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Research report

Secondary traumatic stress increases expression of proteins implicated in peripheral and central sensitization of trigeminal neurons

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ABSTRACT

The pathology of migraine, a common neurological disease, involves sensitization and activation of trigeminal nociceptive neurons to promote hyperalgesia and allodynia during an attack. Migraineurs often exhibit characteristics of a hyperexcitable or hypervigilant nervous system. One of the primary reported risk factors for development of a hyperexcitable trigeminal system is chronic, unmanaged stress and anxiety. While primary traumatic stress is a commonly cited risk factor for many pain conditions, exposure to secondary traumatic stress early in life is also thought to be a contributing risk factor. The goal of this study was to investigate cellular changes within the spinal trigeminal nucleus and trigeminal ganglion mediated by secondary traumatic stress. Male Sprague Dawley rats (sender) were subjected to forced swim testing (primary traumatic stress) and were then housed in close visual, olfactory, and auditory proximity to the breeding male and female rats, pregnant female rats, or female rats and their nursing offspring (all receivers). In response to secondary stress, levels of calcitonin gene-related peptide, active forms of the mitogen activated protein kinases ERK, JNK, and p38, and astrocyte expression of glial fibrillary acidic protein were significantly elevated in the spinal trigeminal nucleus in day 45 offspring when compared to naïve offspring. In addition, increased nuclear expression of ERK and p38 was observed in trigeminal ganglion neurons. Our results demonstrate that secondary traumatic stress promotes cellular events associated with prolonged trigeminal sensitization in the offspring, and provides a mechanism of how early life stress may function as a risk factor for migraine.

1. Introduction

Migraine, prevalent neurological disorder, affects >18% of the general population in the United States, with the prevalence highest in women of childbearing age (Buse et al., 2013a). Migraineurs are thought to have a hyperexcitable nervous system characterized by a heightened sensitivity towards external stimuli that may be responsible for triggering a migraine attack (Burstein et al., 2015; Dodick and Silberstein, 2006). Sensitization and activation of trigeminal nerves, which provide a nociceptive pathway from the peripheral dural tissues to the spinal trigeminal nucleus, is implicated in the underlying pathology of migraine (Pietrobon and Moskowitz, 2013). A lowering of the activation threshold of trigeminal nociceptive neurons that occurs during neuronal sensitization or a primed state is associated with increased neuron-glia interactions and cellular changes in the expression of ion channels and signal transduction receptors (Dodds et al., 2016; Hucho and Levine, 2007). These cellular changes are mediated by increases in neuronal and glial expression of signal transduction proteins in the trigeminal ganglion and spinal trigeminal nucleus. In particular, members of the mitogen-activated protein kinase (MAPK) family mediate intracellular signaling cascades that promote neuron-glia interactions and maintain a hyperexcitable state of nociceptive neurons (Crown, 2012; Ji et al., 2009; Ji et al., 2013). The neuropeptide calcitonin gene-related peptide (CGRP), whose expression is regulated by MAPK and is strongly implicated in migraine pathology, can promote sensitization of primary and secondary trigeminal nociceptive neurons and activation of satellite glial cells in the ganglion and astrocytes and microglia in the spinal cord (Durham, 2016; Iyengar et al., 2017; Seybold, 2009).

The most-cited risk factor for migraine pathology is stress, which if unmanaged can lead to a variety of anxiety disorders including but not limited to major depressive disorders, Post-Traumatic Stress Disorder (PTSD), and panic disorders (Buse et al., 2013b). Symptoms of stress include clenching and grinding of teeth, frequent headaches, and neck pain (Slade et al., 2016). While primary stress is the direct exposure of an organism to a traumatic event outside of normal daily experiences, secondary stress, which is commonly referred to as compassion stress, is

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caused by exposure to victims of a primary traumatic stress (Klaric et al., 2013). Exposure to traumatic events or situations, especially early in life, can lead to dysmodulation between the responses of the sympathetic and parasympathetic nervous system and the HPA axis leading to increased pain sensitivity (Mooney-Leber and Brummelte, 2017; van Bodegom et al., 2017). Clinically, pain threshold and tolerance is used to define the normal functioning of the nervous system in response to stressors. Frequent or severe stress can mediate cellular changes leading to the development of a hypervigilant nervous system, which becomes dysregulated and maladaptive to future allostatic load (Maleki et al., 2012). Hypervigilance is characterized as a heightened state of sensory nerve excitability and pain sensitivity due to an enhanced response to inflammatory molecules that leads to a lower pain threshold and lower pain tolerance. Migraine-associated allodynia, which is defined as a nociceptive response to normally non-painful stimuli, like brushing one's hair involves activation of the trigeminal system and development of central sensitization (Burstein et al., 2015). An individual with a maladaptive hypervigilant nervous system, which can arise from unmanaged anxiety caused by early life stress, is at an increased risk for development of persistent central sensitization as reported in migraine pathology (Aurora and Wilkinson, 2007; Borsook et al., 2012).

Although there appears to be a link between childhood maltreatment in the forms of emotional or physical abuse and the risk of developing migraine (Brennenstuhl and Fuller-Thomson, 2015; Tietjen et al., 2012; Tietjen, 2016), the impact of secondary traumatic stress on the developing trigeminal system has not been investigated. Results from our study provide evidence that exposure of parent rats and nursing offspring to secondary traumatic stress is sufficient to cause a sustained increase in the expression of proteins implicated in peripheral and central sensitization of trigeminal nociceptive neurons in young adult rats.

