Neuroscience 267 (2014) 30–45

PARVALBUMIN IMMUNOREACTIVITY AND EXPRESSION OF $GABA_A$ RECEPTOR SUBUNITS IN THE THALAMUS AFTER EXPERIMENTAL TBI

N. HUUSKO [a*] **AND A. PITKÄNEN** [a,b]

[a] *Department of Neurobiology, A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, PO Box 1627, FI-70211 Kuopio, Finland*

[b] *Department of Neurology, Kuopio University Hospital, PO Box 1777, FI-70211 Kuopio, Finland*

Abstract—Traumatic brain injury (TBI) causes 10–20% of acquired epilepsy in humans, resulting in an ictogenic region that is often located in the cerebral cortex. The thalamus provides heavy projections to the cortex and the activity of thalamocortical pathways is controlled by GABAergic afferents from the reticular nucleus of the thalamus (RT). As rats with TBI induced by lateral fluid-percussion injury (FPI) undergo epileptogenesis, we hypothesized that damage to the parvalbumin (PARV)-immunoreactive (ir) neurons in the RT is associated with seizure susceptibility after lateral FPI. To address this hypothesis, adult Sprague–Dawley rats ($n = 13$) were injured with lateral FPI. At 6 months post-TBI, each animal underwent a pentylenetetrazol (PTZ) seizure susceptibility test and 2 weeks of continuous video-electroencephalography (EEG) monitoring for detection of the occurrence of spontaneous seizures. Thereafter, the brain was processed for PARV immunohistochemistry. We (a) estimated the total number of PARV-ir neurons in the RT using unbiased stereology, (b) measured the volume of the ventroposteromedial (VPM) and ventroposterolateral (VPL) nuclei of the thalamus, which receive PARV-ir inputs from the RT and project to the perilesional cortex, (c) quantified the density of PARV-ir terminals in the VPM–VPL, and (d) studied the expression of $GABA_A$ receptor subunits in a separate group of rats using laser-dissection of the thalamus followed by Real-Time polymerase chain reaction (RT-PCR) array studies. At 6 months post-TBI, only 64% of PARV-ir neurons were remaining in the RT ipsilaterally ($p < 0.001$ as compared to controls) and 84% contralaterally ($p < 0.05$). Accordingly, the volume of the ipsilateral RT was 58% of that in controls ipsilaterally ($p < 0.001$) and 90% contralaterally ($p > 0.05$). Also, the volume of the VPM–VPL was only 51% of that in controls ipsilaterally ($p < 0.001$) and 91% contralaterally ($p < 0.05$). The density of PARV-ir axonal labeling was remarkably increased in the lateral aspects of the VPM and VPL (both $p < 0.001$). Expression of the ε- and θ-subunits of the $GABA_A$ receptor was down-regulated (0.152, $p < 0.01$ and 0.302, $p < 0.05$, respectively), which could relate to the inclusion of the hypothalamus into the tissue analyzed with RT-PCR arrays. In controls, the lower the number of PARV-ir neurons in the RT, the higher the seizure susceptibility in the PTZ test. Rats with TBI showed seizure susceptibility comparable to that in controls with the lowest number of PARV-ir neurons in the RT. Our data show that the RT and VPM–VPL undergo remarkable degeneration after lateral-FPI which results in reorganization of PARV-ir terminals in the VPM–VPL. The contribution of RT damage to seizure susceptibility and post-traumatic epileptogenesis deserves further studies.

Key words: epileptogenesis, lateral fluid-percussion injury, parvalbumin, pentylenetetrazol, reticular nucleus, seizure susceptibility.

