J Mol Neurosci
DOI 10.1007/s12031-017-0911-3

Possible Reversal of PTSD-Related DNA Methylation by Sympathetic Blockade

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Received: 4 February 2017 / Accepted: 9 March 2017

Abstract Studies have shown that brain-derived neurotrophic factor (BDNF) level increase is associated with post-traumatic stress disorder **(**PTSD) risk. BDNF may be a "missing-link" that mediates the interaction between genetics, environment, and the sympathetic system. Trauma has been shown to induce DNA methylation that in turn can increase BDNF concentration due to increased gene expression. Therapies that focus on the reduction of beta-NGF (BNGF) levels may impact PTSD symptoms. The focus of this paper is to discuss possible effect of stellate ganglion block (SGB) on epigenetic changes noted with PTSD mediated by BDNF and NGF. Stellate ganglion block has recently shown significant therapeutic efficacy for treatment of PTSD symptoms. Previously reported theoretical mechanisms of SGB impact on PTSD have focused on likely reduction of NGF, leading to eventual loss of extraneous sympathetic nerve growth, eventually leading to reduction of secondary norepinephrine level, which in turn is hypothesized to reduce PTSD symptoms. We used PUBMED to obtain available data following a search for the following: DNA, neurotrophic factors, post-traumatic stress disorder, and demethylation following local anesthetic application. A number of articles meeting criteria were found and reviewed. Based on the evidence summarized, trauma can lead to DNA methylation, as well as BNGF/NGF level increase, which in turn starts a cascade of sympathetic sprouting, leading to increased brain norepinephrine, and finally symptomatic PTSD. Cascade reversal may occur in part by demethylation of DNA caused by application of local anesthetic to the stellate ganglion.

Keywords Post-traumatic stress disorder · PTSD · Stellate ganglion block · SGB · Anxiety · Cervical sympathetic chain · Autonomic dysfunction · Brain-derived neurotrophic factor · BNGF · DNA methylation · Nerve growth factor · NGF

Background

Post-traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) is a chronic anxiety disorder caused by seeing or experiencing traumatic events. The symptoms of PTSD may lead to significant distress, and potentially, to suicide. While advances have been made in understanding and treating this syndrome, barriers to care continue to exist, such as ineffectiveness of treatments, side effects of pharmaceutical treatments, the difficulty of completing cognitive behavioral treatment, and the stigma attached to having a mental health issue (vs a real biologic or medical issue).

The Sympathetic Nervous System

The sympathetic nervous system (SNS) is part of the autonomic nervous system. Its role is to mobilize body's resources and induce the fight-or-flight response. It is constantly active at a basal level in order to maintain homeostasis. In large part, the activation of the SNS is associated with catecholamine increase, mainly epinephrine and norepinephrine. The role of norepinephrine in the brain has been reported of being that

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Published online: 31 March 2017

of a neurotransmitter, rise in its concentration being associated with increased arousal, and vigilance (Southwick et al. 1999).

Norepinephrine (NE) concentrations in cerebrospinal fluid (CSF) have been reported as significantly higher in patients diagnosed with PTSD than among healthy controls, as well as NE increase has been correlated with the severity of PTSD symptoms (Geracioti et al. 2001). Above summarized increase in noradrenergic activity among PTSD patients suggests that reducing CNS noradrenergic activity could be effective (Taylor et al. 2006).

Brain-Derived Neurotrophic Factor, Nerve Growth Factor, Stress, DNA, mRNA, and Sympathetic System

Brain-derived neurotrophic factor (BDNF), is considered a neurotrophin, which plays important roles in the development and physiology, as well as anxiety and PTSD (Zhang et al. 2016); it is expressed in a number of tissues and cell types (Dell'Osso et al. 2009). Brain-NGF (BNGF) has been shown to be released by nerve growth factor (NGF) (Sarchielli et al. 2011), which concentration has been shown to increase with chronic stress and acute stress (Smith 1996; Alleva et al. 1996). NGF levels have been shown to increase the sympathetic fiber density proportional to NGF messenger RNA (mRNA) levels (Tsukada and Shooter 1992). NGF translocation is also known to affect DNA (Yankner and Shooter 1979). Above demonstrates interactions of BNGF, NGF, mRNA, and DNA.

