and shocked group.

MATERIAL AND METHODS

A. Organotypic hippocampal slice cultures

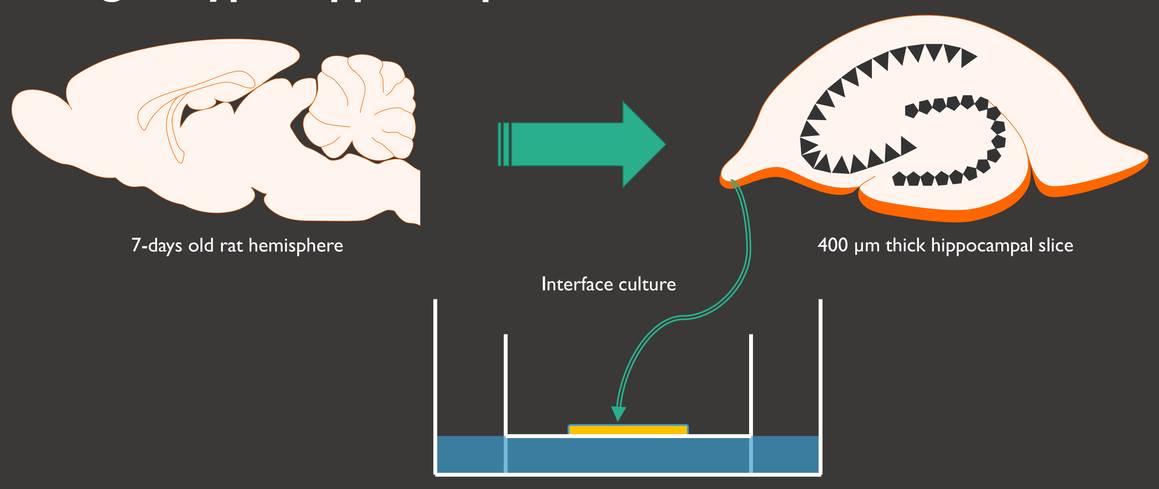


Figure 1. Diagram showing the preparation of organotypic hippocampal slice cultures. 7 days-old rat hemispheres are dissected and both hippocampi isolated. Hippocampi are cut into 400 µm thick slices which are laid onto the membrane of a culture insert. The latter is placed into a culture well previously filled with warm culture medium. This culture technique is called "interface method".

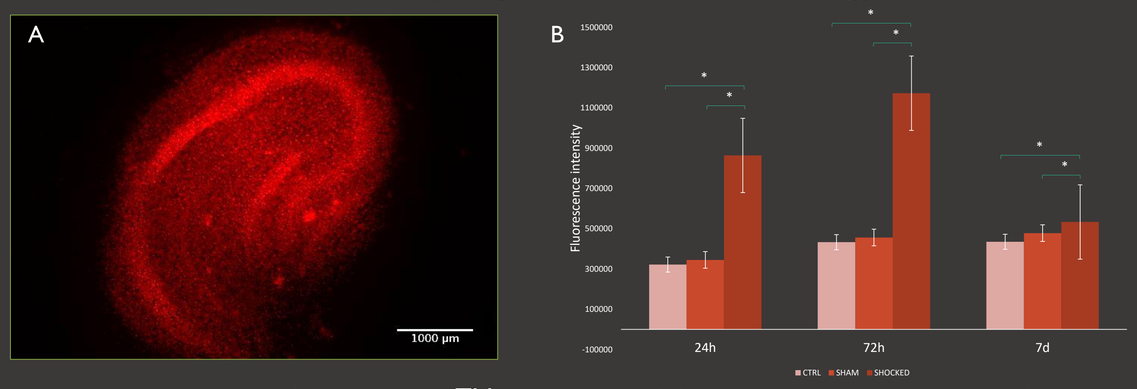
B. Model of TBI

Figure 2. Hand-crafted « coilgun » inertia-driven model used to accelerate the 6-well module to 12,5 m/s reproducing an acceleration/deceleration injury. The module is in plexiglass to reduce friction forces as well as to reduce weight. Coilgun is made of a copper coil crossed by a 220V current previously loaded in 6 capacitors. The strong current crossing the coil generates a powerful magnetic field pushing the piston which accelerates the module on the track.

RESULTS

A. Cell death assessment

Necrosis (Propidium iodide labelling)



Apoptosis (CellEvent™ Caspase 3/7 Detection Reagent)

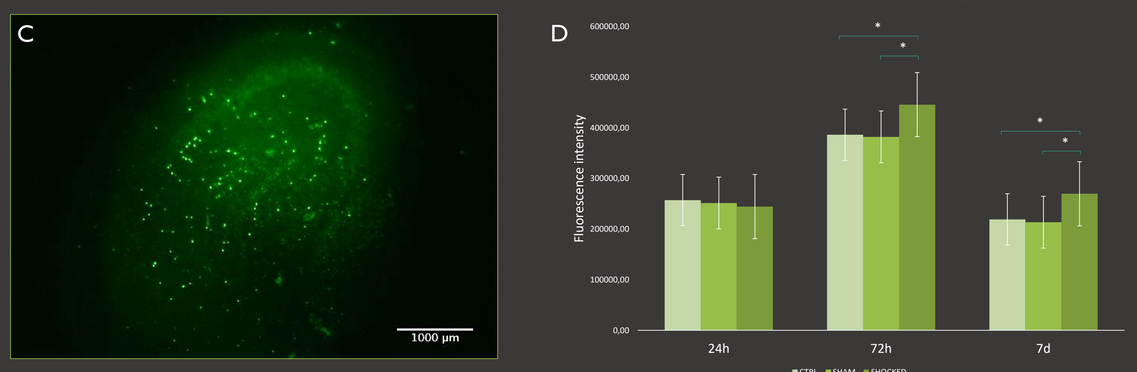


Figure 3. Live imaging of cell death assessment showing (A) necrosis with Propidium Iodide labelling and (C) apoptosis with CellEvent Caspases 3/7 green fluorescence assay. (B) Analysis showed a significant increase in necrosis after 24h and 72h compared to control and sham groups. (D) Analysis showed a significant increase in apoptosis after 24h and 72h compared to control and sham groups. No differences were found between control and sham groups. Statistics were performed using Two-Way Repeated Measure ANOVA followed by a Holm-Sidak groups comparison test. "*" = p<0,05.

