Brain, Behavior, and Immunity xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Lung-injury depresses glutamatergic synaptic transmission in the nucleus tractus solitarii via discrete age-dependent mechanisms in neonatal rats

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ARTICLE INFO

Article history:
Received 17 October 2017
Received in revised form 20 March 2018
Accepted 26 March 2018
Available online xxxx

Keywords:
Viscerosensory
Microglia
Neuroinflammation
Developmental plasticity
Developmental transition period
Lung injury
GluR2
Calcium impermeable AMPA receptor
Nucleus tractus solitarii
Bleomycin

ABSTRACT

Transition periods (TPs) are brief stages in CNS development where neural circuits can exhibit heightened vulnerability to pathologic conditions such as injury or infection. This susceptibility is due in part to specialized mechanisms of synaptic plasticity, which may become activated by inflammatory mediators released under pathologic conditions. Thus, we hypothesized that the immune response to lung injury (LI) mediated synaptic changes through plasticity-like mechanisms that depended on whether LI occurred just before or after a TP. We studied the impact of LI on brainstem 2nd-order viscerosensory neurons located in the nucleus tractus solitarii (nTS) during a TP for respiratory control spanning (postnatal day (P) 11–15). We injured the lungs of Sprague-Dawley rats by intratracheal instillation of Bleomycin (or saline) just before (P9–11) or after (P17–19) the TP. A week later, we prepared horizontal slices of the medulla and recorded spontaneous and evoked excitatory postsynaptic currents (sEPSCs/eEPSCs) *in vitro* from neurons in the nTS that received monosynaptic glutamatergic input from the tractus solitarii (TS). In rats injured before the TP (pre-TP), neurons exhibited blunted sEPSCs and TS-eEPSCs compared to controls. The decreased TS-eEPSCs were mediated by differences in postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid receptors (AMPAR). Specifically, compared to controls, LI rats had more Ca^{2+}-impermeable AMPARs (CI-AMPARs) as indicated by: 1) the absence of current-rectification, 2) decreased sensitivity to polyamine, 1-Naphthyl-acetyl-spermine-trihydrochloride (NASPM) and 3) augmented immunoreactive staining for the CI-AMPAR GluA2. Thus, pre-TP-LI acts postsynaptically to blunt glutamatergic transmission. The neuroimmune response to pre-TP-LI included microglia *hyper*-ramification throughout the nTS. Daily intraperitoneal administration of minocycline, an inhibitor of microglial/macrophage function prevented *hyper*-ramification and abolished the pre-TP-LI evoked synaptic changes. In contrast, rat-pups injured after the TP (post-TP) exhibited microglia *hypo*-ramification in the nTS and had increased sEPSC amplitudes/frequencies, and decreased TS-eEPSC amplitudes compared to controls. These synaptic changes were not associated with changes in CI-AMPARs, and instead involved greater TS-evoked use-dependent depression (reduced paired pulse ratio), which is a hallmark of presynaptic plasticity. Thus we conclude that LI regulates the efficacy of TS → nTS synapses through discrete plasticity-like mechanisms that are immune-mediated and depend on whether the injury occurs before or after the TP for respiratory control.

Abbreviations: nTS, nucleus tractus solitarii; TS, tractus solitarii; EPSC, excitatory post synaptic current; TS-eEPSC, TS-evoked EPSC; GluA2, $GluR_2$-containing AMPA receptors; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CI-AMPARs, Ca^{2+}-impermeable AMPA receptors; CP-Ca, AMPARspermeable AMPARs; Bleo, Bleomycin; LI, lung injury; pre-TP LI, lung injury induced before transition period; post-TP LI, lung injury induced after transition period; IT, intratracheal; IP, intraperitoneal; S+S, Saline IT + saline IP; S + M, saline IT+ minocycline IP; B + S, Bleomycin IT + saline IP; B + M, Bleomycin IT + minocycline IP; NASPM, 1-Naphthyl-acetyl-spermine-trihydrochloride; NMDAR, N-methyl-D-aspartate receptors; IL-1β, Interleukin 1 beta; BEP, branch end points; aCSF, artificial cerebrospinal fluid; TP, transition period; BALF, bronchial alveolar lavage fluid; PBST, phosphate buffered saline + triton-x; CNS, central nervous system; BEP, branch endpoints; RI, rectification index; AP, area postrema; DMNX, dorsal motor nucleus of the vagus; Gr, gracile nucleus; LTD, long term depression; LTP, long term potentiation; PPR, paired-pulse ratio.

