Chapter 1

Neurobiological Aspects of Post-traumatic Epilepsy: Lessons from Animal Models

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Introduction

Traumatic brain injury (TBI) is a common cause of emergency room visits for both adults and the pediatric populations, accounting for almost 2.5 million visits in the United States, a third of which are children.[1] TBI causes diverse brain pathological changes, whether direct or secondary to the initial injury, which include cell death, axonal and mitochondrial injury, inflammation, gliosis, neurodegeneration and synaptic reorganization that have been associated with epileptogenesis.[2–6] Seizures may occur immediately after the TBI (immediate or acute seizures) or during the first post-TBI week (early seizures). However, it is the occurrence of late spontaneous seizures, appearing after the first post-TBI week that establishes the diagnosis of PTE.[7–10] Post-traumatic epilepsy (PTE) may develop in 2–50% of patients that experience TBI. The rate of PTE increases with severity of TBI and with longer observation periods.[11, 12] Skull fracture, parenchymal brain lesions and intracranial hemorrhage, long period of post-TBI loss of consciousness, prolonged amnesia, age at TBI and – in several studies – acute post-TBI seizures have been linked with the development of PTE.[13–16] In addition to PTE, TBI can be associated with cognitive, neurologic or psychiatric comorbidities, like depression or anxiety that also greatly impact the quality of life and medical care needs.[17–23] PTE affects the patient's quality of life due to epileptic seizures, deficits in memory and cognition,[24–26] sleep disorders,[27] post-traumatic stress disorder, depression, anxiety and social behavioral changes.[28, 29] PTE is a devastating disease that still today remains without prevention or cure.[6] The latent period for the development of epilepsy following TBI seems to be important as a time window for prophylaxis pharmacotherapies to prevent the development of epilepsy.[6, 30, 31] The validation of methods that identify the time window for treatment and efficacy of drug effects is essential to translate to clinical trials. In this regard, studies with animal models are critical to elucidate the underlying mechanisms and pharmacological targets for therapeutic interventions in controlled experiments prior to clinical trial with epilepsy patients.

Here, we will review different animal models for TBI, with special emphasis on those that describe PTE development in rodents, highlighting the importance of each of them in the biological aspects post-trauma, comprehension of PTE development and preclinical trials for pharmacotherapies that may prevent and/or cure epilepsy following TBI.

A Comparison of Developmental Stages in Rodents and Humans

Rodents have a significant shorter life span, accelerated maturation and live in a different social environment than humans. The duration of gestation in rodents lasts 23 days opposed to 40

weeks in humans; eye opening occurs in postnatal days 13–15 (P13–15) rodents yet human newborns already have their eyes open; rodents start ambulating at 2 weeks whereas humans are able to do so at around 1 year of life; weaning from the dam in rodents takes place at P21 when breastfeeding in humans is usually during the first 6 months; life expectancy for rodents is up to 2 years whereas humans in the USA live approximately 80 years.[32, 33] Comparisons of brain developmental stages in rodents and humans has produced different results, depending on the criteria used to stage these processes. Using the maturation of the hypothalamic–pituitary–gonadal axis as a criterion,[32, 33] P0–6 are thought to correspond to the neonatal stage in rodents and P7–21 to infancy. Puberty begins around P32–36 in females and P35–45 in males, while adulthood is thought to begin after P60.[34, 35] The observation that around P8–10 the rate of growth of the brain and its DNA, cholesterol and water contents in rodents resembles that of newborn human babies had led to considering the P-10 rodents as equivalent to human newborn babies.[36, 37] However, discrete processes (timing of neurogenesis, synaptogenesis, gliogenesis, oligodendrocyte maturation, age-dependent behaviors, molecular and biochemical changes) mature with asynchronous trajectories within the brain across species challenging the concept that a single across-species staging method could apply for all developmental processes.[32, 33, 38–41]

Rodent Models of PTE

Fluid Percussion Injury

In the fluid percussion injury (FPI) model, a craniotomy is created at a specific skull region and when the animal starts recovering from anesthesia, it is connected to the fluid percussion device where a controlled pressure fluid pulse is delivered through the craniotomy, over an intact dura to generate TBI. Optimization and standardization of the conditions (anesthesia, size and location of craniotomy, pulse pressure) aim to simulate TBI of various severity levels and target specific brain regions. Acute mortality and acute post-TBI responses (apnea duration, first pain response, righting reflex), neurological exam outcome and acute post-TBI seizures are often used to describe the model characteristics. The FPI model reproduces several aspects observed in patients that suffered closed-head TBI with focal-diffuse injury.[17, 42] Table 1.1 summarizes some of those findings in animal models.

