ALPHA-LINOLENIC ACID TREATMENT REDUCES THE CONTUSION AND PREVENTS THE DEVELOPMENT OF ANXIETY-LIKE BEHAVIOR INDUCED BY A MILD TRAUMATIC BRAIN INJURY IN RATS

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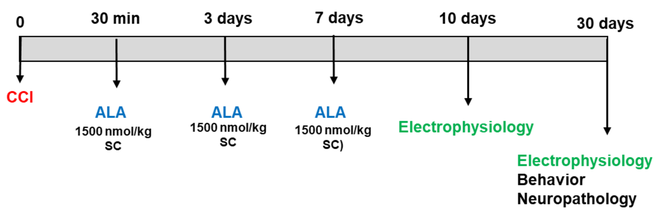
ABSTRACT

Approximately, 1.7 million Americans suffer a TBI annually and TBI is a major cause of death and disability. The majority of the TBI cases are of the mild type and while most patients recover completely from mild TBI (mTBI) about 10% result in persistent symptoms and some result in lifelong disability. Anxiety disorders are the second most common diagnosis post-TBI. Of note, TBI-induced anxiety disorders are difficult to treat and remain a chronic condition suggesting that new therapies are needed. Previous work from our laboratory demonstrated that a mild TBI induced an anxiety-like phenotype, a key feature of the human condition, associated with loss of GABAergic interneurons and hyperexcitability in the basolateral amygdala (BLA) in rodents seven and thirty days after a controlled cortical impact (CCI) injury. We now confirm that animals display significantly increased anxiety-like behavior 30 days after CCI. The anxiety-like behavior was associated with a significant loss of GABAergic interneurons and significant reductions in the frequency and amplitude of spontaneous and miniature $GABA_A$-receptor mediated inhibitory postsynaptic currents (IPSCs) in the BLA. Significantly, subchronic treatment with alpha-linolenic acid (ALA) after CCI prevents the development of anxiety-like behavior, the loss of GABAergic interneurons, the hyperexcitability in the BLA and reduces the impact injury. Taken together, administration of ALA after CCI is a potent therapy against the neuropathology and pathophysiological effects of mTBI in the BLA.

INTRODUCTION

Many human studies have been conducted to ascertain the beneficial effects of ALA and emphasize the possible role of ALA deficiency in the diet that may increase one's risk for certain neurodegenerative disorders [1-5]. In animal studies, ALA is a highly efficacious therapy either as a pretreatment or post-treatment against several models of neurodegenerative disorders including status epilepticus, spinal cord injury and stroke [6-11; 3; 1; 12-16]. However, the therapeutic efficacy of ALA in a model of TBI is obscure. One study showed an anti-inflammatory effect of ALA in the controlled cortical impact model of TBI with improved functional outcome [17].We have recently discovered that, in an experimental model of mTBI, mild controlled cortical impact (mCCI), there is a reduction in $GABA_A$ receptor-mediated inhibitory synaptic transmission in the BLA, a loss of GABAergic interneurons, and a decrease in the surface expression of $GABA_A$ receptors [18]. These results suggest that a reduced inhibitory tonus in the amygdala could significantly contribute to the hyperexcitability and associated emotional deficits observed in mTBI patients. This finding is an important guide to developing novel therapeutic approaches for mTBI, in that it directs efforts towards protecting the brain against loss of GABAergic neuronal function, and the resultant reduced inhibitory tonus, that occur after TBI. Here, we used the CCI model of mild TBI to determine the therapeutic efficacy of ALA on the impact injury, the number of GABAergic interneurons and inhibitory tonus in the BLA and on anxiety-like behavior.

METHODS



RESULTS

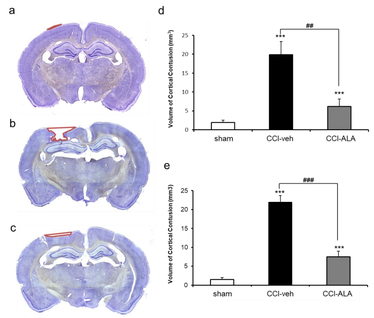


Figure 1. Treatment with α-Linolenic acid at 30 min, 3 days and 7 days after CCI reduces the volume of the cortical contusion quantified 10 and 30 days after CCI. A-C Representative photomicrographs of a coronal brain slice showing the tracing of sham [A] controlled cortical impact-vehicle (CCI-veh) [B] and controlled cortical impact treated with α-linolenic acid (CCI-ALA) [C] animals. Top bar graph [D] shows average and standard errors of cortical contusion volume 10 days after CCI (n = 6-8 group), and bottom bar graph [E] shows average and standard errors of cortical contusion volume 30 days after CCI (n=6- 8 group). ** p<0.01, ***p<0.001 ANOVA, LSD post-hoc test.

