Journal of Neurology & Neurophysiology

Epps and Allen, J Neurol Neurophysiol 2017, 8:6
DOI: 10.4172/2155-9562.1000454

Review Article Open Access

Neurovascular Coupling: A Unifying Theory for Post-Concussion Syndrome Treatment and Functional Neuroimaging

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Received date: November 28, 2017; **Accepted date:** December 21, 2017; **Published date:** December 24, 2017

Abstract

Post-concussion syndrome (PCS) occurs in a significant percentage of concussion patients and is defined as having a history of traumatic brain injury with persistence of three or more symptoms. Standard structural clinical neuroimaging studies show no abnormal findings for the majority of PCS patients as opposed to functional MRI, which often reveals irregularities in the blood-oxygen level dependent (BOLD) signal. This suggests that dysregulation of neurovascular coupling, which causes abnormal BOLD signals, plays a significant role in PCS pathology. However, compared to the pathophysiologic mechanisms occurring in acute concussion, the underlying neuropathophysiology of chronic concussive sequelaeor PCS is less understood, though becoming clearer with emerging research. We present a treatment approach grounded in the physiological theory presented here called Enhanced Performance in Cognition (EPIC), which has shown strong clinical success. Dysregulation of neurovascular coupling (NVC), along with disruptions in cerebrovascular reactivity (CVR) and autonomic nervous system (ANS) dysregulation are the targets of EPIC treatment. Success of the approach itself tentatively supports the hypothesis that these three features figure prominently in the neuropathophysiology of PCS. The aim of this report is to provide a theory of the underlying mechanisms of PCS pathology and its treatment that is in accord with the current corpus of research and explains the recent therapeutic success seen in PCS patient using the EPIC treatment. We propose a novel theory concerning the mechanisms by which NVC dysregulation is normalized. This includes focused, intense and repetitive neurocognitive challenges during post-exercise cognitive boost and the avoidance of intracerebral steal in the setting of restored and re-regulated CVR and ANS. It is intended that both the theory and treatment approach are presented explicitly enough to generate empirical studies with clear hypothetical predictions from the theory as well as clinical innovations with significant relevance to improve current practices.

Keywords: Post-concussion syndrome; Post-concussion syndrome; Autonomic nervous system; Cerebral blood flow; Cerebral vascular reactivity; Neurovascular coupling; Functional MRI

Introduction

Mild traumatic brain injury (mTBI) represents approximately 70-90% of traumatic brain injuries in the United States with an incidence of 600 in 100,000 people per year [1,2]. Though the terms mTBI and concussion are often used interchangeably, concussion represents a variety of mTBI characterized by the absence of structural brain damage, though clinical manifestations may be similar [3]. Concussive symptoms typically resolve in 7 to 10 days (sports-related concussions) or within 3 months (non-athletes) [4]. However, approximately 33% of patients will have persistence of symptoms with 30% of those patients meeting post-concussion syndrome (PCS) criteria 6 months out from time of injury [5-7]. PCS is defined as having a history of traumatic brain injury with persistence of three or more symptoms (i.e., headache, dizziness, fatigue, irritability and insomnia, difficulty in concentration or memory and intolerance of stress, emotion or alcohol) [8].

Standard structural clinical neuroimaging studies have no abnormal findings for the majority of PCS patients as the clinical presentations of PCS are thought to be caused by cerebrovascular dysregulation and neuronal dysfunction [9-11]. However, functional MRI (fMRI), which uses blood oxygen-level dependent (BOLD) signaling, has shown abnormalities in patients with PCS [12,13]. The BOLD signal detected in fMRI has been shown to be a reliable, indirect measure of neuronal activity in the form of vascular responsiveness to neuronal activity or neurovascular coupling (NVC). In the healthy brain, it has demonstrated a high level of consistency [14,15]. Therefore, when an abnormal BOLD signal is observed, the problem is with dysfunctional neural activation, dysfunctional NVC or both. Considering PCS, which by definition lacks gross structural injury, the observed BOLD abnormalities are more likely a result of dysfunctional NVC rather than dysfunctional neural activation and likely the predominant source of its symptoms. Further recent studies confirm a central role of NVC disruption in PCS [16-22].