INTRODUCTION

Traumatic brain injury (TBI) is recognized as a major public health problem worldwide (Finfer and Cohen, 2001; Hyder et al., 2007; Maas et al., 2008). According to the World Health Organization (WHO), TBI will surpass many other diseases as the major cause of death and disability by the year 2020 (http://www.who.int/violence_injury_prevention/road_traffic/activities/neurotrauma/en/). After the initial brain damage caused by the direct mechanical force to the head, secondary brain damage develops over the following days to weeks to months, consisting of molecular changes that underlie the reorganization of cellular networks; some leading to disabilities and some to functional recovery (Pitkänen and McIntosh, 2006; Loane and Faden, 2010; McAllister, 2011; Pitkänen and Lukasiuk, 2011; Fernández-Espejo and Owen, 2013).

One of the functional consequences of cellular plasticity is post-traumatic epilepsy (PTE). Previous epidemiology studies have shown that the 30-year cumulative incidence of epilepsy is 2.1% for mild, 4.2% for moderate, and 16.7% for severe injuries. Some TBI patients present with penetrating head injury (e.g., bullet wounds), of which up to 53% develop epilepsy. From those who eventually develop epilepsy following TBI, 80% do so within 2 years of the injury and 10–20% of

*Corresponding author. Tel: +358-40-355-3670; fax: +358-17-16-3030.
E-mail address: noora.huusko@uef.fi (N. Huusko).

Abbreviations: asf, area sampling fraction; BOLD, blood oxygenation level dependent imaging; CE, coefficient of error; ED, epileptiform discharge; EEG, electroencephalography; FPI, fluid-percussion injury; ir, immunoreactive; LCM, laser capture microdissection; MD, mediodorsal; MRI, magnetic resonance imaging; PARV, parvalbumin; PTE, post-traumatic epilepsy; PTZ, pentylenetetrazol; RGB, red, green and blue color model; ROI, region of interest; RT, reticular nucleus of the thalamus; RT-PCR, Real-Time polymerase chain reaction; ssf, section sampling fraction; TBI, traumatic brain injury; tsf, tissue sampling fraction; TUNEL, terminal deoxynucleotidyl transferase dUTP Nick-End Labeling; VL, ventrolateral; VM, ventromedial; VPL, ventroposterolateral; VPM, ventroposteromedial.

http://dx.doi.org/10.1016/j.neuroscience.2014.02.026
0306-4522/

acquired epilepsy is caused by TBI (Annegers et al., 1998). One of the great challenges in neurology is to provide therapies that improve the outcomes of patients with TBI, including prevention of epileptogenesis without compromising recovery. Current understanding of the mechanisms of post-traumatic epileptogenesis is far from being complete, and the identification of neuronal networks to which molecular analyses of post-traumatic epileptogenesis should be focused are urgently needed.

Previous histological studies have demonstrated remarkable damage to various nuclei of the thalamus in humans with TBI (Ross et al., 1993; Thompson et al., 2005). Ross et al. (1993) showed up to 75% reduction in the density of neurons in the reticular nucleus of the thalamus (RT). Neuroimaging studies have revealed ongoing thalamic damage and microglial reactivity even at 17 years post-TBI (Ramlackhansingh et al., 2011). Like in humans, histological and magnetic resonance imaging (MRI) studies in the lateral fluid-percussion injury (FPI) model of human closed head injury have revealed remarkable progressive thalamic damage over a one-year follow-up (Kharatishvili et al., 2007; Hayward et al., 2010; Niskanen et al., 2013). Therefore, animal models reproduce at least some aspects of the thalamic pathology in humans with TBI, and provide an opportunity to investigate the mechanisms and functional consequences of post-TBI thalamic abnormalities in more detail (Kawai et al., 1995; Ross et al., 1995; Böttiger et al., 1998).