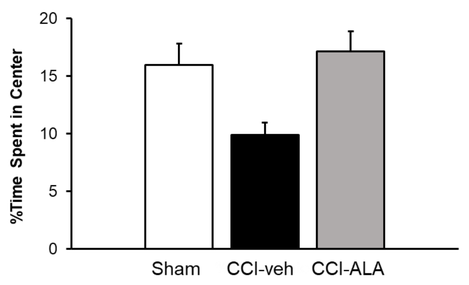


Figure 2. Treatment with Alpha Linolenic Acid 30 min, 3 days and 7 days after Mild Traumatic Brain Injury prevents long term increased anxiety like behavior in the open filed test. (A) CCI-veh (n=18) rats spent significantly less % time in the center of the open field 30 days after the injury compared to CCI-ALA (n= 18) and sham (n=18) rats. No significant differences were found between sham, CCI-veh and CCI-ALA rats in distance traveled (B), vertical activity (C), and movement time (D). *p<0.05

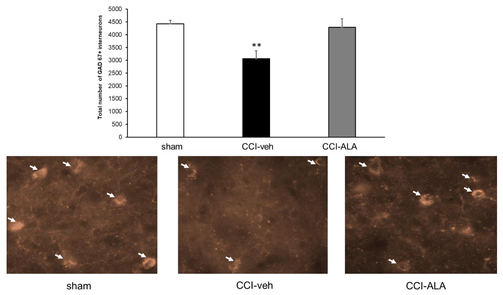


Figure 3. Treatment with Alpha Linolenic Acid 30 min, 3 days and 7 days after mTBI prevents loss of GABAergic inhibitory interneurons in the BLA. (Top) Group data showing the mean and standard error of the stereologically estimated total number of GAD-67 positive cells in the BLA 30 days after the injury. Only CCI-veh rats show a significant reduction in GAD-67 positive cells compared to sham or CCI-ALA rats (**p<0.01, n=4-5 per group). (Bottom) Representative photomicrograph of GAD-67 immunohistochemically stained GABAergic interneurons in the BLA of sham, CCI-veh and CCI-ALA rats 30 days after the injury. Total magnification is 63X, scale bar 50µm.

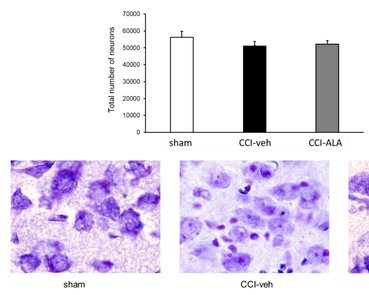


Figure 4. Mild TBI does not cause a significant loss of neurons in the BLA 30 days after injury. (Top) Representative photomicrographs from Nissl-stained sections showing BLA cells from sham, CCI-veh, and CCI-ALA animals, respectively. Total magnification is 630X; scale bar, 50 µm. (Bottom) Group data (mean ± SE; n = 4-5 for each group) of stereological estimation of the total number of Nissl-stained neurons in the BLA.

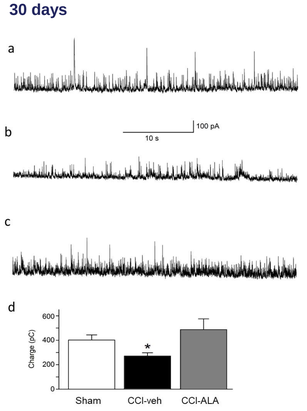


Fig. 5 Effect of ALA on sIPSCs of the principle neurons in the BLA 30 days after mTBI. (A) sIPSCs recorded from BLA pyramidal neuron from sham male rat. (B) sIPSCs recorded from BLA pyramidal neuron from CCI-veh male rat. (C) sIPSCs recorded from BLA pyramidal neuron from CCI-ALA male rat. A, B, C: holding potential + 30 mV. (D) Ordinate axis: charge transferred by sIPSCs measured in pico-Coulombs (pC). Abscissa: columns illustrating the amount of charge transferred by sIPSCs during 40 s at Vh = +30 mV for different animals in different conditions. There was a significant reduction in amount of charge transferred by sIPSCs in CCI-veh rats 30 days after the injury. (two-sample t-test; * p = 0.05).