In short, it is becoming increasingly clear that NVC alterations, along with cerebrovascular reactivity (CVR) disruptions and autonomic nervous system (ANS) dysregulation, play a significant role in PCS sequelae. The Enhanced Performance in Cognition (EPIC) treatment shows clear promise in the efforts to target NVC alterations (as well as CVR disruptions and ANS dysregulation) in the treatment of PCS [23]. However, a unifying theory describing how this treatment normalizes the pathologic state of PCS and is consistent with relevant research has yet to be proposed. Therefore, the aims of this article are two-fold. First, we propose a unifying theory of the pathologic state of

PCS that is consistent with recent research findings by describing the nature of NVC dysfunction, CVR disruption and ANS dysregulation, including cerebral dysautoregulation (CD) and the role they play in the pathologic state of PCS. Second, we seek to explain the mechanisms by which EPIC treatment (or any similar approach) normalizes the pathologic state of PCS. In particular, we propose a novel theory concerning how EPIC or EPIC-style treatment helps to re-regulate NVC in PCS patients.

The significance of such a proposed theory should not be understated as it holds both clinical and research implications. Clinically, there is no gold standard for the diagnosis or treatment of PCS and, despite the given definition, diagnostic criteria are based on subjective symptoms and lack general agreement and specificity. Given this, there are even differences in the estimated prevalence of PCS. Concerning treatment, PCS patients traditionally are educated and prescribed rest, neurocognitive rehabilitation and antidepressants, all without much evidence of success [24]. Some researchers, however, have focused on developing PCS diagnostic and treatment approaches based on the physiologic theory of concussion [25]. However, though individual components of the physiologic dysfunction occurring in PCS have been investigated (such as ANS dysfunction and CD), there lacks a proposed universal theory of the pathologic state of PCS. This is in stark contrast to the pathophysiologic mechanisms of acute concussion, which Giza et al. [9,11] describe and which theory has become largely accepted.

Our proposed theory of the pathologic state of PCS also has significant research implications. Because EPIC treatment was designed to target specific aspects of the pathologic state of PCS, as this approach shows very strong initial positive outcomes, a theory proposing how this treatment normalizes PCS pathology may function as a platform from which the true pathophysiologic processes underlying PCS may be reversed-engineered. That is, by proposing a theory of how the EPIC treatment works, hypotheses and studies to support or refute those hypotheses, can be generated testing individual aspects of the theory; thus, clarifying our understanding of PCS in both clinical and mechanistic terms.

The Pathologic State of PCS

Overview

An overview of our theory of the pathologic state leading to abnormal BOLD signal in PCS can be found in Figure 1.

