Valproic acid effects in the hippocampus and prefrontal cortex in an animal model of post-traumatic stress disorder

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HIGHLIGHTS

• The predator exposure model of PTSD elevates inflammation and oxidative stress.
• The predator exposure model of PTSD modulates neurotransmitters and increases anxiety.
• Valproic acid diminishes HDAC activity, NF-κB transcription and inflammatory cytokines.
• Valproic acid corrects neurotransmitter aberrancies by modulating rate-limiting enzymes.
• Valproic acid increases fear extinction by increasing serotonin and decreasing catecholamines.

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ABSTRACT

Reactive oxygen species (ROS) and pro-inflammatory cytokines (PIC) are upregulated in post-traumatic stress disorder (PTSD). Histone deacetylase inhibitors (HDACi) modify genetic transcription and can diminish ROS and PIC escalation. They can also modulate levels of neurotransmitters such as catecholamines and serotonin (5-HT). Thus, this study sought to analyze the effects of the HDACi valproic acid (VA) on oxidative stress, inflammation, and neurotransmitter modulation via a predator exposure/pyschosocial stress animal model of PTSD. PTSD-like effects were induced in male Sprague-Dawley rats (n = 8/group × 4 groups). The rats were secured in Plexiglas cylinders and placed in a cage with a cat for 1 h on days 1, 11, and 40 of a 40-day stress regimen. PTSD rats were also subjected to psychosocial stress via daily cage cohort changes. At the conclusion of the stress regimen, the treatment group (PTSD + VA) and control group (Control + VA) rats were given VA in their drinking water for 30 days. The rats were then euthanized and their brains were dissected to remove the hippocampus and prefrontal cortex (PFC). Whole blood was collected to assess systemic oxidative stress. ROS and PIC mRNA and protein elevation in the PTSD group were normalized with VA. Anxiety decreased in this group via improved performance on the elevated plus-maze (EPM). No changes were attributed to VA in the control group, and no improvements were noted in the vehicle groups. Results indicate VA can attenuate oxidative stress and inflammation, enhance fear extinction, and correct neurotransmitter aberrancies in a rat model of PTSD.

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1. Introduction

Post-traumatic stress disorder (PTSD), an anxiety disorder recently reclassified as a trauma- and stressor-related disorder, can develop in response to real or perceived life-threatening situations and results in a prolonged stress response [1]. According to the Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5), a diagnosis of PTSD necessitates exposure to a traumatic event, intrusive recollections, avoidance of associated stimuli, negative cognitions/mood, hyperarousal, and a significant social impairment. These symptoms must persist for at least 30 days and not be due to illness, medication, or substance abuse [2]. To date, no definitive diagnostic biomarkers have been identified for PTSD. Current research, however, points toward physiological abnormalities in
the hypothalamic–pituitary–adrenal (HPA) axis, sympathetic adrenal medullary system, immune system, and brain neurotransmitters that may be responsible for the progression of the disorder [3–6]. Many chronic conditions such as hypertension, heart failure, and metabolic syndrome perpetuate in a state of inflammation and oxidative stress [7–9]. In PTSD, inflammation and oxidative stress also persist. Our lab recently demonstrated ROS and PICs were elevated in the brain and systemic circulation during PTSD progression [10].

Inflammation is a critical component of the immune response, but chronic inflammation can damage cellular mechanisms. There are a host of triggers initiating the inflammatory response, many of which are initiated via Toll-like receptor-4 (TLR4) and subsequent nuclear factor (NF)-κB activation. Transcription by NF-κB requires DNA and chromatin remodeling, which enables access to the pertinent genomic sequences. Gene expression is regulated via highly controlled acetylation/deacetylation of histone N-terminal tails, which either increases or decreases gene availability [11]. Acetylation/deacetylation is accomplished by histone acetyltransferases (HAT) and histone deacetylases (HDAC), which enable and restrict genome access, respectively. When oxidative stress and inflammation are increased, upregulated PICs can correspond with heightened HDAC activity and NF-κB transcription, resulting in perpetual PIC production [12]. Although HDACs restrict access for transcription and should depress PIC production, the exact opposite may be the case. The effects of HDAC inhibitors (HDACi) extend to non-histone proteins that are reversibly acetylated, which markedly affects their function [13,14]. This functional shift means HDACs may actually enhance the inflammatory response. In addition to histone modification, HDACs may also modulate neurotransmitters by modifying levels of pertinent rate-limiting enzymes [15]. For example, tyrosine hydroxylase, the rate-limiting enzyme for dopamine (DA) and norepinephrine (NE) synthesis, is depressed by HDACi [16]. Studies have also shown antidepressant effects of HDACi [17], possibly due to epigenetic modifications of tryptophan hydroxylase, the rate-limiting enzyme for serotonin (5-HT) synthesis.