Studies that have utilized the FPI model for young rodents at different ages (PN17 and PN28) reported that the youngest animals appeared to have longer apnea period, higher mortality rate and hemodynamic changes than adult rodents.[41, 43] At older ages, the FPI is characterized by substantial structural damage to the impacted cortex (left parietal or right frontoparietal) and subcortical structures.[6] The FPI model has also been applied to neonate (PN1–5) and juvenile (3–4 weeks old) pigs for the investigation of the different cerebral hemodynamic responses after TBI.[41, 44]

Several behavioral comorbidities are observed in FPI model that are similar to what is experienced in TBI patients (Table 1.2). In both rats and mice of both sexes, FPI results in cognitive deficits,[18, 19, 21, 45–52] sensorimotor deficits,[18, 50, 53–55] sleep abnormalities,[56] anxiety[18, 21, 50, 52, 53, 57] or depressive symptomatology.[18, 19, 50, 57]

Cellular and molecular post-FPI changes that have been investigated for their role in epileptogenesis include alterations in the immune system, such as increased cytokine

Table 1.1 Rodent models of PTE

Model	Sex, species, age	Induction method (anesthesia, method)	Gross pathology	Acute post-TBI seizure-like events and mortality
		Fluid percussion injury models: lateral (LFPI), rostral parasagittal (rpFPI),		
cFPI	*Male – SDR, adult*	Day 1: Pentobarbital, midline 4.8 mm. Day 2: Methoxyflurane, FPI 2.1–3.8 atm	- hemorrhages in corpus callosum, fibria hippocampi and thalamus; scattered petechial hemorrhages in brainstem	- acute mortality increases with pressure (10–10 - acute seizure ra higher in nonsurvivors - EEG: after disch of spikes 40–60 after FPI and suppression for 10–60 min post
LFPI	Male – *Harlan SDR,* adult	Pentobarbital, LFPI 4 mm, severe (2.6–3.3 atm)		30% of rats have acute TBI seizure-like events
LFPI	Male – *LE Hooded rats, adult*	Isoflurane, LFPI 5 mm,	- MRI changes in cortex, hippocampus,	

Table 1.1 (cont.)

Model	Sex, species, age	Induction method (anesthesia, method)	Gross pathology	Acute post-TBI seizure-like events and mortality
		moderate, severe, (3.2–3.5 atm)	thalamus, amygdala in LFPI rats - Large deformation high dimensional mapping of hippocampal morphometry differentiates PTE rats (lateral hippocampal region increase) from non-PTE (ventral and medial hippocampal decrease) - FDG-PET changes in ipsilateral hippocampus, 1 week to 3 months, may predict PTE rats	
LFPI	Male – C57BL/6 mice, adult	Day 1: Pentobarbital, lateral parietal craniotomy 3 mm Day 2: Isoflurane, LFPI 2.9 ± 1.1 atm	- Cortical and hippocampal injury - Hippocampal injury in 56% of LFPI mice - No difference in Timm staining between controls and LFPI animals	- 10% acute morta

rpFPI	Male – *SDR*, P33–35	Halothane, rpFPI 3 mm, 3.25–3.5 atm	-	Animals with focal GFAP staining under electrodes were excluded	11% acute mortality

Table 1.1 (cont.)