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https://doi.org/10.1016/j.bbi.2018.03.031
0889-1591/

1. Introduction

In many central nervous system (CNS) circuits, the mechanisms governing synaptic plasticity of glutamatergic synapses transition during development. Stimuli applied before versus after these transitions can alter synaptic efficacy through distinctly different mechanisms (Nosyreva and Huber, 2005; Bellone and Nicoll, 2007; Corlew et al., 2007; Ho et al., 2007). The timing of these transitions periods (TPs) often coincides with transient shifts in glutamate-receptor subunit stoichiometry and the closure of critical periods for CNS-circuit development (Crair and Malenka, 1995; Heynen et al., 2003; Bellone and Nicoll, 2007). The plasticity expressed before the transitions can confer heightened sensitivity to sensory-relevant stimuli, but may also render CNS-circuits more vulnerable to lasting maladaptive changes caused by pathologic stimuli such as hypoxia, injury and inflammation (Berardi et al., 2000; Hensch, 2005; Levelt and Hubener, 2012; Bavis and MacFarlane, 2017).

In the current study we investigate the immune response to a neonatal lung injury (LI) and its impact on brainstem (medulla oblongata) viscerosensory function to determine whether discrete synaptic plasticity mechanisms are recruited when the injury occurs before versus after a TP for the neural control of respiratory function (Bavis and MacFarlane, 2017). We examined the impact of LI on viscerosensory projections of the tractus solitarii (TS), which consist of vagal, glossopharyngeal and facial nerve pathways that synapse with 2nd-order neurons in the nucleus tractus solitarii (TS → nTS synapses). We elected to study these glutamatergic synapses because: I) they exhibit synaptic plasticity for a variety of pathologic respiratory stimuli, which raises the possibility of LI-evoked changes (Chen et al., 2003; Kline et al., 2007; Sekizawa et al., 2008; Zhang et al., 2008; Kline, 2009; Pozo and Goda, 2010); II) they are modulated by proinflammatory cytokines, which suggests the immune response to LI may regulate synaptic efficacy (Xu et al., 2006; Marty et al., 2008; Jacono et al., 2011; Rogers and Hermann, 2012; Razavi-Azarkhiavi et al., 2014; Skurikhin et al., 2015); III) They are developmentally regulated during the TP, in part through abrupt shifts in the stoichiometry of low conductance Ca^{2+}-impermeable (CI) $GluR_2$ containing AMPA receptor (GluA2s) (Liu and Wong-Riley, 2005; Balland et al., 2006). Based on work within other CNS sites, this suggests the timing of the TP may serve as a point of demarcation between immature and mature synaptic plasticity mechanisms in the nTS (Nosyreva and Huber, 2005; Ho et al., 2007; Henley and Wilkinson, 2016); and IV) The timing of the GluA2 shift also coincides with a window of heightened vulnerability to respiratory challenges (P11-15), which suggests the transition process may underlie the enhanced vulnerability (Bavis and MacFarlane, 2017). Taken together, this raises the possibility that if an LI is present during the TP, synaptic efficacy changes may occur through impairment of developmentally regulated processes such as postsynaptic GluA2 insertion/removal.

Our preliminarily evidence showed that LI evoked in rat-pups by intratracheal Bleomycin (Bleo) just before the TP (P9-11, pre-TP) augmented GluA2s in the nTS 7d after injury (P16-18) (Litvin et al., 2016). Several recent studies have reported that GluA2-mediated synaptic plasticity in some CNS-circuits can be evoked by peripheral and/or central inflammation (Park et al., 2009; Zhang et al., 2014; Riazi et al., 2015; Sullivan et al., 2017). Because Bleo LI can potentiate proinflammatory cytokine production in the peripheral circulation or in the nTS, it raised the possibility that this immune response could evoke GluA2-mediated synaptic plasticity at TS → nTS synapses (Xu et al., 2006; Jacono et al., 2011; Razavi-Azarkhiavi et al., 2014; Skurikhin et al., 2015). Thus, we hypothesized that the immune response to pre-TP-LI contributed to a GluA2-dependent synaptic plasticity mechanism that was not active in post-TP-LI rat-pups.