Currently, HDACi have proven beneficial as anticonvulsants and mood-stabilizers and have received attention as treatment for some forms of cancer [18]. They have also been shown to modify receptors and other neuronal cellular mechanisms that have a role in synaptic plasticity and learned behavior [19]. Research has demonstrated that administration of HDACi enhanced synaptic plasticity, dendritic growth, and the extinction of learned behavior in a drug-induced animal model [20]. A recent study revealed that the HDACi valproate enhances fear extinction [21]. Since fear extinction is an important component of PTSD therapy, it follows that HDACi administration in PTSD may prove beneficial in diminishing negative learned behavior and enhancing resiliency. Our lab recently demonstrated the efficacy of valproic acid (VA) in diminishing ROS and PIC escalation in hypertension [7]. Oxidative stress and inflammation can damage neurons and negatively influence synaptic plasticity, so we hypothesized that attenuating this effect may diminish PTSD symptoms and improve overall resiliency behavior.

Based on this information, this study investigated whether chronic HDACi administration could modulate behavior, redox imbalances, inflammation, and neurotransmitters during the progression of PTSD, using a predator exposure/psychosocial stress animal model.

### 2. Materials and methods

#### 2.1. Ethics statement

This study was carried out in accordance with the recommendations of the Institute for Laboratory Animal Research’s 2011 Guide for the Care and Use of Laboratory Animals, under the auspices of an animal care and use protocol approved by the Louisiana State University Institutional Animal Care and Use Committee.

#### 2.2. Animals

Naïve male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were used in all experiments. Rats were the same age (12 weeks) and weight upon delivery. Rats were pair-housed in standard plastic cages with access to food and water ad libitum. The vivarium room was kept on a 12-h light:12-h dark cycle, temperature was maintained at 20 ± 1 °C, and humidity ranged from 23% to 42%. After a 1-week acclimation period, the mean weight of all rats was 347.9 ± 4.5. Two cats, a male and a female (Harlan Laboratories, Indianapolis, IN) were used for all predator exposures. They were housed in an open room (15 in. × 15 in.) in the vivarium with access to food, water, and enrichment devices ad libitum. The cat room was on the same light/dark cycle and maintained at similar temperature and humidity.

#### 2.3. Stress induction

The predator exposure/psychosocial stress regimen is designed to induce a PTSD-like syndrome as true PTSD is clinically defined as a human disorder. Following the acclimation period, rats were weighed, ear-tagged, tail-marked, and 250–500 μL of blood was drawn from the tail vein. The rats were then randomly assigned to the PTSD or control group and returned to the vivarium for 24 h. The following day, PTSD rats were started on a predator exposure/psychosocial stress regimen as previously described [6,10,22,23]. Briefly, PTSD rats were isolated in cylindrical containers and canned cat food was smeared on the outside of the cylinders. Rats were then placed in a stainless steel cage with a cat for one hour. The first cat exposure was conducted during the light cycle (07:00–19:00). Ten days later, a second cat exposure was conducted during the dark cycle (19:00–07:00). The third exposure was during the light cycle. Rats were also subjected to daily cage cohort changes. The regimen continued for 40 days, after which certain PTSD and control group rats were administered valproic acid (VA) for 30 days. Rats were then euthanized, perfused with a phosphate buffered solution, and the brains were removed. The hippocampus and PFC were dissected and flash-frozen in liquid nitrogen.

#### 2.4. Elevated plus-maze

Elevated plus-maze (EPM) testing was conducted as previously described [6,10]. Animals were placed on the maze facing an open arm and allowed to roam freely for 5 min. Movement was monitored via an overhead camera and captured with a software program. The primary measurements were the total number of arm entries and the time spent in the open vs. closed arms.