Model	Sex, species, age	Induction method (anesthesia, method)	Gross pathology	Acute post-TBI seizure-like events and mortality
			Controlled cortical impact model	
CCI	Male – *SDR, adult*	Isoflurane, CCI right parietal, 4 m/sec, 100 msec, 2.5 mm depth	- Ipsilateral necrotic cavities - Cell loss at cortex and hippocampus - Astrogliosis - Abnormal Timm staining in CCI rats	- 12.3% acute pos seizures

CCI	*Male – C57BL/6 mice, adult*	Pentobarbital, left lateral parietotemporal craniotomy 5 mm. CCI done 45 min later (3 mm tip, 0.5 mm depth, 5 m/sec, dwell time 100 msec)	- Cortical and hippocampal injury - Hippocampal injury in 60% of CCI mice - Abnormal Timm staining in hippocampus (83%) - Increased mossy fiber sprouting in mice with spikes	- 10% acute mortali
CCI	Male – *CD-1 mice, adult*	Isoflurane, left lateral craniotomy 5 mm, CCI 3 mm impact tip, 2 mm depth, 5 m/sec, 100 msec	- Cortical and hippocampal injury - Mossy fiber sprouting	- 31% acute seizure

Table 1.1 (cont.)

Model	Sex, species, age	Induction method (anesthesia, method)	Gross pathology	Acute post-TBI seizure-like events and mortality
CCI	*Male – Harlan CD-1 mice, adult*	Isoflurane, lateral craniotomy 4 mm, CCI 3 mm tip, 0.5–1 mm depth, 3.5 m/sec, 400 msec duration	- Ipsilateral cortical injury, somatosensory cortex	
CCI	*Male – C57BL/6 J mice, P21*	2,2,2-tribromoethanol ip or isoflurane, CCI **Severe**: 4.5 m/sec, 1.73 mm depth, 150 msec **Moderate**: 4 m/sec, 1.2 mm depth, 150 msec	- Astrogliosis hippocampal	
			Other models	
Blast TBI	*Male – C57BL/6 mice, adult*	Ketamine, dexmedetomidine, blast injury, 14.6 psi, Repeated TBI 3×	Increased Iba1, tau and tau phosphorylation in the brain	- 50% acute or early seizures

Penetrating TBI	*Male – Charles River SDR, adult*	Isoflurane, Steel burr 1.5 mm diameter 1000 revolutions/min, pushed in brain 8 mm below surface; copper or stainless steel wire or nothing placed in lesion	Lesion across tract (2 mm). Rats with seizures had more extensive lesion to ventral areas, amygdala and piriform Copper wire resulted in larger lesions and discoloration with copper precipitation	**Early seizures, days 3–5 post (nonconvulsive):** - Copper group: 70 - Stainless steel group: 83%

CCI: controlled cortical impact; cFPI: central fluid percussion injury; FPI: fluid percussion injury; LE: Long Evans; LFPI: lateral FPI; PTE: parasagittal FPI; SDR: Sprague Dawley rat; TBI: traumatic brain injury.

Table 1.2 Models of TBI with comorbidities

Model	Sex, species, age at TBI	Cognitive, behavioral comorbidities
LFPI	Rats, adult	- Visuo-spatial learning (MWM), sensorimo task), anxiety (elevated plus maze) prese - Cognitive deficits did not predict PTE
LFPI	Rats, P17–19	Cognitive deficits Depression-like behavior Anxiety-like behavior Sleep abnormalities Locomotor impairment
cFPI	*Male – SDR, adult*	Locomotor deficits 4–8 days post-TBI
CCI	*Male – SDR rats / adult*	Spatial learning and memory deficits (MWM Motor deficits
CCI	*Female – C57BL/6 mice, adult*	Persisting impairment in spatial learning (M Transient motor deficits (elevated narrow be
CCI	*Male – SDR rats, P28*	Spatial memory deficits (MWM) Short term memory (novel object recognitio Increased impulsive-like and anti-anxiety beh maze)
CCI	Male – SDR rats, P7 vs. P17	P7: MWM deficits on day 11 post-TBI with m P17: MWM deficits on day 11 post-TBI with a
CCI	*Male – C57BL/6* J Mice / P21	Hyperactivity. Spatial memory deficits Social dysfunction