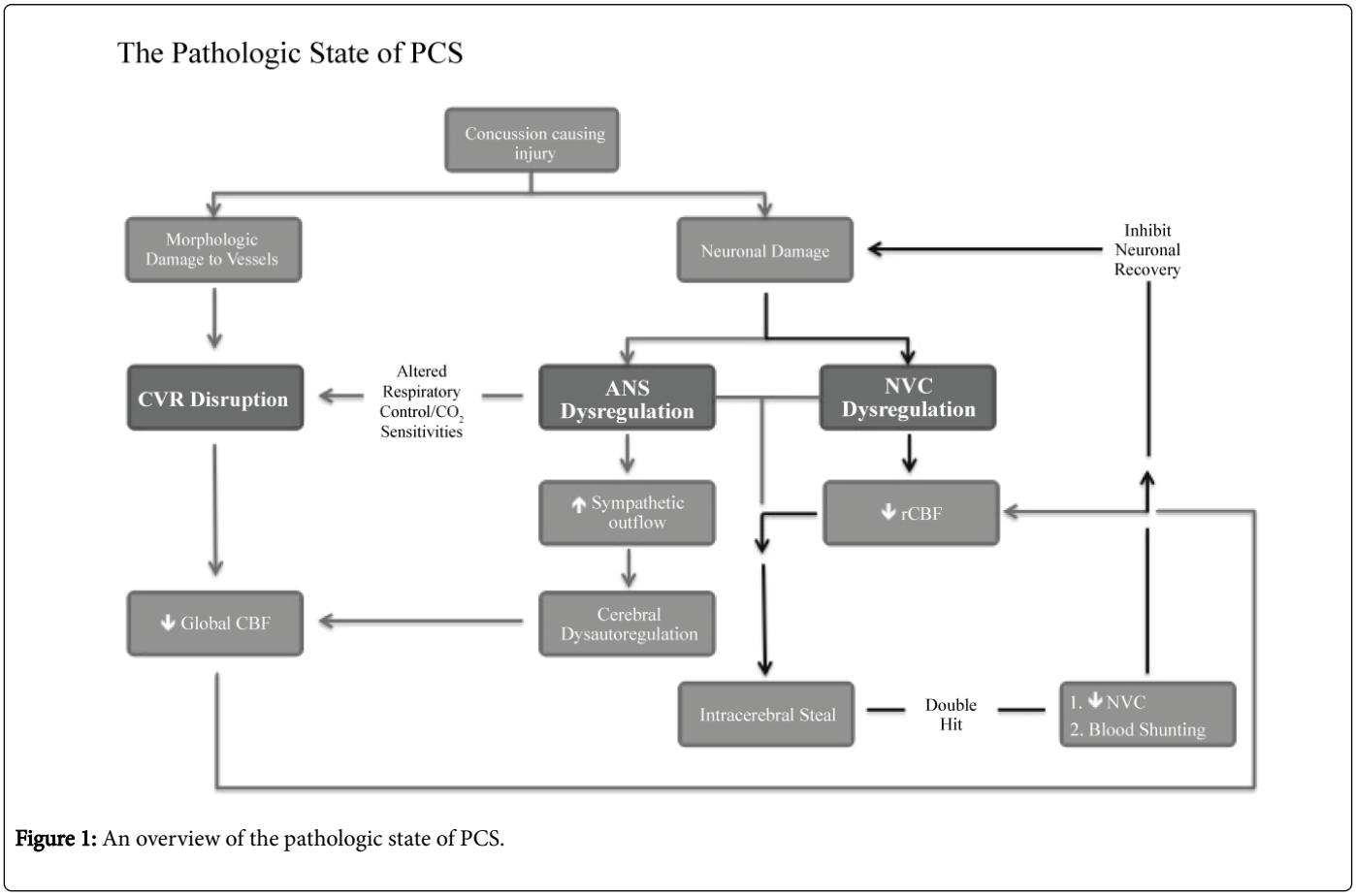

Figure 1: An overview of the pathologic state of PCS.

Three key mechanisms implicated in PCS are CVR disruption, ANS dysregulation (leading to CD) and NVC dysregulation. Injury chronically impairs neurons and vessels functionally and micro-structurally, including neurons that control ANS, biasing towards sympathetic activation and decreasing global CBF. The sum of disruption in CVR also plays a role in the reduction of CBF. NVC dysfunction leads to reduced blood flow to relatively localized areas of neural tissue, affecting rCBF. ANS (including CD) and NVC dysregulation contribute to intracerebral steal, wherein blood flow is shunted away to non-activated neural regions. This enters the brain into a vicious cycle (Bolded lines) in which damaged neurons responsible for PCS pathology need blood to rehabilitate; yet that

blood is chronically diverted. Overall, the key elements of PCS pathology and its subsequent BOLD signal abnormalities include ANS dysregulation with subsequent CD, CVR disruption and NVC dysregulation. Secondary influential elements include CBF, rCBF and intracerebral steal.

