NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION
RESEARCH ARTICLE
M. E. Jones et al. / Neuroscience xxx (2018) xxx–xxx

Chemogenetic Manipulation of Dorsal Hippocampal Astrocytes Protects Against the Development of Stress-enhanced Fear Learning

Meghan E. Jones, Jacqueline E. Paniccia, Christina L. Lebonville, Kathryn J. Reissner and Donald T. Lysle *

Department of Psychology and Neuroscience, Behavioral and Integrative Neuroscience Program, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Abstract—Maladaptive behavioral outcomes following stress have been associated with immune dysregulation. For example, we have previously reported that stress-induced dorsal hippocampal interleukin-1β signaling is critical to the development of stress-enhanced fear learning (SEFL). In parallel, astroglial signaling has been linked to the development of post-traumatic stress disorder (PTSD)-like phenotypes and our most recent studies have revealed astrocytes as the predominant cellular source of stress-induced IL-1β. Here, we used chemogenetic technology and morphological analyses to further explore dorsal hippocampal astrocyte function in the context of SEFL. Using a glial-expressing DREADD construct (AAV8-GFAP-hM4Di(Gi)-mCherry), we show that dorsal hippocampal astroglial G_i activation is sufficient to attenuate SEFL. Furthermore, our data provide the first initial evidence to support the function of the glial-DREADD construct employed. Specifically, we find that CNO (clozapine-n-oxide) significantly attenuated colocalization of the G_i-coupled DREADD receptor and cyclic adenosine monophosphate (cAMP), indicating functional inhibition of cAMP production. Subsequent experiments examined dorsal hippocampal astrocyte volume, surface area, and synaptic contacts (colocalization with postsynaptic density 95 (PSD95)) following exposure to severe stress (capable of inducing SEFL). While severe stress did not alter dorsal hippocampal astrocyte volume or surface area, the severe stressor exposure reduced dorsal hippocampal PSD95 immunoreactivity and the colocalization analysis showed reduced PSD95 colocalized with astrocytes. Collectively, these data provide evidence to support the functional efficacy of the glial-expressing DREADD employed, and suggest that an astrocyte-specific manipulation, activation of astroglial G_i signaling, is sufficient to protect against the development of SEFL, a PTSD-like behavior. © 2018 Published by Elsevier Ltd on behalf of IBRO.

Key words: SEFL, PTSD, fear learning, astrocyte, stress, hippocampus.

INTRODUCTION

Neural immune signaling can modulate a wide range of complex behaviors, including maladaptive responses to stress such as depression, anxiety, and PTSD (Goshen et al., 2007; Goshen and Yirmiya, 2009; Bull et al., 2014; Hutchinson and Watkins, 2014). Previous work from our laboratory has shed light on the importance of one pro-inflammatory cytokine, interleukin-1β (IL-1β), in the development of a PTSD-like behavior, stress-enhanced fear learning (SEFL) (Rau et al., 2005). We reported that severe stress induces a time-dependent increase in IL-1β immunoreactivity and mRNA in the dorsal hippocampus (DH) and that site-specifically blocking dorsal hippocampal IL-1 signaling through an intra-DH infusion of IL-1 receptor antagonist (IL-1RA) protects against the development of SEFL (Jones et al., 2015, 2017). Interestingly, IL-1β has been linked to PTSD in clinical populations as well, in that several groups have reported upregulated peripheral cytokines in PTSD patients (Gill et al., 2009; Guo et al., 2012; Gola et al., 2013; Lindqvist et al., 2014; Passos et al., 2015; Wang and Young, 2016), and have suggested cytokine expression be explored as a biomarker for affected individuals following trauma (Cohen et al., 2011). While cytokines can be expressed by multiple cell types in the brain, astrocyte-derived cytokines, including IL-1β, have been implicated in stress response mechanisms (Sugama et al., 2011a,b). Consistent with this, our most recent data suggest that dorsal hippocampal astrocytes are the cellular source of stress-induced IL-1β (Jones et al., 2017). While traditionally studied as support cells of the central nervous system (CNS), astrocytes are now known to be

*Corresponding author. Address: University of North Carolina at Chapel Hill, Department of Psychology and Neuroscience, CB# 3270, Chapel Hill, NC 27599-3270, United States. Fax: +1-919-962-2537.
E-mail address: dlysle@email.unc.edu (D. T. Lysle).