#### 2.5. Valproic acid

Rats were pair-housed and administered VA in their water bottles. Vehicle (water) or valproic acid (VA, 0.71% (w/v), Sigma) dissolved in water were prepared and provided daily for 30 days as previously described [7].

#### 2.6. Electron paramagnetic resonance spectroscopy

Superoxide and total ROS (n = 6/group) were measured in whole blood via electron paramagnetic resonance (EPR) as previously described [10]. Superoxide and total ROS levels were compared between all four groups. Briefly, blood was incubated at 37 °C with CMH (200 μM) for 30 min for ROS measurement, PEG-SOD
(50 U/μl) for 30 min, then CMH (200 μM) for an additional 30 min for O2•− measurement. Aliquots of incubated probe media were then taken in 50-μl disposable glass capillary tubes for determination of O2•− or total ROS production.

2.7. Real-time PCR analysis

Semi-quantitative real-time RT-PCR (n=6/group) was used to determine the mRNA levels of TLR4, NALP3, IL-1β, and IL-18 in the PFC and hippocampus. The primer sequences used for real-time PCR are given (Table 1). Total RNA isolation, cDNA synthesis and RT-PCR were performed as previously described [10,24].

2.8. Western blot analysis

Tissue homogenates from the PFC and hippocampus were subjected to western blot (WB) analysis (n=6) for the determination of protein levels of TLR4, NALP3, IL-1β, IL-18, TH, TPH, and β-actin. The extraction of protein and WB was performed as previously described [6,10,24]. Primary antibodies: IL-1β, IL-18, and β-actin, 1:1000 dilution (SC-7884, SC-7954, and SC-1616R respectively, Santa Cruz Biotechnology, Santa Cruz, CA); NALP3, 1:1000 dilution (ORB-101128, Bioryt, San Francisco, CA), TLR4, 1:1000 dilution, TH, 1:200 dilution (ab13556 and ab112 respectively, ABAM, Cambridge, MA), and TPH, 1:1000 dilution (AB1541, Millipore, Billerica, MA). Secondary antibodies: anti-rabbit and anti-mouse, 1:5000 dilution (SC-2004 and SC-2314 Santa Cruz Biotechnology, Santa Cruz, CA).

2.9. High-performance liquid chromatography (HPLC)

Neurotransmitter concentrations were detected using Eicom HTEC 500 HPLC system. The standard solutions of NE (MW 337.3), DA (MW 158.17), 5-hydroxytryptamine (5-HT; MW 212.68) and isoproterenol (internal standard; MW 247.7) were each 1 ng/μl concentrations. Sample preparations were carried out as previously described [6,10,25].

2.9.1. HPLC-detection of neurotransmitters

HPLC system working conditions: isocratic elution; mobile phase (citrate buffer in methanol with EDTA and sodium octane sulfonate); Eicom Pak SC-30DS (ID 3.0 x 100 mm) column; flow rate 340 μl/min; graphite working electrode WE-3G (Gasket GS-25) (+750 mV versus Ag/AgCl electrode); temperature 25 °C.

2.9.2. HPLC-mobile phase

Citic acid monohydrate (8.84 g; mol wt. 210.14), and 3.10 g of sodium acetate (mol. wt. 82.03) in 800 ml of MilliQ Ultrapure fresh water (>18.2MΩ/cm) and 200 ml of HPLC grade methanol were added. EDTA (mol. wt. 372.24; 0.005 g) and sodium octane sulfonate (0.220 g), both from Dojin Laboratories, Rockville, MD, were added.

2.10. HDAC activity analysis

Nuclear extracts of PFC and hippocampus tissue were obtained with a Nuclear Extraction Kit (K266-100, BioVision, Milpitas, CA). Nuclear extracts were then analyzed for HDAC activity with a Colorimetric HDAC Activity Assay Kit (K331-100, BioVision), both according to the manufacturer’s instructions.

2.11. Assessment of NF-κB activity

An ELISA kit (ab133112, Abcam, Cambridge, MA) was used to assess the activity of NF-κB in the nuclear extracts of the PFC and hippocampus according to the manufacturer’s instructions.

