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# Role of microglia in a mouse model of paediatric traumatic brain injury

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ABSTRACT

The cognitive and behavioural deficits caused by traumatic brain injury (TBI) to the immature brain are more severe and persistent than TBI in the mature brain. Understanding this developmental sensitivity is critical as children under four years of age sustain TBI more frequently than any other age group. Microglia (MG), resident immune cells of the brain that mediate neuroinflammation, are activated following TBI in the immature brain. However, the type and temporal profile of this activation and the consequences of altering it are still largely unknown.

In a mouse model of closed head weight drop paediatric brain trauma, we characterized i) the temporal course of total cortical neuroinflammation and the phenotype of *ex vivo* isolated CD11B-positive microglia/macrophage (MG/MΦ) using a battery of 32 markers, and ii) neuropathological outcome 1 and 5 days post-injury. We also assessed the effects of targeting MG/MΦ activation directly, using minocycline a prototypical microglial activation antagonist, on these processes and outcome.

TBI induced a moderate increase in both pro- and anti-inflammatory cytokines/chemokines in the ipsilateral hemisphere. Isolated cortical MG/MΦ expressed increased levels of markers of endogenous reparatory/regenerative and immunomodulatory phenotypes compared with shams. Blocking MG/MΦ activation with minocycline at the time of injury and 1 and 2 days post-injury had only transient protective effects, reducing ventricular dilatation and cell death 1 day post-injury but having no effect on injury severity at 5 days.

This study demonstrates that, unlike in adults, the role of MG/MΦ in injury mechanisms following TBI in the immature brain may not be negative. An improved understanding of MG/MΦ function in paediatric TBI could support translational efforts to design therapeutic interventions.



## 1. Introduction

Traumatic brain injury (TBI) is the most common injury leading to significant lifelong disability that occurs in children (Stanley et al., 2012). Unfortunately, the cognitive and behavioural deficits caused by traumatic brain injury (TBI) to the immature brain are more severe and persistent than those observed following comparable injuries to the mature (adult) brain (Anderson et al., 2005; Ewing-Cobbs et al., 2006; Hessen et al., 2007; Rivara et al., 2012) (reviewed in (Giza et al., 2007)) with injury in an experimental setting progressing into a chronic brain disorder (Ajao et al., 2012; Kamper et al., 2013). This is in contrast to Kennard's Principle that the immature brain has superior potential for repair (Bennet et al., 2013). This is of particular concern as children under the age of

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four years sustain TBI more frequently than any other age group (Koepsell et al., 2011) and in children under the age of 2 years, the rates of TBI serious enough to require intensive care support are as high as 50 per 100,000 (Keenan et al., 2003). A developmental sensitivity to TBI as seen in humans is also observed in a rodent model of TBI, where within the first 30 days of life, injury is maximal when TBI is caused at postnatal day 7 (P7) (Bittigau et al., 1999). In addition, during the first three postnatal weeks, rodents display a heightened sensitivity to excitotoxicity (Ikonomidou et al., 1999). In mouse and humans this period is when developmental processes such as maximal brain growth, synaptogenesis and myelination occur.

In the paediatric population, TBI is caused by injuries and insults, which include acceleration/deceleration injuries (shaken baby syndrome) and contusion injuries (direct skull impact) (Pinto et al., 2012). Contusion injuries are the prevailing form of non-inflicted injuries and also represent a large proportion of inflicted injuries (Pinto et al., 2012). The primary injury process in TBI is mechanical damage (i.e. shear forces inducing vascular damage and bleeding), followed immediately by mast cell degranulation (Stokely and Orr, 2008), and secondary pathological processes, including excitotoxicity, ischemia, mitochondrial dysfunction, activation of matrix metalloproteinases (MMPs) and activation of caspases leading to apoptosis (Xiong et al., 2013). These secondary injury processes induce neuroinflammation, which itself has the potential to be neurotoxic (Hagberg et al., 2012), but which is poorly understood in the immature brain following TBI.

Microglia (MG) are the central regulators of neuroinflammation, involved in the pathological processes of the majority of acute and chronic brain injuries, such as stroke, Alzheimer's disease and multiple sclerosis (for review see (Prinz et al., 2011)). Thus MG are logical candidates to mediate neuropathological changes following TBI in the immature brain. MG possess enormous functional plasticity that allows them to participate in both injury and repair, as reviewed in (Colton and Wilcock, 2010; Ransohoff and Perry, 2009). The nomenclature of these functional activation states (phenotypes) of MG has been simplified to facilitate their description and a common nomenclature includes classic pro-inflammatory or cytotoxic, anti-inflammatory or reparatory/regenerative and immunomodulatory phenotypes.