Abbreviations: BDNF, brain-derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; CNO, clozapine-n-oxide; CNS, central nervous system; DG, dentate gyrus; DH, dorsal hippocampus; DMSO, dimethyl sulfoxide; FGF2, Fibroblast growth factor-2; FGFR, Fibroblast Growth Factor Receptor family; GDNF, glial-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; IL-1β, interleukin-1β; PSD95, postsynaptic density 95; PTSD, post-traumatic stress disorder; ROI, region of interest; SEFL, stress-enhanced fear learning.

https://doi.org/10.1016/j.neuroscience.2018.07.015
0306-4522/

Please cite this article in press as: Jones ME et al. Chemogenetic Manipulation of Dorsal Hippocampal Astrocytes Protects Against the Development of Stress-enhanced Fear Learning. Neuroscience (2018), https://doi.org/10.1016/j.neuroscience.2018.07.015

critically involved in the development and disease of the CNS (Barres, 2008). The goal of the experiments reported herein was to gain a better understanding of hippocampal astrocyte function in the context of SEFL.

Several recent reports have shed light on glial-dependent mechanisms that improve behavioral outcomes following severe stress (Ben Menachem-Zidon et al., 2011; Xia et al., 2013; Levkovitz et al., 2015). Fibroblast growth factor-2 (FGF2) has been shown to alleviate PTSD-like behaviors and to prevent stress-induced changes in glial fibrillary acidic protein (GFAP) expression following Single Prolonged Stress (Xia et al., 2013). In addition, astrocytes have been hypothesized to influence fear learning through an IL-1β-dependent mechanism in that the fear-conditioning deficits traditionally observed in an IL-1 receptor knockout line are rescued by the introduction of neural precursor cells which ultimately differentiated into astrocytes (Ben Menachem-Zidon et al., 2011). Effective antidepressants, which can be prescribed to alleviate PTSD symptoms, have also been associated with gliotrophic effects (Czeh et al., 2007; Banasr et al., 2010; Niciu et al., 2014), and a recent report by Iwata and colleagues showed that the protective effect of imipramine in a model of learned helplessness was blocked by fluorocitrate, a reversible astrocyte inhibitor, infused directly into the hippocampus (Iwata et al., 2011). Lastly, Zhang and colleagues demonstrated that gastrodin, a compound shown to protect against depressive-like phenotypes, acts by enhancing astrocyte-derived brain-derived neurotrophic factor (BDNF) (Zhang et al., 2014). Collectively, evidence from multiple rodent paradigms of stress-induced depressive or anxiety-like behavior suggests that astrocyte function may be important in understanding the behavioral consequences of stress.