2.12. Statistical analysis

Data are presented as mean ± SEM. Statistical analysis conducted by one-way ANOVA with a Bonferroni post hoc test, unpaired Student’s t-tests, and four-parameter logistic regression for curve fit. p-values less than 0.05 were considered significant. Statistical analyses were performed using Prism (GraphPad Software, Inc; version 5.0).

3. Results

3.1. Elevated plus-maze performance

Immediately following the stress regime, the PTSD group spent considerably less time in the open vs. closed arms, t(10) = 3.99, p < 0.001. Overall ambulations, however, were not affected, t(10) = 0.88, p > 0.05. After the 30-day VA treatment, the PTSD + VA group spent significantly more time in the open arms vs. the PTSD + Veh group, F(3,22) = 41.93, p < 0.01. The control group showed no difference between the control + VA and control + Veh groups, F(3,22) = 5.17, p > 0.05 (Fig. 1A). No differences were found in overall ambulations between or within groups, F(3,22) = 1.52, p > 0.05 (Fig. 1B). Repeated measures were also conducted to analyze anxiety changes within the same groups over time. No changes were found in the control group between pre-treatment, control + Veh, and control + VA, F(2,21) = 3.07, p > 0.05 (Fig. 1C). In the PTSD group, however, significant differences were noted between pre-treatment, PTSD + Veh, and PTSD + VA, F(2,27) = 14.88, p < 0.0001 (Fig. 1D).

3.2. Oxidative stress analysis

Analysis of the EPR data revealed superoxide was significantly elevated in whole blood in the PTSD group vs. controls, and it diminished with VA, F(3,18) = 2.54, p < 0.05. No difference was noted in the control + VA vs. the control + Veh groups, F(1,8) = 0.07, p > 0.05 (Fig. 2A). Total ROS was also significantly elevated in the PTSD group vs. controls, and it was also decreased with VA, F(3,18) = 3.13, p < 0.05. No difference was noted in the control + VA vs. the control + Veh groups, F(1,8) = 0.64, p > 0.05 (Fig. 2B).

3.3. Brain inflammatory markers

The PTSD group demonstrated elevated mRNA levels of TLR4, F(3,12) = 6.37 and 7.14, p < 0.05 (Fig. 3A and B); NALP3, F(3,12) = 5.69 and 17.10, p < 0.05 (Fig. 3C and D); IL-1β, F(3,12) = 6.08 and 2.85, p < 0.05 and p > 0.05 (Fig. 3E and F); and IL-18, F(3,12) = 44.84 and 5.45, p < 0.05 (Fig. 3G and H). Valproic acid administration normalized the upregulated mRNA to levels similar as the untreated controls. In the PFC (Fig. 3I) and hippocampus (Fig. 3J), both regions also demonstrated elevated protein levels of TLR4, F(3,4) = 30.52 and 454.70, p < 0.05, NALP3, F(3,4) = 9.42 and 27.67, p < 0.05, IL-1β, F(3,4) = 12.03 and 12.83, p < 0.05, and IL-18, F(3,4) = 130.0 and 50.18, p < 0.05. Valproic acid administration also attenuated the upregulated protein to levels similar as the untreated controls.
3.4. HDAC activity

Analysis of HDAC activity in the PFC revealed increased HDAC in the untreated PTSD group vs. controls, which was subsequently attenuated with VA (Fig. 4A), F(3,17) = 3.282, p < 0.05. HDAC activity in the hippocampus was elevated in the untreated PTSD vs. control group (did not reach significance), but it was significantly downregulated in the PTSD+VA group (Fig. 4B), F(3,17) = 2.006, p < 0.05.

3.5. NF-κB transcription

The transcription factor NF-κB was significantly elevated in the PFC in the untreated PTSD group vs. controls, which was normalized with VA (Fig. 4C), F(3,17) = 13.86, p < 0.0001. In the hippocampus, NF-κB was also upregulated in the untreated PTSD group vs. controls, which was subsequently downregulated with VA (Fig. 4D), F(3,17) = 16.66, p < 0.0001.