2. Results

2.1. Secondary stress increases CGRP and MAPK expression in the spinal trigeminal nucleus

Initially, brainstem tissue containing the spinal trigeminal nucleus (STN) was isolated and stained with the fluorescent nuclear dye DAPI to visualize the distribution of neuronal and glial cells. The number and pattern of nuclear staining within the STN was not altered by secondary stress when compared to tissues obtained from naïve control animals (data not shown). Similarly, the same tissues were immunostained with the neuronal specific nuclear protein NeuN to visualize the distribution of neurons within the outer laminae. Within the STN, the location and relative number of neurons appeared similar when comparing tissues obtained from naïve control animals and those obtained from animals subjected to prenatal and postnatal secondary stress (n = 4 for all conditions; data not shown). As a control for antibody specificity, tissues were incubated with secondary antibodies alone and no specific staining pattern was detected in the absence of primary antibodies (data not shown).

To determine the effects of secondary stress on proteins associated with the development and maintenance of central sensitization, cellular changes in the STN were investigated using immunohistochemistry. An increased level of staining intensity of the neuropeptide CGRP was observed in neurons and glia in the outer lamina region of the STN in animals subjected to secondary stress when compared to naïve control (Fig. 2, n_{naive} = 4, $n_{stressed}$ = 3). The relative immunostaining intensity was significantly elevated in samples from stressed offspring (1.94 ± 0.08; Cohen's d = 5.42; t = −5.416; df = 4; CI: −1.424, −0.459; P = 0.006) as compared to levels detected in animals from naïve litters (1.00 ± 0.16).

Similar to CGRP, an increase in the intensity of immunostaining for the phosphorylated, active forms of ERK (P-ERK;), p38 (P-p38), and JNK (P-JNK) was detected in laminae I-III of the STN (n_{Naive} = 4, $n_{Stressed}$ = 4). Low levels of P-ERK were detected in neurons and glia of control tissues (Fig. 3). However, in stressed offspring, neuronal P-ERK staining was readily observed in a punctate pattern in the outer laminae and the intensity was significantly increased (1.73 ± 0.11; Cohen's d = 3.6; t = −4.408; df = 6; CI: −1.142, −0.327; P = 0.005) as compared to levels detected in naive animals (1.00 ± 0.18). Similarly, the expression of the active form of p38 was significantly elevated in neurons localized in the medullary region of the STN in offspring exposed to secondary stress (2.43 ± 0.27; Cohen's d = 4.64; t = −5.685; df = 6; CI: −2.052, −0.817; P = 0.001) when compared to levels in control animals (Fig. 4). Low levels of P-JNK staining were detected in neurons and glia of control tissues (Fig. 5); however, in tissues obtained from stressed animals, a diffuse pattern of P-JNK staining was observed in the outer lamina of the medullary horn. When compared to naïve control animals (1.00 ± 0.18), there was a significant increase in staining intensity after exposure to secondary traumatic stress (2.03 ± 0.18; Cohen's d = 3.85; t = −4.712; df = 6; CI: −1.563, −0.495; P = 0.003).

2.2. Effect of secondary stress on astrocytes

A low level of GFAP expression was detected in laminae I-III in naïve offspring (Fig. 6, n_{Naive} = 4, $n_{Stressed}$ = 3). In contrast, the intensity and number of GFAP positive astrocytes was significantly elevated in tissues obtained from offspring exposed to secondary stress (2.56 ± 0.64; Cohen's d = 2.74; t = −3.058; df = 5; CI: −2.879, −0.249; P = 0.028) as compared to those observed in naïve tissues (1.00 ± 0.26).

2.3. Increased expression of P-ERK and P-p38 in trigeminal ganglion of stressed offspring

In naïve animals, a low level of P-ERK immunostaining was detected in neurons, but not satellite glial cells, in the V1/V2 region of the trigeminal ganglion (Fig. 7, n_{Naive} = 4, $n_{Stressed}$ = 8). While the average percentage of neurons exhibiting nuclear localization of P-ERK was 20.79% ± 2.32 in naïve animals, the average number of P-ERK positive neurons was significantly increased in the V1/V2 region (57.61% ± 3.60; Cohen's d = 4.28; t = −6.77; df = 10; CI: −48.93, −24.70; P < 0.001) in stressed animals. Increased nuclear expression of P-ERK was observed in both large diameter Aδ and small diameter C fiber neurons in ganglion from animals exposed to early life secondary stress. Similar to P-ERK expression, naïve animals exhibited low levels of P-p38 in the nucleus of neuronal cell bodies (33.07% ± 5.49) but significantly increased nuclear levels in Aδ and C fiber neurons in the V1/V2 region (74.28% ± 1.14; η^2 = 0.671; U < 0.001; P = 0.004) in stressed animals (Fig. 8). In addition, an increase in the number of satellite glial cells exhibiting P-ERK and P-p38 staining was observed in stressed offspring when compared to naïve animals. Unlike the result in the STN, CGRP levels in the ganglion were not increased in response to early life stress (data not shown).

3. Discussion

A major finding of our study was that exposure of parent rats and nursing pups to secondary early life stress resulted in sustained changes in the levels of proteins associated with central and peripheral sensitization of trigeminal neurons of young adult