There is considerable evidence P11-15 represents a TP for breathing control in the rat-pup. Firstly, during this period excitatory and inhibitory transmission systems within several brainstem respiratory sites (including the nTS) undergo abrupt changes towards adult neuronal expression levels (Wong-Riley and Liu, 2008; Liu and Wong-Riley, 2010a, 2012, 2013; Turner and Johnson, 2015; Bavis and MacFarlane, 2017), which are similar to TPs in other CNS sites (Kumar et al., 2002; Tyzio et al., 2007; Brill and Huguenard, 2008; Roberts et al., 2009; Isoo et al., 2016). Secondly, the ventilatory responses to acute hypoxia or hypercapnia become transiently blunted between P12 and P15 and exposure to chronic hypoxia during this period can prolong the hypoxic insensitivity; this suggests the TP may be an important step towards the development of some ventilatory functions (Liu et al., 2009; Teran et al., 2014; Bavis and MacFarlane, 2017). Finally, several forms of respiratory plasticity that are present in juvenile and adult rats become functional just after the end of this TP (Dutschmann et al., 2009, 2014; Fuller et al., 2009; Bavis and MacFarlane, 2017). We therefore induced LI in rat-pups at P9-11, which was just before the presumed onset of the TP (at P11), so that progressively worsening LI would develop during the TP (Kaminski et al., 2000; Borzone et al., 2001; Cutillo et al., 2002; Babin et al., 2011). We contrasted this with the impact of LI initiated after the TP by inducing injury at P17-19. In these more mature rat-pups, the LI progressively worsened during a time period when: I) several forms of mature respiratory plasticity are present (Dutschmann et al., 2009, 2014; Fuller et al., 2009; Bavis and MacFarlane, 2017), II) neurotransmitter and receptor expression changes in brainstem respiratory sites become stabile (Liu and Wong-Riley, 2005, 2010a, 2010b, 2013; Wong-Riley and Liu, 2008; Dufour et al., 2010), and III) the vagally-mediated Hering Breuer inflation reflex and inspiratory off-switch are active, indicating increased maturation of respiratory control (Dutschmann et al., 2009, 2014).

We now report that LI weakens TS → nTS synaptic transmission efficacy before and after the TP. However, distinctly different synaptic plasticity mechanisms are responsible for this loss of efficacy depending on the temporal relationship of the injury to the TP. In pre-TP-LI rat pups, the loss of efficacy occurs through a *post*-synaptic CI-AMPAR-dependent mechanism that is concurrent with microglia *hyper*-ramification in the nTS, all of which can be reversed by the microglia/macrophage inhibitor minocycline. Alternatively, rats injured after the TP (post-TP) exhibit microglia *hypo*-ramification and a loss of synaptic efficacy that is CI-AMPAR-independent and consistent with *pre*-synaptic plasticity (Kline, 2009). Together, these data indicate mechanisms governing LI-evoked synaptic plasticity of viscerosensory transmission undergo a developmental switch during the TP. This switch is analogous to that exhibited by some forms of long term synaptic plasticity mechanisms; whereby a stimulus (i.e. electrical, pharmacological or environmental) applied before versus after a TP can alter synaptic efficacy at different sites (pre- vs. post-synaptically) or through different mechanisms (LTD vs. LTP) depending on the temporal relationship of the stimulus to the TP (Nosyreva and Huber, 2005; Bellone and Nicoll, 2007; Corlew et al., 2007; Ho et al., 2007).

2. Materials and methods

2.1. Animals

Sprague Dawley dams with litters containing 10 cross-fostered male pups were purchased from Envigo (Indianapolis, IN, USA), and delivered at least 24 h prior to the induction of LI. Rats were delivered pathogen free, and housed under specific-pathogen free con-

Please cite this article in press as: Litvin, D.G., et al. Lung-injury depresses glutamatergic synaptic transmission in the nucleus tractus solitarii via discrete age-dependent mechanisms in neonatal rats. Brain Behav. Immun. (2018), https://doi.org/10.1016/j.bbi.2018.03.031