3.6. Neurotransmitter modulation

In the PFC, the catecholamines NE (Fig. 5A) and DA (Fig. 5C) were significantly higher in the PTSD group vs. controls, F(3,17) = 8.66 and F(3,17) = 36.32, p < 0.05, respectively. Conversely, 5-HT (Fig. 5E) was significantly lower in the PTSD group vs. controls, F(3,17) = 7.41, p < 0.05. Valproic acid administration lowered catecholamine levels and increased 5-HT to levels similar as the untreated controls. In the hippocampus, NE (Fig. 5B) was significantly higher in the PTSD group vs. controls, F(3,17) = 58.57, but no significant difference was found in DA levels, F(3,17) = 1.56, p > 0.05. The level of 5-HT (Fig. 5E) was lower in the PTSD group vs. controls, F(3,17) = 4.05, p < 0.05.

Fig. 1. Elevated plus-maze performance. The PTSD group spent considerably less time in the open vs. closed arms. After VA treatment, the PTSD + VA group spent significantly more time in the open arms vs. the PTSD + Veh group. The control group showed no difference between the control + VA and control + Veh groups (A). No differences were found in overall ambulations between or within groups (B). Repeated measures found no difference in the control group between pre-treatment, control + Veh, and control + VA (C). In the PTSD group significant differences were noted between pre-treatment, PTSD + Veh, and PTSD + VA (D). All data are presented as mean ± SEM. **p < 0.01, ***p < 0.001 relative to the control group. ##p < 0.001, ###p < 0.0001 relative to the treatment group.

Fig. 2. Reactive oxygen levels. Superoxide (A) and total ROS (B) were significantly elevated in the circulating blood in the PTSD group. The elevation was normalized to control group levels with VA. All data are presented as mean ± SEM. *p < 0.05 relative to the control group. #p < 0.05, ##p < 0.001 relative to the treatment group.

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**4. Discussion**

The present study sought to analyze the effects of HDACi administration on the behavior and physiology associated with PTSD by employing a predator exposure/psychosocial stress regimen. Multiple animal models designed to create PTSD-like effects are reported, but a particular model that seems to meet face, predictive, and construct validity is the predator exposure/psychosocial stress regimen [26]. The model by Zoladz et al. has been shown to cause heightened anxiety, exaggerated startle response, impaired cognition, and increased cardiovascular reactivity [22,23], all of which are common symptoms reported in humans with PTSD [27,28]. In addition, our lab recently demonstrated that the predator exposure model produced neurotransmitter modulation and altered catecholamine and 5-HT rate-limiting enzymes [6]. Although HDACi are currently prescribed for certain neurologic disorders and show efficacy as anti-inflammatory agents, their utility in PTSD treatment has been largely unexplored. We have successfully obtained data with this model indicating HDACi (VA) may be a promising pharmacologic therapy for PTSD patients. Three novel and important findings emerged from this study. First, increased oxidative stress and inflammation in the brain and blood in response to psychological stress are attenuated with VA. Second, the aberrant neurotransmitter profile of increased catecholamines and decreased 5-HT was normalized with VA. Lastly, VA seems to enhance resiliency behavior as indicated by reduced anxiety levels in the PTSD + VA group.

Histone deacetylases (HDAC), along with histone acetyltransferases (HAT), modulate gene expression by decreasing or increasing gene availability, respectively, HDACs and HATs act in concert with one another, maintaining homeostasis during normal cellular functions [11]. During abnormal states, however, this imbalance is tilted in favor of HDAC activity, resulting in increased oxidative stress and inflammation [29–31]. It is an oversimplification to state that acetylation increases and deacetylation decreases gene expression, as research has shown that non-histone proteins...
can be reversibly acetylated, which drastically affects their function [32]. Current research has demonstrated that elevation of PIC expression can increase HDAC activity, which is directly proportional to heightened NF-κB transcription and further production of PICs [12]. As a result, HDAC inhibition may be relevant to treatment of neuroinflammatory disorders [33]. Our findings that a PTSD-like syndrome increases NF-κB expression and HDAC activity in the stressed animals positively correlate with the increased ROS and PIC levels. In addition, the attenuation of inflammatory mediators in the PTSD + VA group confirms previous findings that HDACs have anti-inflammatory properties.