The three particular mechanisms of interest in the pathologic state of PCS are CVR disruption, ANS dysregulation (leading to CD) and NVC dysregulation. First, a concussion-causing injury occurs, which chronically damages neurons (and vessels) functionally and micro-structurally. Proposed mechanisms of functional damage to neurons include ionic flux and metabolic disturbances leading to chronic energy impairment, inappropriate protease activation and an abnormal functioning system of protein degradation all resulting in chronic cell death [11].

Proposed mechanisms of micro-structural damage include neurofilament phosphorylation, proteolytic damage to subaxolemmal spectrin and mechanical damage to microtubules [11]. When the neurons responsible for ANS regulation are involved, subsequent dysregulation occurs [26]. The dysregulated ANS is biased towards sympathetic activation and contributes to the disturbance in normal cerebral autoregulation (CA) or CD. Effects of CD include decreased anterior and posterior cerebral circulation and decreased global cerebral blood flow (CBF) [27-30]. The sum of disruption in CVR or a vessel's ability to respond to vasoactive signaling, also plays a role in the reduction of CBF [18,22]. Though largely unelucidated, CVR is disrupted in PCS and is most likely due to the morphologic damage of the cerebral vessels. CVR differs from NVC, as NVC is the signaling system by which neural activation induces vessel reaction [21].

NVC acts as a more local operator of blood flow control than do the ANS or CVR. NVC dysregulation, as occurs with chronic neuronal damage in PCS, leads to reduced blood flow to localized areas of neural tissue or regional cerebral blood flow (rCBF) [29,30]. Also, NVC dysregulation leads to the abnormal BOLD signal seen in PCS patients. Lastly, ANS (including CD) and NVC dysregulation contribute to intracerebral steal. That is, in normal function, noradrenergic signals are automatically sent to vessels in the outer periphery of the site of neural activation in order to constrict vessels that might divert blood flow. However, in the ANS-dysregulated state of PCS, this does not occur efficiently and blood flow is shunted away to non-activated neural regions [31]. This enters the brain into a vicious cycle in which damaged neurons responsible for PCS pathology need blood to rehabilitate, but that blood is continually being shunted away. Overall, the key elements of PCS pathology and its subsequent BOLD signal abnormalities include ANS dysregulation with subsequent CD, CVR disruption and NVC dysregulation. Secondary influential elements include CBF, rCBF and intracerebral steal. The following discussion will examine each mechanism in detail.

Autonomic nervous system dysregulation and cerebral dysautoregulation

The mechanisms of ANS neuronal damage that contribute to CD, whether functional or microstructural, in the sub-acute or chronic post concussive state as compared to the acute concussive state, remain relatively undetermined [11]. However, it is proposed that functional damage occurs secondary to mitochondrial Ca^{2+} sequestration, which leads to chronic energy deficiency, oxidative-damaged proteasome proteins (increasing toxin accumulation) and activated intracellular proteases signaling the apoptotic cascade, all of which leads to eventual cell death [11]. Micro-structural damage occurs secondary to mechanical damage to microtubules, phosphorylation of neurofilament side armsand proteolytic damage to subaxolemmal spectrin, all of which contribute to structural collapse [11]. Supporting these proposed mechanisms are the gross pathologic findings, including cortical and hippocampal atrophy, ventriculomegaly and cavum septum pellucidum, seen in the brains of boxers and others exposed to repeated concussions [32].

Regardless of the mechanism, ANS dysregulation and CD occur in patients with PCS, partly due to damaged neurons responsible for sympa- and para-sympathetic outflow [24,33]. Specifically, there is an over-bias towards sympathetic activation [26]. In a healthy brain, the orbitofrontal cortex along with the medial prefrontal cortex tonically inhibit the amygdala with coordinated disinhibition of the amygdala's central nucleus, which is thought to be the major efferent source of modulation for autonomic responses [34,35]. In the pathologic state of PCS, however, inhibitory nuclei are damaged leading to a net increase in sympathetic activity [34,35].