The thalamus is a major relay station for peripheral information entering the cerebral cortex (Sherman and Guillery, 2006). RT is a shell-like structure that is located at the interface between the thalamocortical and corticothalamic pathways (Guillery et al., 1998; Jones, 2009). All RT neurons are GABAergic and also immunopositive for calcium-binding protein parvalbumin (PARV) (Houser et al., 1980; Seto-Ohshima et al., 1989; Arai et al., 1994; Ross et al., 1995). Consequently, it is the major provider of GABAergic inputs to the thalamocortical relay neurons. The RT can be divided into functional sectors based on its connectivity (Shosaku et al., 1989; Guillery et al., 1998; Pinault and Deschênes, 1998; Jones, 2009). Each RT sector sends axons to a particular thalamic first order and/or higher order nucleus of a given modality (visual, auditory, somatosensory, motor, limbic). The RT itself receives afferents from the axon collaterals of its thalamocortical target cells as well as from layers V and VI of the cortex of a given modality (Carman et al., 1964; Shosaku et al., 1989; Guillery et al., 1998; Pinault and Deschênes, 1998; Jones, 2009).

Considering the well-documented damage to the thalamus both in experimental and human TBI, and the importance of the RT in control of thalamic information flow, we hypothesized that the greater the damage to PARV-immunoreactive (ir) neurons in the RT, the greater the seizure susceptibility. To address this hypothesis, we induced TBI with lateral FPI and quantified the number of PARV-positive neurons in the RT. We also quantified the density of PARV-ir axons in the ventroposteromedial (VPM)–ventroposterolateral (VPL) that is the origin of thalamocortical pathways to part of the lesional and perilesional cortex at 6 months post-TBI. Then we assessed whether changes in PARV-ir elements were associated with seizure susceptibility in the pentylenetetrazol (PTZ) test. In addition, we prepared a separate group of rats to investigate whether the chronic changes in GABAergic innervation are associated with altered expression of $GABA_A$ receptor subunits in the thalamus.

EXPERIMENTAL PROCEDURES

Study design is summarized in Fig. 1.

Animals

A total of 25 adult male Sprague–Dawley rats (body weight 298–368 g at the time of injury; Harlan

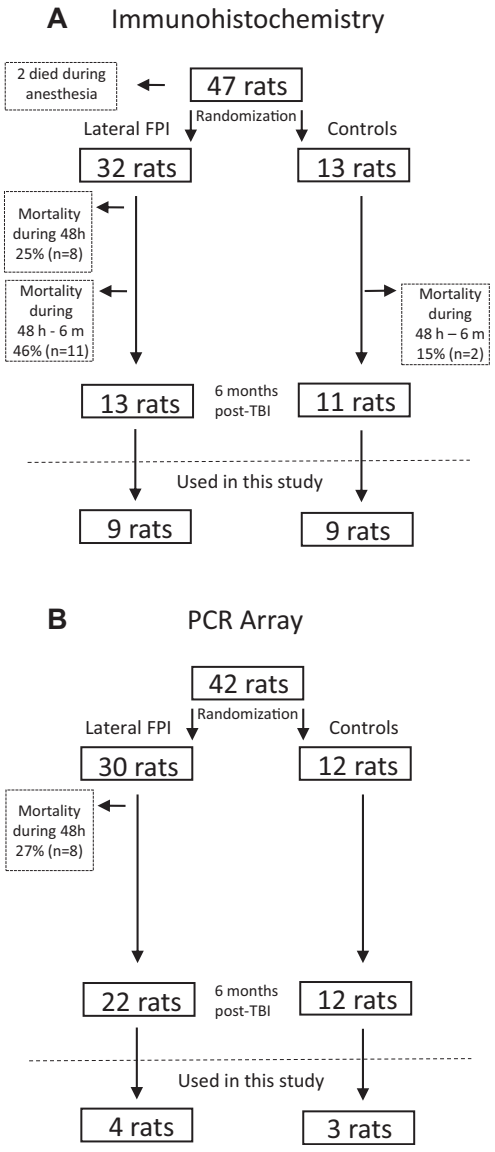

Fig. 1. Flow-chart of animals included in (A) immunohistochemical or (B) RT-PCR array study. For histology we chose rats with a high-quality video-EEG recording available. Rats for PCR array analysis were randomly sampled from the cohort.