Our first approach to study hippocampal astrocyte function in this context was to directly manipulate astrocytes following the severe stressor of SEFL. G-protein-coupled receptor (GPCR) signaling in astrocytes is a viable target for such experiments in that IL-1β, mentioned above, is known to be regulated by G_i signaling (Cogswell et al., 1994; Ye, 2001; Jin et al., 2014). Furthermore, morphine, a systemic treatment known to reduce SEFL (Szczytkowski-Thomson et al., 2013) and to attenuate stress-induced IL-1β (Jones et al., 2015), activates G_i-coupled signaling via activation of the μ opioid receptor (Convertino et al., 2015). As such, we used glial-expressing designer receptors exclusively activated by designer drugs (DREADDs) to selectively manipulate dorsal hippocampal astroglial G_i signaling within the SEFL paradigm. While five groups have shown that manipulating astrocytes in the CNS directly influences behavioral outcomes (Agulhon et al., 2013; Bull et al., 2014; Scofield et al., 2015; Yang et al., 2015; Adamsky et al., 2018), glial-expressing DREADDs are still new, and only one effect has been reported with GFAP-hM4Di to date (Yang et al., 2015). Yang and colleagues' virus-specific and CNO-specific enhancement of feeding supports the validity of GFAP-hM4Di, however, there are no published data reported to directly confirm that CNO activates G_i-coupled signaling in GFAP-hM4Di-infused animals. To provide support for the validity of this important tool in neuroscience, we also used high-resolution confocal microscopy to measure the colocalization of the mCherry signal in adeno-associated virus serotype 8 (AAV8)-GFAP-hM4Di-mCherry-tranduced dorsal hippocampal astrocytes with cyclic adenosine monophosphate (cAMP), a G_i-dependent signal.

Our second approach to studying hippocampal astrocyte function in this context was to examine changes in astrocyte morphology induced by stress. The morphometric properties of astrocytes are important to investigate because astrocyte morphology and synaptic contact can directly influence astrocyte and neuronal function via regulation of glutamate homeostasis, synaptic remodeling, neurotrophic factor secretion, or synaptic strength (Montgomery, 1994; Scofield and Kalivas, 2014; Blanco-Suarez et al., 2016; Colombo and Farina, 2016). Current studies that have examined astrocyte morphology following stress have been limited by the reliance on GFAP or S100β immunoassays (Tynan et al., 2013; Xia et al., 2013; Choi et al., 2016; Saur et al., 2016). GFAP constitutes only about 15% of the total volume of an astrocyte, is limited to a subset of astrocytes (Benediktsson et al., 2005; Rajkowska and Stockmeier, 2013), and therefore cannot provide information regarding how fine processes of glial cells that make synaptic contacts are altered following stress (Scofield et al., 2016). Dr. Reissner and colleagues have optimized a method to isolate and quantify astrocyte volume and synaptic contacts throughout a 3-dimensional reconstruction of an individual cell (Scofield et al., 2016). With their method, an adeno-associated virus serotype 5 (AAV5), AAV5-GFAP-LcK-GFP, is used to label astrocytes in a membrane-dependent manner such that entire astrocytes, including the most distal perisynaptic processes, can be visualized and quantified. Double fluorescence immunohistochemistry is used to visualize the colocalization of the astrocyte with synaptic markers, such as postsynaptic density 95 (PSD95), and high-resolution confocal microscopy and Bitplane Imaris analysis can produce thorough measures of the volume, surface area, and synaptic colocalization (Scofield et al., 2016). This method leads to both reliable and reproducible results and provides rich detail regarding astrocyte morphology that reveals more information than previous methods allowed. Here, we employed this technology to examine how the severe stressor of SEFL alters the morphometric properties of astrocytes.

To measure synaptic colocalization, we examined expression of PSD95, an integral protein of the postsynaptic density of primarily excitatory synapses which is associated with stabilization of a dendritic spine/synaptic contact (Mir et al., 2014; Taft and Turrigiano, 2014; Berry and Nedivi, 2017). Importantly, a reduction in PSD95 in pyramidal neurons is strongly associated with spine retraction, and, even in adulthood, plasticity and learning involve a degree of spine turnover (Yang et al., 2009; Woods et al., 2011; Hayashi-Takagi et al., 2015). Both susceptibility to a depression-like phenotype following either social defeat stress or chronic unpredictable mild stress and anxiety-like behavior have

Please cite this article in press as: Jones ME et al. Chemogenetic Manipulation of Dorsal Hippocampal Astrocytes Protects Against the Development of Stress-enhanced Fear Learning. Neuroscience (2018), https://doi.org/10.1016/j.neuroscience.2018.07.015