The role increased sympathetic activity plays in peripheral vascular tone is of particular interest to PCS treatment. That is, sympathetic activity leads to varying degrees of increased arterial wall stiffness via vasomotor tone augmentation as seen in studies characterizing the contours of the arterial pulse wave in sympathetic activation [28]. Subsequently, this leads to decreased vessel lumen diameter and increased vascular resistance above basal tone [36]. The effected vessels of interest in the pathologic state of PCS include the bilateral internal carotid and vertebral arteries, providing anterior and posterior circulation to the brain respectively. Therefore, a major effect of a dysregulated ANS with increased sympathetic activation is decreased CBF. Decreased CBF leads to inadequate blood flow to damaged neurons creating a disadvantageous environment for neuronal rehabilitation and the PCS brain enters into a vicious cycle. That is, ANS dysregulation decreases CBF which in turn hinders the recovery of neurons responsible for ANS regulation.

CA is the homeostatic process that preserves CBF despite fluctuating blood pressure (BP). It is facilitated by four proposed mechanisms: myogenic, neurogenic, metabolic and endothelial [37]. Note however, that our usage of CA is more narrowly defined as myogenic mechanisms as well as neurogenic components relating solely to ANS regulation. Metabolic and endothelial mechanisms are subsumed under our construct of CVR. Finally, neurogenic signaling related specifically to cognitive and motor operations is considered the primary mechanisms of NVC.

CA mechanisms are critical in matching CBF with peripheral cardiovascular changes. In the healthy brain under physiologic conditions, an increase in the cerebral perfusion pressure (mean arterial pressure–intracranial pressure) - occurring with increased physical stress will result in vasoconstriction to decrease CBF leading to an appropriate decrease in intracranial pressure [37]. In PCS, however, autoregulatory mechanisms become pathologic (CD) as CBF is constantly dampened and reduced as if the brain is under an unrelenting physical stressor.

Why these autoregulatory mechanisms become pathologic is yet to be fully elucidated. For example, one study found CD (when reduced CBF becomes dependent on mean BP) in patients with mTBI whereas another study had no similar observation [38,39]. One theory involves the neural misinterpretation of heart rate increases associated with the

increased sympathetic output from a dysregulated ANS. An intricate neurovisceral relationship between the heart and the pre-frontal cortex has been proposed [34,35]. In research, heart rate variability (HRV) has successfully been used to investigate this relationship, as there is a known, though not fully understood, relationship between the ANS and subcortical regulatory systems and HRV [26]. People with high HRV have shown better performance on neurocognitive tasks compared to those with lower HRV [40]. Further, HRV is adversely affected by any degree of traumatic brain injury (mild or severe) [41]. ANS dysregulation occurs in patients with PCS leading to decreased CBF. However, decreased HRV with sustained elevated heart rates may play a role in perpetuating the ANS dysregulation and exacerbating CD.

Overall, in the pathologic state of PCS, neurons responsible for the regulation of sympathetic and para-sympathetic outflow are damaged leading to an increase in sympathetic output and subsequent CD with a resulting decrease in CBF. Neuronal rehabilitation is hindered due to an inadequate blood supply. Also, as ANS dysregulation directly affects CA, it indirectly affects rCBF and intracerebral steal as will be discussed in later sections.

Cerebrovascular reactivity

CVR may be defined as the change in CBF in response to a measured vasoactive stimulus [18]. Physiologically, a true independent vasoactive stimulus is the partial pressure of CO_2 in arterial blood ($PaCO_2$). In research, however, increases in the end-tidal partial pressure may be used as a surrogate measure. Therefore, using BOLD signaling in fMRI as a surrogate for CBF, CVR may be defined as the percentage of change in BOLD intensity per mmHg change in $PaCO_2$, measured in the absence of a concurrent cognitive or sensorimotor task [22]. It is important to differentiate CVR from NVC. NVC may be defined as increases in CBF caused by neural activation, stimulated either by cognitive or sensorimotor tasks [42]. Quantification of NVC is commonly performed using fMRI to measure percentage change of Oxy-/Deoxy-hemoglobin ratios as a result of CBF and CBF velocity increase from baseline upon activation. In short, in NVC, the neuron plays an active role in triggering its own oxygen supply via vasoactive signaling whereas CVR is simply the intracerebral vessel's ability to react to a given stimulus, positive or negative.