The roles of oxidative stress and inflammation in pathological conditions such as cardiovascular disease, diabetes mellitus, metabolic syndrome, and neurological diseases are well established [9,24,34,35]. We recently demonstrated increased oxidative stress and inflammation in the brain and systemic circulation of rats subjected to the predator exposure model [10]. Reactive oxygen and nitrogen radicals have unpaired electrons that can cause damage by oxidizing proteins, lipids, nucleic acids, and other cellular components. The damage caused by ROS can activate transcription factors such as NF-κB, leading to increased production of cytokines and chronic inflammation [36]. Immunomodulating cytokines function to transmit information concerning inflammatory responses to the CNS [37–40]. The CNS then participates in negative feedback regulation of the peripheral immune response by releasing pituitary hormones and norepinephrine (NE) which increase sympathetic drive. In chronic stress-related conditions such as PTSD, however, a sustained sympathoexcitatory state can alter the Ti1/Ti2 cell balance and actually increase PIC production [41].

A primary initiator of the inflammatory response is the inflammasome, a multi-protein complex that upregulates PICs. In our previous work, we demonstrated that the NALP3 inflammasome was upregulated in the predator exposure/psychosocial stress PTSD model [10]. NALP3, possibly in a two-stage process with TLR4, utilizes caspase-1 to cleave pro-IL-1β and pro-IL-18 to their active forms, leading to upregulation of other PICs and inflammatory components. A few of these components have been implicated in PTSD pathophysiology. Studies show that PTSD was exacerbated by increased levels of NO and other ROS, causing cellular damage in the hippocampus [3]. Other research discovered DNA breakage in the hippocampus of rats subjected to stress [42]. Inflammasomes are primarily found in resident macrophages and granulocytes, but they are also key inflammatory mediators in non-myeloid cells [43], which may explain the increase in PICs in the blood and periphery during PTSD progression.

One of the diagnostic criteria for PTSD is hyperarousal, which includes an exaggerated startle response and heightened anxiety [2]. Previous research with the predator exposure model also demonstrated memory-related impairments of the hippocampus during anxiety testing [44]. To measure anxiety levels, we used the elevated plus-maze (EPM). Rodents have a natural tendency to explore novel environments, but open areas or alleys invoke a greater fear and avoidance response [45]. The EPM is widely used as a measure to test anxiety and has been extensively validated for use in rats [46,47]. Entry into the open areas is associated with increased freezing behavior as well as increased plasma corticosterone levels, indicating heightened anxiety [47]. Anxiogenic compounds or procedures can increase avoidance of the fear-provoking open arms, whereas anxiolytic compounds or procedures can increase open arm exploration [47]. The primary criteria correlated with anxiety levels are total arm entries and the percent time spent in the open vs. closed arms. We found that VA had an anxiolytic effect, as rats in the PTSD + VA group displayed an increase in open arm exploration vs. the PTSD + Veh group.

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Fig. 5. Neurotransmitter modulation. In the PFC, the catecholamines NE (A) and DA (C) were significantly higher in the PTSD group vs. controls, F(3,17)=8.66 and F(3,17)=36.32, p<0.05, respectively. Conversely, the monoamine 5-HT (E) was significantly lower in the PTSD group vs. controls, F(3,17)=7.41, p<0.05. VA administration lowered catecholamine levels and increased 5-HT to levels similar as the untreated controls. In the hippocampus, NE (B) was significantly higher in the PTSD group vs. controls, F(3,17)=58.57, but no significant difference was found in DA (D) levels, F(3,17)=1.56, p>0.05. The level of 5-HT (F) was lower in the PTSD group vs. controls, F(3,17)=30.65, p<0.05. Valproic acid administration lowered NE levels and increased 5-HT to levels similar as the untreated controls. **p<0.001, ***p<0.0001 relative to the control group.

The modulation of various neurotransmitters observed with the predator exposure/psychosocial stress model is in concert with many of the neurotransmitter changes seen in human PTSD patients [48–51]. Previous research has shown that stress blocks hippocampal long-term potentiation (LTP) and impairs its function [52]. The hippocampus is the primary region for spatial and long-term memory storage and expresses all of the 5-HT receptor families, which reflects the overall serotonergic functions relating to cognition and mood in this region [53]. In addition, the hippocampus has the highest density of glucocorticoid receptors compared to other brain regions [54]. During stress, glucocorticoid production can reduce the excitability of hippocampal neurons, and 5-HT may have a protective effect against such damage by activating 5-HT1A receptors [55]. Persistent activation of the HPA axis and excessive production of glucocorticoids, however, may directly reduce hippocampal 5-HT levels and adversely affect normal serotonergic transmission, thus contributing to heightened fear, depressed mood, and reduced resilience. The hippocampus also contains multiple NE receptors which, when activated during stress, may contribute to the reinforcement of long-term memories [56]. In a study by Geraci et al. involving male combat veterans with PTSD, CSF concentrations of NE were significantly higher vs. controls [50]. This finding could possibly explain why memories formed during extremely stressful events persist over time.