As background, gene expression is defined as manufacturing a protein, which is a process that requires two steps. Step 1, called transcription, involves DNA information transfer to a mRNA. Step 2 is called translation, where mRNA information is used to build a protein molecule; in the case under discussion, this is NGF. Mammals respond to stress by increasing the synthesis and secretion of NGF by *target organs of sympathetic fibers* such as the amygdala (Tsukada and Shooter 1992; Badowska-Szalewska et al. 2016), an organ long known to be activated in patients diagnosed with PTSD (Liberzon and Martis 2006). The secretion of NGF leads to sympathetic fiber growth also known as sprouting (Ruit et al. 1990). Sympathetic fiber growth remains dependent on NGF for survival and maintenance of dendritic geometry into old age and may occur on mature neurons (Ruit et al. 1990). Sympathetic sprouting can occur as a response to elevated NGF in the absence of injury (Kawaja and Crutcher 1997). The underlying neurobiology of sprouting is likely to be an NGF signal promoting changes of the axon cytoskeleton (Zhou et al. 2004).

Cervical Sympathetic Ganglion and the Amygdala Connections

A connection of the sympathetic cervical ganglion to the limbic system has been shown by Dr. Westerhaus, where he and Dr. Loewy injected pseudorabies virus into the stellate ganglion and observed pseudorabies makers in the substance of the brain. This approach demonstrated a connection from the stellate ganglion to the amygdala (Westerhaus and Loewy 2001).

Cervical Sympathetic Ganglion, mRNA, and Inducible and Reversible Changes

Original theory attempting to explain effect of SGB in the treatment of PTSD focused on the NGF reducing effect of stellate ganglion block, leading to reduction of NGF levels required to maintain sympathetic nerve sprouting (Lipov et al. 2009). The loss of sprouting was postulated to reduce NE levels, reducing PTSD symptoms (Lipov et al. 2009). Malleable nature of sympathetic sprouting as well as related reduction of NE proposed above has been experimentally demonstrated by Dr. Kong. In his study of myocardial ischemic (MI) injury in a rat model, he was able to demonstrate dysregulated expression of a specific RNA (sRNA) in cervical sympathetic ganglia. He went on to use interfering RNA (iRNA) against sRNA, which reversed myocardial ischemia-induced RNA changes in the cervical sympathetic ganglion (Kong et al. 2013). Dr. Kong further evaluated the effect of iRNA on nerve sprouting and serum NE and epinephrine (EPI) levels. He reported nerve sprouting markers to be significantly increased in the MI group; however, the same markers were reduced in the MI rats following treatment with iRNA. Similar changes, mainly reduction of serum NE and EPI, were noted in MI rats when treated by sRNA (Kong et al. 2013).

The above evidence strongly supports the following hypothesis:

Cervical sympathetic ganglion has inducible mRNA; reversal of the mRNA induction may reverse sympathetic nerve sprouting; and reversal of sympathetic nerve sprouting can reduce serum NE.

TrkA Receptor and NGF and TrkA Receptor Complex

TrkA receptors are a tyrosine kinase family that regulates synaptic plasticity and growth cone steering in the nervous system of mammals. TrkA receptors can affect neuronal survival and differentiation through various cascades; it has a high affinity for the NGF. NGF is known to have multiple functions; among them are the following: regulation of growth cones, gene encoding regulation, and modulation of enzymes for neurotransmitters (Suter and Forscher 2001) (Fig. 1).

NGF and TrkA Receptor Complex

NGF-TrkA complex can deliver NGF signal from axon terminals to cell bodies of sympathetic neurons (Riccio et al. 1997), such as the stellate ganglion. NGF can bind TrkA in endocytic organelles (endocytosis = the taking in of matter by a living cell by invagination of its membrane to form a vacuole) (Fig. 1). NGF can induce the formation of signaling endosomes containing

