

## Research report

# Glial cell responses in a murine multifactorial perinatal brain injury model

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## ABSTRACT

The impact of traumatic brain injury during the perinatal period, which coincides with glial cell (astrocyte and oligodendrocyte) maturation was assessed to determine whether a second insult, e.g., increased inflammation due to remote bacterial exposure, exacerbates the initial injury's effects, possibly eliciting longer-term brain damage. Thus, a murine multifactorial injury model incorporating both mechanisms consisting of perinatal penetrating traumatic brain injury, with or without intraperitoneal injection of lipopolysaccharide (LPS), an analog of remote pathogen exposure has been developed. Four days after injury, gene expression changes for different cell markers were assessed using mRNA *in situ* hybridization (ISH) and qPCR. Astrocytic marker mRNA levels increased in the stab-alone and stab-plus-LPS treated animals indicating reactive gliosis. Activated microglial/macrophage marker levels, increased in the ipsilateral sides of stab and stab-plus LPS animals by P10, but the differences resolved by P15. Ectopic expression of glial precursor and neural stem cell markers within the cortical injury site was observed by ISH, suggesting that existing precursors and neural stem cells migrate into the injured areas to replace the cells lost in the injury process. Furthermore, single exposure to LPS concomitant with acute stab injury affected the oligodendrocyte population in both the injured and contralateral uninjured side, indicating that after compromise of the blood-brain barrier integrity, oligodendrocytes become even more susceptible to inflammatory injury. This multifactorial approach should lead to a better understanding of the pathogenic sequelae observed as a consequence of perinatal brain insult/injury, caused by combinations of trauma, intrauterine infection, hypoxia and/or ischemia in humans.

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## 1. Introduction

Individuals suffering brain injuries during the last trimester of gestation and the early postnatal period often develop conditions such as periventricular leukomalacia (PVL) and white matter damage, which in many cases lead to cognitive deficits and/or cerebral palsy (CP) later in childhood (Sewell et al., 2014; Titomanlio et al., 2015). In humans, these perinatal outcomes have been associated with prematurity, low birth weight, maternal systemic infections

and/or placental deficiency, neonatal encephalopathy, ischemic stroke, and traumatic injury (Bloch, 2005; Volpe, 2009b), suggesting that the brain is particularly susceptible to injury during this critical phase. A combination of more than one insult may elicit an even more detrimental brain response (Girard et al., 2009; Girard et al., 2012). Several possibilities have been suggested to explain why this period is particularly vulnerable for injury (McClure et al., 2008; Semple et al., 2013). The gliogenesis process is still incomplete; astrocyte precursor migration and maturation has just begun and most oligodendrocyte maturation and myelination occurs postnatally (Back et al., 2001; Clancy et al., 2001, 2007; Workman et al., 2013). In addition, the blood-brain barrier and brain immune systems are underdeveloped (Melville and Moss, 2013; Moretti et al., 2015). Research efforts examining perinatal brain injury in animal models to assess cellular and molecular mechanisms of injury with the objective of devising therapies that lead to brain repair and regeneration are expanding (Titomanlio

**Abbreviations:** PVL, periventricular leukomalacia; LPS, lipopolysaccharide; CP, cerebral palsy; TBI, traumatic brain injury; FISH, mRNA fluorescent *in situ* hybridization; BrdU, 5-Bromo-2'-deoxyuridine; i.p., intraperitoneal; P, post-natal day; WM, white matter; VZ, ventricular zone;  $\Delta\Delta Ct$  method, comparative cycle threshold method.

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et al., 2015; Yager, 2015). Animal models focusing on perinatal asphyxia, hypoxia-ischemia, placental insufficiency, intracerebral hemorrhage, and gestational inflammation have been reported in several species (ferret, rabbit, rat, sheep, pig, primate) (McAdams et al., 2017; Yager, 2015). Although 50% of CP cases occur in normal weight babies and 10% are related to early postnatal infection, accidental brain injury, and/or drowning (Sewell et al., 2014; Titomanlio et al., 2015), no neonatal traumatic injury model has been developed reflecting these etiologies.

From the clinical perspective, traumatic brain injury (TBI) elicits more severe responses during perinatal periods than in adults (Adelson and Kochanek, 1998; Levin et al., 1982). Concussive head trauma in infant rats has been shown to cause extensive neuronal cell death at the trauma site followed by degeneration of other distant neurons (Bayly et al., 2006; Bittigau et al., 1999; Ikonomidou et al., 1996; Pohl et al., 1999). Furthermore, as brain development progresses, there is decreased susceptibility to trauma-induced apoptosis, possibly related to downregulation of the caspase-3 pathway (Yakovlev et al., 2001) and to changes in glutamate homeostasis (Lea and Faden, 2001). In humans and in other animal models, oligodendrocyte loss and astrogliosis after perinatal brain injury have been reported (Marin-Padilla, 1997, 1999; Robinson et al., 2005), but significant questions, such as whether glial precursors are recruited after injury to become mature and/or hypertrophic astrocytes, how oligodendrocytes and their precursors respond to astroglial activation and how proliferation is regulated after injury, remain unanswered. Furthermore, analyses of the responses of astrocytes and their precursors after perinatal stab-wound brain injury are lacking even though these cells may be expected to be key players in controlling glutamate metabolism after injury and in reestablishing the damaged blood-brain barrier. Previously, we established that a stab injury in the developing chick embryo brain leads to many of the phenotypes associated with PVL and that an early response to such injury involves accelerated astrocyte maturation without proliferation, as well as an acute loss of neurons and oligodendrocyte precursors (Domowicz et al., 2011). Unfortunately, avian models lack markers with which to adequately assess the inflammation response and blood-brain-barrier integrity. Thus, we have developed a multifactorial murine model of traumatic stab-wound injury in P6 mouse brain cortex, with and without accompanying induced inflammation. Since inflammation is likely a major risk factor in many forms of perinatal brain damage (maternal systemic infections and bacterial infections in preterm newborns) (Fleiss et al., 2015; Hagberg et al., 2015), we use intraperitoneal LPS injections at the time of brain injury as a model of remote bacterial infection, allowing us to assess near-term effects on the brain and, eventually, to follow long-term consequences for behavioral development.