Other evidence of catecholamine dysregulation in PTSD includes elevated urine catecholamine excretion, exaggerated biochemical responses to yohimbine, and clinical efficacy of adrenergic blockers [57]. Noradrenergic modulation was also noted with previous experiments utilizing the predator exposure/psychosocial stress animal model and treatments with selective serotonin reuptake enhancers (SSRE), α2 agonists, and tricyclic antidepressants [58]. Although we found no significant difference in DA levels in the hippocampus, we noted a reduction in homovanillic acid (HVA) levels (not shown), which was consistent with current human PTSD research [49]. HVA is a downstream product of DA metabolism, and traumatic stress may impede CNS release of DA from the substantia nigra and ventral tegmental area (VTA) [59], the primary CNS regions of dopaminergic neurons, thus reducing metabolite concentration.

The PFC, responsible for executive functions such as consequences, drive, and social "control," is highly innervated by serotonergic neurons from the raphé nuclei and also expresses an abundance of 5-HT receptors. The 5-HT1A and 5-HT2A receptors are a key modulator of the PFC-amygda/ corticobulbic circuit involved in threat and emotional responses [60]. PTSD-related aberrancies in this serotonergic system may cause inappropriate or incomplete extinction of conditioned fear. The PFC also contains NE receptors and receives input from NE neurons from the
locus coeruleus, which are activated during the stress response [61]. Pathological or stress–related elevations of NE in the PFC, however, may inhibit working memory and performance [62]. Current neuroimaging research indicates the PFC is hyporesponsive during symptomatic PTSD states and responsiveness is inversely proportional to symptom severity [63]. In contrast to unchanged DA levels in the hippocampus, DA levels were significantly increased in the PFC, which was consistent to the similar CSF and urine DA elevations seen in humans with PTSD [51,64,65]. In a similar manner to NE, stress–related elevations of DA may also impair working memory and performance. The PFC is densely innervated by dopaminergic neurons from the VTA, and dopamine release can be achieved via VTA or local stimulation. A recent study by Butts et al. demonstrated that stress-induced glucocorticoid stimulation of DA neurons caused a local release of DA in the PFC [66]. These data support the theory that overstimulation of the HPA axis and the resulting elevation in glucocorticoid activity can directly modulate DA and other neurotransmitters. Taken together, our findings that VA was able to increase 5-HT and decrease catecholamine levels in the brain, apparently by modulating levels of the rate-limiting enzymes for the respective neurotransmitters, represents a completely novel finding in an animal model of PTSD and demonstrates a valid justification for VA as an alternative to currently accepted pharmacotherapy.

5. Conclusions

This study was conducted to test alternative pharmacologic therapies for PTSD, as the currently approved selective-serotonin reuptake inhibitors (SSRIs) have proven nominally effective [67]. Based on their mechanism of action, SSRIs should increase 5-HT levels and attenuate many of the detrimental effects of lowered 5-HT. This concept has proven effective in the treatment of depression [68,69]. In PTSD, however, SSRI efficacy can be classified as questionable at best. In a study by Davidson et al., they demonstrated decreased severity of symptoms and an overall increase in functioning in the PTSD patients vs. controls [70]. This study, however, had uneven gender, racial, and traumatic event distribution. The data showed a 45% increase in symptom improvement in the treatment group, but also a 36% increase in the placebo group. Taken together, the numbers indicate a significant portion of the noted improvement may be due to a placebo effect. The study did not perform physiological testing and therefore could not anticipate modulation of other neurotransmitters in response to serotonin reuptake. We have achieved such data and have successfully demonstrated that VA produces both physiological and behavioral changes in animals with a PTSD-like syndrome.

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