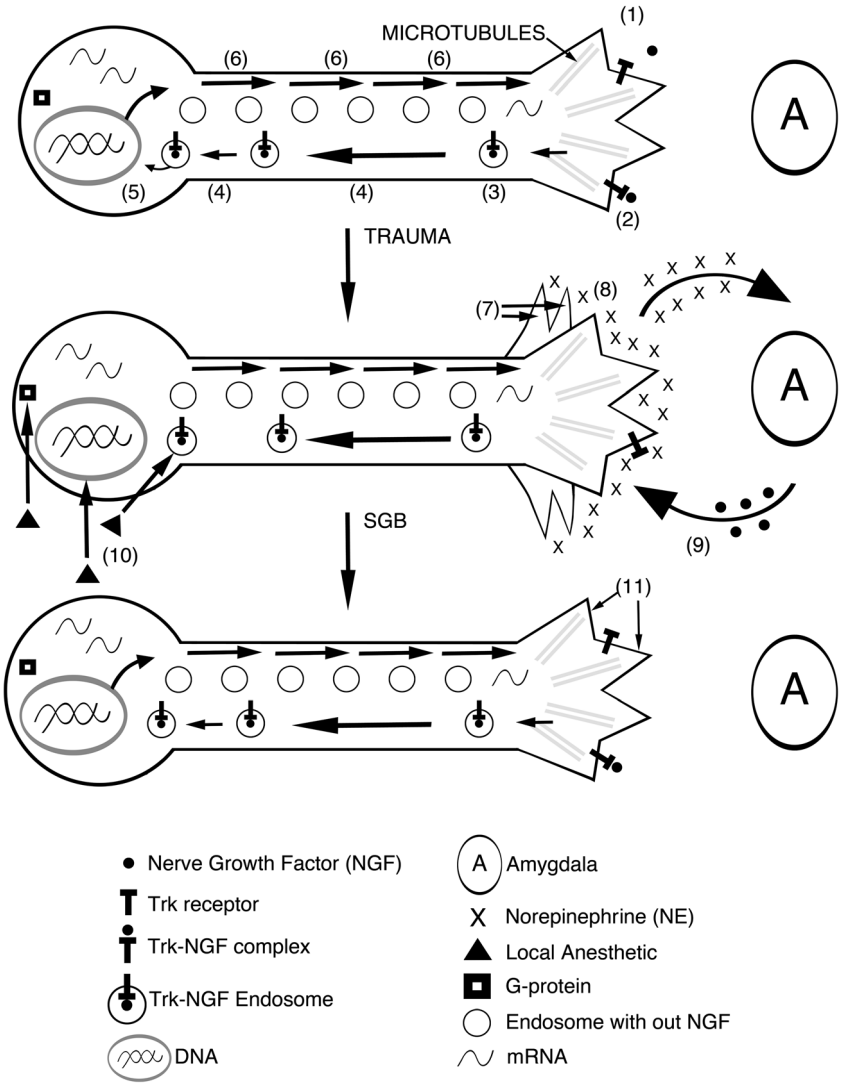

Fig. 1 Multiple effects of NGF increase. *1* release of nerve growth factor; *2* Trk-NGF complex; *3* Trk-NGF signaling endosome; *4* retrograde support; *5* DNA/mRNA modulation via Trk-NGF endosome; *6* anterograde transport; *7* sympathetic nerve sprouting; *8* norepinephrine increase; *9* nerve growth factor increase; *10* stellate ganglion block, local anesthetic effect; *11* reversal of sprouting, effect on Trk, DNA, and G protein

activated TrkA (Grimes et al. 1996), as well as sustained, long-term activation of TrkA (Vivas et al. 2014). NGF binding TrkA can also lead to mRNA induction in the sympathetic nuclei (Tsukada and Shooter 1992).

NGF, BNGF, History of Trauma, PTSD and Genetic Changes, BDNF, and PTSD Connection

Increased levels of BDNF, in peripheral blood plasma, have been shown to be associated with PTSD risk and exaggerated startle reaction (Zhang et al. 2016).

Effects of Previous Trauma in Developing PTSD

Epidemiologic studies have shown early adverse experiences place children at an increased risk of developing depression, anxiety, or both (Heim and Nemeroff 2001). Similarly, multiple trauma exposure, particularly in childhood, may result in a multiple symptoms that include PTSD (Cloitre et al. 2009).

The impact of the trauma discussed above is likely to induce NGF, BNGF, and sympathetic sprouting as well as epigenetic changes in DNA.

BDNF and PTSD Epigenetic Changes

A relatively new science of epigenetics is starting to have significant impact on explaining the DNA changes following trauma. Epigenetics is a study of gene expression modification that results from external or environmental factors, that leads to activation or deactivation of genes (Moore 2015). One of the epigenetic mechanisms is the DNA nucleotide methilization. In a study by Dr. Zhang, trauma has been shown to induce methilization of a DNA nucleotide responsible for BNGF expression (Zhang et al. 2016). More specifically, a substitution of methionine (Met) for valine (Val) at position 66 (Val66Met) can influence human memory (Zhang et al. 2016) and susceptibility to PTSD (Zhang et al. 2006). Clinical PTSD symptoms seem to correlate with higher levels

of BNDF in the peripheral blood plasma (Zhang et al. 2016; Hauck et al. 2010) as well as increased level of DNA methilization; BDNF levels in Met carriers are higher than in Val/Val homozygotes (Harris et al. 2006) and the frequency of the Met/Met in BDNF gene was greater among those with PTSD than those without PTSD (Zhang et al. 2016).