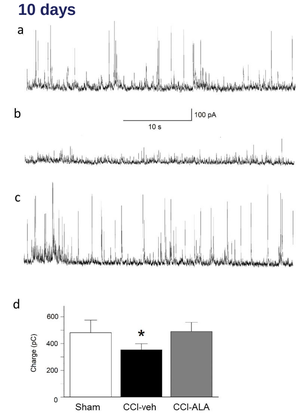


Fig. 6 Effect of α-Linolenic acid on sIPSCs of the principle neurons in the BLA 10 days after mTBI. a sIPSCs recorded from BLA pyramidal neuron from sham male rat. b sIPSCs recorded from BLA pyramidal neuron from CCI-veh male rat. c sIPSCs recorded from BLA pyramidal neuron from CCI-ALA male rat. a, b, c Holding potential + 30 mV. D Ordinate axis: charge transferred by sIPSCs measured in pico-Coulombs (pC). Abscissa: columns illustrating the amount of charge transferred by sIPSCs during 40 s at Vh = + 30 mV for different animals in different conditions. There was a significant reduction in amount of charge transferred by sIPSCs in CCI-veh rats 30 days after the injury. * p < 0.05compared with other groups

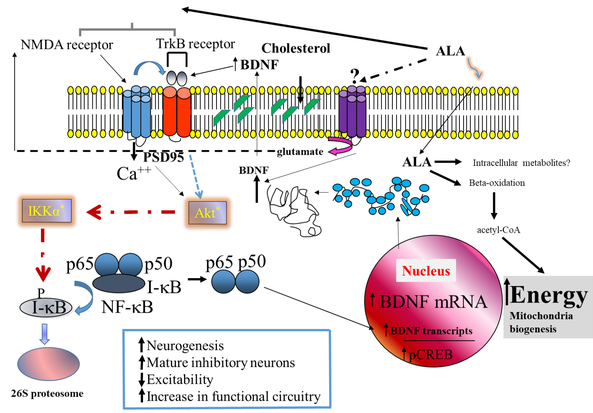


Fig. 7 Possible mechanism of α-linolenic acid-induced increase in inhibitory neurons in the amygdala. Administration of ALA by subcutaneous injection at 30min, 3 and 7 days after CCI may increase the synthesis and release of mature brain-derived neurotrophic factor (BDNF) as shown previously [33]. The increased release of mature BDNF binds to and activates its cognate receptor TrkB rapidly and efficiently due to enhanced lipid raft formation by ALA. Activated N-methyl-D-aspartate (NMDA) and TrkB receptors lead to the activation (phosphorylation*) of Akt, a major pro-survival kinase. Activation of Akt results in the activation (phosphorylation*) of I-κB kinase, the enzyme that phosphorylates I-κB, the inhibitor that prevents the nuclear factor kappa B (NF-κB) dimer from translocating into the nucleus. When the phosphorylated form of I-κB dissociates from the p65/p50 dimer, NF-κB translocates to the nucleus to activate gene transcription. One gene activated by NF-κB along with activated cyclicAMPresponse element binding protein (CREB) is BDNF [17]. We also hypothesize that there is an increased release of glutamate after CCI that binds to and activates NMDA receptors to promote survival [21, 22]. Enhanced mature BDNF protein increases neurogenesis in the amygdala in a similar fashion as we demonstrated in the subgranular zone of the dentate gyrus after soman-induced brain damage [23, 53] and along with metabolism of ALA via β-oxidation to increase energy production and possibly mitochondria biogenesis results in the increase of inhibitory neurons and the absence of the anxiety-like behavior, reduced excitability and restoration of functional circuitry.

DISCUSSION AND CONCLUSION

Traumatic brain injury is responsible for over five million deaths annually throughout the world and is a leading cause of disability and death especially in young adult males [25; 26]. TBI is often bilateral and affects limbic structures such as the amygdala and hippocampus, frontotemporal and basal ganglia resulting in behavioral, emotional, and cognitive impairment. Long-term consequences include neurological and neuropsychiatric disorders; anxiety disorders rank second only after depression. The most common diagnosis within the anxiety disorders is general anxiety disorders. While treatments for these long-term consequences are available, they are difficult to treat and no formal clinical trials have been conducted in those that develop these disorders post-TBI [27-28; 29; 30]. In addition, general anxiety disorders develop into chronic conditions within the mild to moderate group of those individuals that sustain a traumatic brain injury [30]. These findings suggest that newer more effective therapies are needed for post-TBI anxiety disorders. ALA, a nutraceutical with pleiotropic properties and a natural product that has a very wide safety margin, should be further developed as a therapy to reduce brain injuries and prevent the long-term anxiety disorders that develop after TBI.

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