B. Fluorescent immunohistochemistry

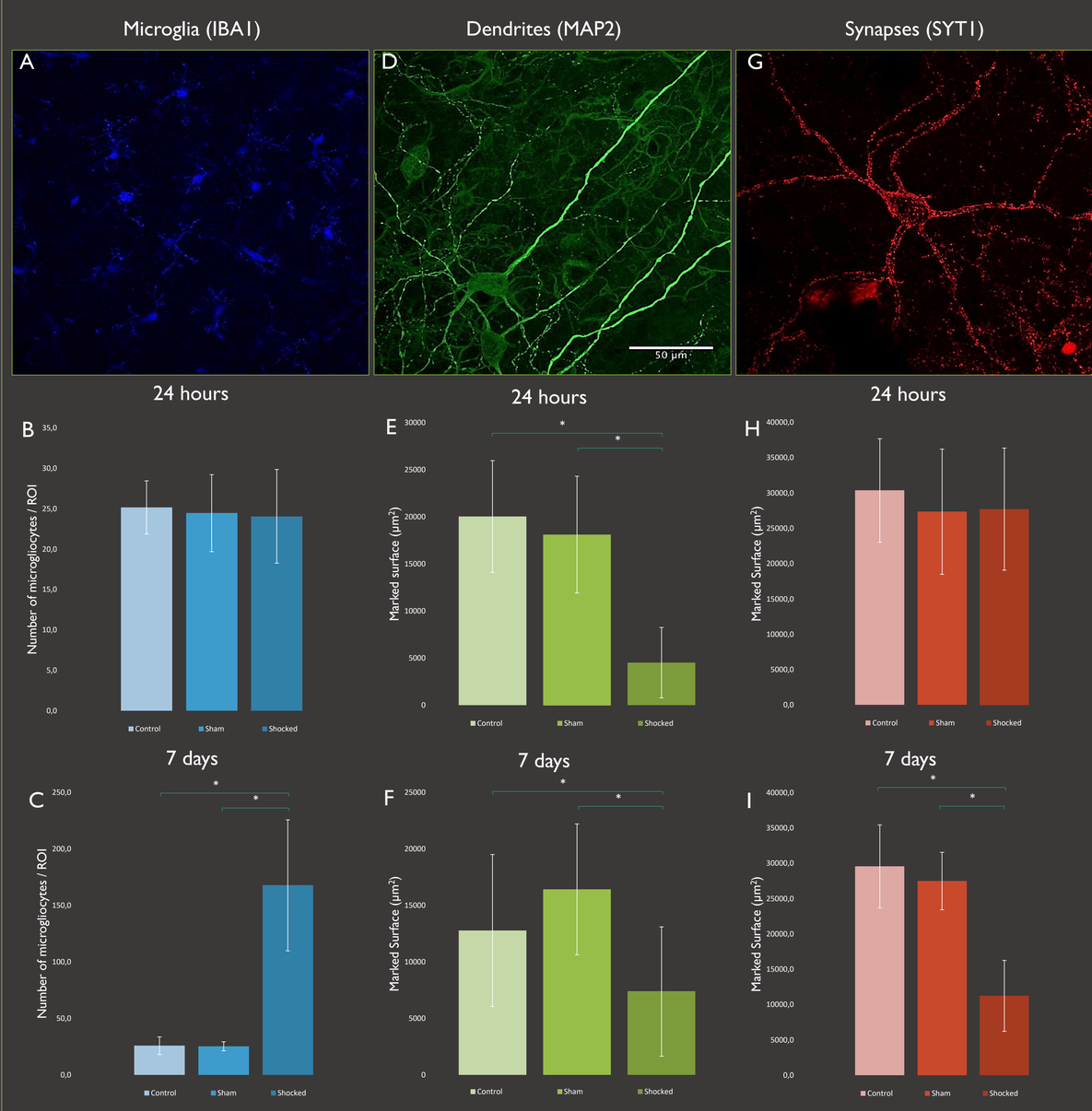


Figure 4. Fluorescent immunohistochemistry targeting (A) IBA1 for activated microglia, (D) MAP2 for dendrites and (G) SYT1 for presynaptic endings. (B) Analysis showed no differences between groups at 24h but (C) a significant increase of the number of activated microgliocytes at 7 days post-injury compared to control and sham groups. (E) Analysis showed a straight diminution of dendrites surface within the shocked slices after 24h compared to control and sham groups (F) which remained at 7 days post-injury. (H) Analysis showed no differences between the groups at 24h but (I) a significant decrease in the presynaptic endings surface in the shocked slices compared to control and sham groups at 7 days post-injury. Statistics were performed using Two-Way ANOVA followed by a Holm-Sidak groups comparison test. "*" = p<0,05.

PERSPECTIVES

These results allow us to validate the model as a relevant traumatic brain injury model which offers the ease of an *in vitro* platform with the tissue complexity of the organotypic cultures. In this context, diffuse axonal injury, clinical hallmark of traumatic brain injury, could be studied in a dynamic and interactive cellular environment.