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STRESS-ALTERED SYNAPTIC PLASTICITY AND DAMP SIGNALING IN THE HIPPOCAMPUS-PFC AXIS; ELUCIDATING THE SIGNIFICANCE OF IGF-1/IGF-1R/CAMKIIα EXPRESSION IN NEURAL CHANGES ASSOCIATED WITH A PROLONGED EXPOSURE THERAPY

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Abstract—Traumatic stress patients showed significant improvement in behavior after a prolonged exposure to an unrelated stimulus. This treatment method attempts to promote extinction of the fear memory associated with the initial traumatic experience. However, the subsequent prolonged exposure to such stimulus creates an additional layer of neural stress. Although the mechanism remains unclear, prolonged exposure therapy (PET) likely involves changes in synaptic plasticity, neurotransmitter function and inflammation; especially in parts of the brain concerned with the formation and retrieval of fear memory (Hippocampus and Prefrontal Cortex: *PFC*). Since certain synaptic proteins are also involved in danger-associated molecular pattern signaling (DAMP), we identified the significance of IGF-1/IGF-1R/CaMKIIα expression as a potential link between the concurrent progression of synaptic and inflammatory changes in stress. Thus, a comparison between IGF-1/IGF-1R/CaMKIIα, synaptic and DAMP proteins in stress and PET may highlight the significance of PET on synaptic morphology and neuronal inflammatory response. In behaviorally characterized Sprague–Dawley rats, there was a significant decline in neural IGF-1 ($p < 0.001$), hippocampal ($p < 0.001$) and cortical ($p < 0.05$) IGF-1R expression. These animals showed a significant loss of presynaptic markers (synaptophysin; $p < 0.001$), and changes in neurotransmitters (VGLUT2, Tyrosine hydroxylase, GABA, ChAT). Furthermore, naïve stressed rats recorded a significant decrease in post-synaptic marker (PSD-95; $p < 0.01$) and synaptic regulator (CaMKIIα; $p < 0.001$). As part of the synaptic response to a decrease in brain CaMKIIα, small ion conductance channel (KCa2.2) was upregulated in the brain of naïve stressed rats ($p < 0.01$). After a PET, an increase in IGF-1 ($p < 0.05$) and IGF-1R was recorded in the Stress-PET group ($p < 0.001$). As such, hippocampal ($p < 0.001$), but not cortical (ns) synaptophysin expression increased in Stress-PET. Although PSD-95 was relatively unchanged in the hippocampus and PFC, CaMKIIα ($p < 0.001$) and KCa2.2 ($p < 0.01$) were upregulated in Stress-PET, and may be involved in extinction of fear memory-related synaptic potentials. These changes were also associated with a normalized neurotransmitter function, and a significant reduction in open space avoidance; when the animals were assessed in elevated plus maze (EPM). In addition to a decrease in IGF-1/IGF-1R, an increase in activated hippocampal and cortical microglia was seen in stress ($p < 0.05$) and after a PET (Stress-PET; $p < 0.001$). Furthermore, this was linked with a significant increase in HMGB1 (Hippocampus: $p < 0.001$, PFC: $p < 0.05$) and TLR4 expression (Hippocampus: $p < 0.01$; PFC: ns) in the neurons. Taken together, this study showed that traumatic stress and subsequent PET involves an event-dependent alteration of IGF1/IGF-1R/CaMKIIα. Firstly, we showed a direct relationship between IGF-1/IGF-1R expression, presynaptic function (synaptophysin) and neurotransmitter activity in stress and PET. Secondly, we identified the possible role of CaMKIIα in post-synaptic function and regulation of small ion conductance channels. Lastly, we highlighted some of the possible links between IGF1/IGF-1R/CaMKIIα, the expression of DAMP proteins, Microglia activation, and its implication on synaptic plasticity during stress and PET.

Key words: IGF-1R, CaMKIIα, IGF-1, synaptic morphology, DAMP, traumatic stress.

INTRODUCTION

Prolonged exposure therapy (PET) has been described as an effective method in the management of traumatic stress symptoms, which has led to enhanced quality of life in some patients (Yehuda et al., 2014; Castillo et al., 2016). PET and other traumatic stress interventions, are aimed at improving emotional, social and stress-related behaviors through fear-extinction learning (Fiorenza et al., 2012; Furini et al., 2014; Jerud et al., 2016; Larsen et al., 2016). Traumatic stress is associated with altered structural and functional connectivity in parts of the brain involved in fear memory processing, notably, the hippocampus, prefrontal cortex (PFC) and amygdala. The brains of traumatic stress patients are often characterized by abnormal white matter structure, reduced brain volume and hippocampal activity (Pang, 2015; Hayes et al., 2016). In this respect, the goal of fear extinction learning is to retrain the brain to prevent the retrieval of

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Abbreviations: CaMKIIα, calcium calmodulin-dependent kinase 2 alpha; HMGB1, high mobility group box protein 1; IGF-1, insulin-like growth factor 1; IGF-1R, IGF-1 receptor type 1; KCa2.2, calcium-dependent potassium channel (SK2 or KCNN2 family); TLR4, toll-like receptor 4.

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aversive memories associated with the post-traumatic stress condition. However, the effectiveness of PET depends on multiple cellular mechanisms such as inflammation, neurotransmitter systems, and synaptic modifications in neural circuits associated with fear memory retrieval (Abraham et al., 2016; Novick et al., 2016).