There are specific differences in the immune and inflammatory responses to injury between neonatal and adult humans and experimental animals (Copland et al., 2004; Giza et al., 2007; Schultz et al., 2004; Zhu et al., 2005), including in microglia responsiveness (Butovsky et al., 2014). Studies of neuroinflammatory profile and MG activation states have recently been published in adult models of TBI (Bye et al., 2007; Kumar et al., 2015) but it is unknown how microglia would respond to a similar injury to the developing brain. As such, this study investigates for the first time the characteristics of MG- driven neuroinflammation in a mouse model of paediatric TBI. Furthermore, as a proof-of-concept, we aimed to assess the effects of modulating MG activity on injury severity using the immunomodulatory tetracycline minocycline. Minocycline reportedly has strongly anti-inflammatory actions and has been used to reduce MG activation and injury with success in numerous pathological models (see Table 1 and review, (Garrido-Mesa et al., 2013)).

## 2. Materials and methods

### 2.1. Animals

Study ethics were approved by the Bichat and Robert Debré Hospital ethics committee (No 2011-14/676-0050) and adhered to the European Union Guidelines for the Care and Use of Animals. Procedures were typically carried out between 10am and 1 pm (light phase: 7am-7 pm daily), all animals were monitored daily during experimentation. A single animal represents an experimental unit with groups spread between and across litters where possible and each litter had an approximate 50–50% spread of males-females. Specifically, data in Fig. 2 are derived from 6 litters; Fig. 3 derived from 24 litters; Figs. 4, 5 and 7 derived from 6 litters each; Fig. 6 derived from 6 litters. Animals were housed in Plexiglas cages (30x18x15 cm) together with littermates and their dam for the whole of the experiment. Animals had access to standard chow and water *ad libitum* and bedding was wood-chips with shredded paper for nesting (Pharmaserv, France).

### 2.2. Traumatic brain injury model and experimental procedure

Postnatal day 7 (P7; weight 4–5 g) OF1 mice (Charles River, L'Arbresle, France) of both sexes were randomly (alternating animals) allocated to TBI, control or TBI+ treatment (phosphate buffered saline [PBS] or minocycline) groups. The study protocol is detailed in Fig. 1. A dose of 45 mg/kg of minocycline was chosen based on its prior use in models of adult TBI, stroke and paediatric excitotoxic lesion, see Table 1. In a separate experimental workspace within the animal facility, mice were anesthetized with isoflurane (8% induction) and subjected to a closed head weight–drop head trauma at P7 in a model as described previously (Kaindl et al., 2007). In brief in a process lasting no more than 3 min, the skull was fixed into a stereotaxic frame, the skull surface exposed with a skin incision and the impact device was oriented parallel to the parietal bone with the centre of the foot plate (2 mm diameter) positioned 2 mm anterior and 1 mm lateral to lambda on the parietal bone. The foot-plate was first allowed to touch the skull and was then further depressed by 0.5 mm. The impact device consisted of a hollow stainless-steel cylinder 20 cm in length, perforated at 1 cm intervals to prevent air compression, and guiding a 10 g weight falling from a height of 10 cm onto the foot-plate (2.0 mm in diameter). The contusion impact was delivered unilaterally to the left side of the skull, the same operator conducted all experiments and cortical contusions were of comparable severity in all animals. Body temperature was kept constant via the use of a heating pad maintained at 37 °C until pups were returned to their dams at approximately 15 min post-TBI. Sham animals were anesthetized and an incision made in their scalp, this was then sutured and animals were recovered after 3 min in line with the time taken for the TBI procedure. Minocycline (45 mg/kg in PBS: Sigma, Lyon, France) (Cai et al., 2006; Dommergues et al., 2003) or PBS alone was injected intraperitoneal immediately following TBI, and at 24 and 48 h post-TBI, depending on the protocol. A group of sham minocycline was not included in this study as the specific aim was to investigate the effects of modulating the microglial activation state associated with TBI. Furthermore, minocycline has been widely reported to have no effect on microglial gene expression in a basal state (Kobayashi et al., 2013; Scholz et al., 2015).

### 2.3. Tissue preparation, and histology

One or five days after TBI, animals were euthanatized via an overdose of pentobarbital and decapitation and brains were immersion fixed (formol 4% for 5 days), embedded in paraffin and coronally sectioned (16 μm) from the frontal pole to the occipital lobes. Ventricular area was determined as described previously (Kaindl et al., 2007; Moretti et al., 2016) on cresyl-violet-stained sections. In short, the border of each lateral ventricle from three serial sections spanning the hippocampus and midstriatum was outlined, then the cross-sectional ventricular areas were