Traditional models of developmental brain injury have used rodents from postnatal day (P) 7–10 as equivalent to a term human infant, based on measurement of postmortem brains (Dobbing and Sands, 1979). More recently, a series of studies have provided a basis to identify equivalent maturational states across 18 mammalian species (see <http://www.translatingtime.net/>), encompassing different brain areas and developmental processes (Clancy et al., 2001, 2007; Workman et al., 2013). In particular, examination of oligodendrocyte maturation in rats and mice suggested white matter development and axonal outgrowth in the rodent CNS at postnatal day 7 is analogous to that seen during 32–36 weeks of gestation in human fetuses (Back et al., 2001), which is the most vulnerable period for brain injury leading to morbidity and mortality in term and pre-term neonates (Lawn et al., 2005). Thus, in order to evaluate the molecular and biochemical changes concurrent with gliogenesis and oligodendrocyte maturation as a consequence of traumatic brain injury in mice, the postnatal window of days 6–10 was chosen for experimentation.

The data obtained with this novel model system indicate that stab injury to the neonatal mouse brain leads to loss of neurons and oligodendrocytes as well as astrocytic precursor activation; while inflammation induced by lipopolysaccharide (LPS) at the time of injury predominantly affects the oligodendrocyte population.

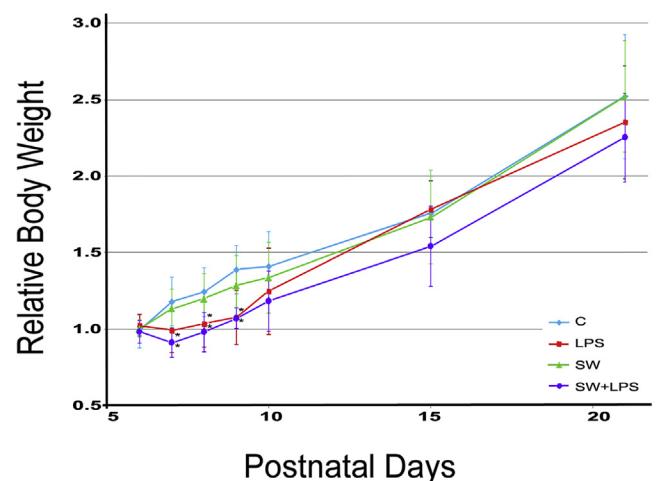
## 2. Results

### 2.1. LPS administration induces transient weight loss in neonates

LPS administration, which mimics septicemia in premature human infants (Mallard and Wang, 2012), has previously been shown to induce sickness behavior and weight loss in adult mice (Lawson et al., 2013; Walker et al., 2013), as well as a pro-inflammatory reaction and weight loss in neonatal rodent CNS (Cardoso et al., 2015). Thus, it was necessary to evaluate the contribution of LPS-only treatment in establishing the TBI model. One day (P7) after single LPS injections administered to either mock-treated stab-injury-control P6 mice or to stab-wound-treated P6 (TBI) mice, delayed neonate growth as measured by weight gain, was observed. This delay continued over the subsequent 3 days, then weight gain started to recover, catching up by day 10 (Fig. 1, inset); non-significant differences in weight loss were observed in LPS treated animals with or without stab wound injury or as late as P15 or P21 (Fig. 1). It should be noted that our use of a single LPS injection was in contrast to previous studies in which multiple-injection regimens administered over several days were observed to cause acute body and brain weight loss, which however were also reversed when LPS administration was terminated (Cardoso et al., 2015). However, multiple injections of LPS in neonates significantly increased mortality, thus all of our experiments were performed with single LPS injections.

### 2.2. CNS cell marker expression after stab-wound injury

Developmental programs of the various brain cell types are incomplete at these neonatal stages, thus the impact of injury on



**Fig. 1.** Relative body weight change after injury. Relative body weight change was calculated as a function of the average body weight at postnatal day 6 (experiment day) for each litter. Mice were mock treated (C), injured in the brain cortex with an 18-gauge needle (SW), treated with LPS (2 µg/g (i.p.)), or subjected to both treatments in succession (SW + LPS). LPS treatment slowed mouse weight gain for three days after the injury. Each point represents an average for 8–16 animals per treatment group. Error bars represent standard deviations for the group data. One-way ANOVA analysis followed by a Tukey's test was performed between the averages for the four groups at each age to assess significance relative to the control group. \* $p < .05$ .

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