Interestingly, in traumatic stress, synaptic IGF-1R signaling has been identified as a major player in the regulation of neurotransmitter activity and prevention of inflammation (Rubovitch et al., 2010; Zhao et al., 2012). Neurotrophic factors, such as insulin-like growth factor 1 and its type 1 receptor (IGF-1R), are known to modulate the synaptic activity of dopamine and glutamate in the developing and adult nervous systems (Mattson, 1990, 2008; Guevara-Aguirre, 1996; Pehar et al., 2010). Moreover, alterations in neural IGF-1 have been described in the cause and progression of several neuropsychiatric disorders associated with development, aging, degeneration and malformation of cytoskeletal proteins in neurons (Guevara-Aguirre, 1996; Pehar et al., 2010; Hwa et al., 2013). Additionally, other reports have shown that loss of neurotrophins, and associated receptors are pivotal to a compromised synaptic integrity, abnormal neurotransmitter signaling events and activation of inflammatory pathways in traumatic stress (Su et al., 2015; Finsterwald et al., 2015).

Generally, IGF-1R activation involves multiple signaling pathways. Notably, Akt/mTOR signaling facilitates an increase in the nuclear transcription of NF-κB. In a separate – but related – mechanism, *Ras* signaling promotes the activation of MAPK/ErK (Chetty et al., 2006; Rubovitch et al., 2010). In support of this proposition, a decrease in IGF-1R signaling has been shown to attenuates danger-associated molecular pattern (DAMP) signaling by blocking the activation of pro-inflammatory molecules such as MAPK/ErK, Akt/mTOR, NF-κB (Zhao et al., 2012; Yu et al., 2012). Additionally, IGF-1/IGF-1R signaling is known to regulate the nuclear translocation of HMGB1, while attenuating a significant part of DAMP signaling in the HMGB1-TLR4 pathway (Gontier et al., 2015). Several other divergent pathways, such as Wnt/β-Catenin signaling, involves IGF-1R and CaMKIIα alterations in synaptogenesis. Aside from its role in the regulation of long-term potentiation (LTP), CaMKIIα holds the ability to block some of the inflammatory and synaptic activities of MAPK/ErK (Bouallegue et al., 2009; Rosso and Inestrosa, 2013). Although previous studies have shown that IGF-1R signaling is involved in the regulation of inflammation and synaptic function, the significance of an event-dependent change in IGF-1R expression – as a response mechanism – in traumatic stress and PET remains unclear.

During stress events – such as predator exposure – a commensurate neural and psychological stress is induced. Since exposure therapy involves recreating the traumatic experience, an additional layer of neural stress is induced in the hippocampus-PFC axis during the retrieval of the associative memory. Thus, since IGF-1/IGF-1R directly modulate the activity of CaMKIIα in inflammation and synaptic function, this study sought to determine whether a change in IGF-1/IGF-1R/CaMKIIα expression may represent specific changes in neural morphology and DAMP signaling in stress, and modifications that may occur in PET.

EXPERIMENTAL PROCEDURES

Animal strain

Adult male Sprague–Dawley rats (Charles River Lab, Wilmington, MA) weighing between 250–300 gm was used for this study. The animals were kept under standard laboratory conditions of 12 h alternating dark and light cycle, and fed ad libitum. All animals handling procedures were in accordance with approved protocols by the Institutional Animal Care and Use Committee (IACUC) of the Louisiana State University School of Veterinary Medicine.

Experimental model of traumatic stress

To induce traumatic stress, we adopted the model previously described by Zoladz et al. (2008). This method involves a combination of *acute* predator exposure events, with *chronic* psychosocial stress events (Fig. 1A). Rats *(n = 30)* were randomly assigned to traumatic stress *(n = 20)* or control *(n = 10)* groups. All animals were maintained in the animal holding facility for the duration of the experiment. Rats were kept in cylindrical holdings (Plexiglas containers) covered with cat chow and were placed in a separate metal cage (76 cm × 76 cm × 60 cm) with an adult cat (7 years old). This allowed for the free movement of the cat around the cylinder (food) but prevented the cat from touching the rat. Predator exposure duration of 1 h was adopted for each exposure event as previously described (Wilson et al., 2014). The first exposure was done on (Day 1) during the daylight cycle (07:00–19:00). After 10 days, a second exposure was done during the dark cycle (Day 11; 19:00–07:00). Between Day 1 to Day 31, rats were subjected to a random daily cohort cage rotation to eliminate any form of social support, and induce chronic psychosocial stress during the period of the experiment. It is important to note that no Cat or Cat material was allowed near the cage rotation cohort. Additionally, the last cage for a rotation was also the first cage, and represents the actual group of the rat (home cage). The control rats *(n = 10)* were kept in the same cages from Day 1- Day 31 and were not subjected to cage rotation or predator exposure events (Fig. 1A).

Prolonged exposure therapy (unrelated stimulus)

The underlying principle employs the re-exposure of naïve traumatic stress rats to an unrelated causative stimulus (Cat meow tone). The tone was played from a pre-recorded audio file through speakers positioned in the conditioning chamber. This is aimed at training the animal to facilitate the extinction of associative fear memory. While traumatic stress was induced through predator exposure and psychosocial stress, for the PET, we used a fear conditioning chamber, and pulses of cat meow tone in the dark for 5 min. As from Day 40, a

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