NGF and Norepinephrine

Norepinephrine has been known to be released during times of severe stress. Specific brain regions that seem to have the highest increase are the hypothalamus, amygdala, and locus coeruleus, in the rat model (Tanaka et al. 2000). One of the NE effects that is not often discussed is the NGF synthesis induction. Dr. Furukawa reported a significant increase in NGF content after treating cells with norepinephrine (Furukawa et al. 1986). Interestingly, NGF can induce a significant increase in NE release; this was demonstrated by infusing rat brain with NGF, followed by a significant NE increase (Isaacson and Billieu 1996). Thus, a continued cycle of NGF and NF increase may be initiated (Fig. 1).

Stellate Ganglion Block and Its Clinical Applications

SGB is an anesthetic injection next to a cervical sympathetic ganglion; it has recently been shown to have great promise as an intervention for PTSD. The first reported use of SGB to treat PTSD was in 2008 (Lipov et al. 2008). Following original report, a number of institutions have utilized SGB for treatment of PTSD; they were Walter Reed National Medical Center (Mulvaney et al. 2014), Tripler Army Medical Center (Alino et al. 2013), and Advanced Pain Centers (Lipov 2015). Preliminary effectiveness rate seems to be 70 to 75% (Navaie et al. 2014). A recent study by Dr. Alkire used a positron-emission tomography (PET) scanner before and after SGB in patients diagnosed with PTSD. The findings were as follows: SGB dramatically reduced PTSD symptoms in three of five (60%) subjects. Brain region deactivation that correlated with the individual Clinician-Administered PTSD Scale (CAPS) score improvement and their functional improvement following SGB centered on the amygdala and hippocampus, primarily in the right hemisphere (Alkire et al. 2015). Above is consistent with previous report of overactivation of the right amygdala (Liberzon and Martis 2006).

Local Anesthetic Effect on the Sympathetic Ganglion

Local Anesthetic Effect on TrkA and NGF-TrkA Complex

Local anesthetics have been shown to inhibit tyrosine kinase activity of TrkA, which can lead to a suppression of NGF-mediated neurite outgrowth (Takatori et al. 2006) (Fig. 1).

Local Anesthetic and DNA Demethylation

Considering prolonged clinical use of local anesthetic (LA) and the common explanation of its effect the impact of LA on the Na ion channel, it may be difficult to conceive of LA as having impact on the DNA. Yet, bupivacaine has been shown to form stable liposomal-like structures upon direct mixing with plasmid DNA (Pachuk et al. 2000). Further, local anesthetics such as procainamide have been shown to have demethylation properties (Scheinbart et al. 1991; Villar-Garea et al. 2003).

We presume that bupivacaine and similar anesthetics may have demethylation effects, thus potentially reversing trauma-induced DNA methylation. This view is reinforced by a report by Dr. Hyunhwa of gene expression being proportional to PTSD symptom severity (Lee n.d.) (Fig. 1).

Local Anesthetic and Effect on G Protein

As unlikely as local anesthetic interaction with DNA can be, it seems another target for its PTSD effect may be G protein. Local anesthetic can affect G protein by interference with Gαq protein function (a subunit of G protein) (Hollmann et al. 2001). Further, G protein can be affected by trauma, where infant maternal separation (in a mouse model), a paradigm of early life stress in rodents, can alter pre-mRNA expression of the α subunit of the heterotrimeric G protein (Bhansali et al. 2007) (Fig. 1).

Conclusion

Based on the evidence summarized, we believe PTSD is mediated in large part via the sympathetic nervous system. A trauma can lead to DNA methylation, as well as BNGF/NGF level increase, which in turn starts a cascade of sympathetic sprouting, leading to increased brain norepinephrine, and finally symptomatic PTSD. Reversal of this cascade by reducing NGF-TrkA complex and demethylation of DNA can occur by application of the local anesthetic to the stellate ganglion, which reduces NGF, reduces sympathetic sprouting, leading to the reduction of the brain norepinephrine, which finally results in resolution of symptoms of PTSD. Of course, further work regarding this mechanism is needed to confirm this hypothesis, though much other work as well as our publications support this hypothesis.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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