To assess CVR in both healthy and diseased patients, various vasoactive stimuli (and neuroimaging techniques to measure the effects of those stimuli) have been used. Vasoactive stimuli include CO_2 inhalation, breath holding, hyperventilation and acetazolamide, all of which have the goal of artificially manipulating $PaCO_2$ in order to observe a passive dilatory response [20]. Commonly used neuroimaging techniques to measure the effects of these vasoactive stimuli include positron-emission tomography (PET), single photon emission computerized tomography (SPECT), XeCT, transcranial Doppler ultrasonography and non-invasive MRI sequencing such as BOLD and ASL. Considering assessment of CVR in research, these neuroimaging techniques have proven to be highly reliable and reproducible whereas the stimuli used in vasoactivation have not shown the same consistency [18]. This may be due to both the fact that applying the same magnitude and duration of stimulus across all study patients can be inherently difficult and other factors, such as the difference in vasodilatory reserve of the vascular bed in question and the flow reserve of the feeding vessels. This is important to consider when critically evaluating research regarding CVR in PCS.

The role of CVR in mTBI and PCS continues to be a matter of research interest and the mechanisms are becoming clearer. Mutch et al. [43] compared CVR in healthy adults to adults with PCS using both BOLD signaling MRI and prospective iso-oxic targeting of end-tidal partial pressure of CO_2 ($PETCO_2$). There was no difference in $PETCO_2$ changes between the subject groups. On the other hand, they showed quantified alterations in CVR that were patient-specific and present in both symptomatic and asymptomatic concussion patients that were not found among the healthy subject group. Though this pilot study only included five control subjects and twelve PCS patients, it suggests a role in CVR disruption in PCS. Chan et al. [44] investigated CVR changes in a single mTBI, female patient longitudinally in both symptomatic and asymptomatic phases of mTBI and compared her to healthy controls. They used BOLD MRI as their measure of CBF changes and breath-holding techniques as their vasoactive stimuli and performed imaging at the time of injury, two months after concussion and one-year post-mTBI. On the initial and two-month scans, mapping of CVR revealed "substantial neurovascular deficits and hemispheric asymmetry within grey and white matter". Interestingly, one-year post-mTBI scans demonstrated normalization of CVR compared to healthy control groups and was associated with symptomatic improvement. As will be discussed later, this suggests a role for CVR restoration in PCS therapy.

Using $PETCO_2$ and BOLD MRI, Mutch et al. [45] investigated CVR in 15 Adolescents with PCS compared to 17 normal controls. They observed reduced CVR in the PCS group as a whole. Considering patient-specific results, qualitative and quantitative disruptions in CVR were observed in all PCS patients individually. Receiver operator curve analysis showed voxels manifesting both CVR responses greater than and lower than the control response. Further, patient-specific alterations in regional resting CBF were seen in the PCS patients. These researchers concluded, "Adolescent PCS is associated with patient-specific abnormalities in regional mean CBF and BOLD CVR that occur in the setting of normal global resting CBF." Lastly, da Costa et al. [16] investigated CVR in PCS patients and its association with clinical symptoms using the Sport Concussion Assessment Tool 2 (SCAT2). Utilizing BOLD MRI and vasoreactive CO_2 as well as a larger prospective cohort of 25 mTBI patients with 18 matched controls, they performed imaging testing and clinical symptom evaluation at a mean follow up time of 63 (first follow-up) and 180 (second follow-up) days. They found a significant correlation between CVR indexes and performance on the SCAT2 that persisted beyond 120 days. This suggests that increases in CVR disruption may lead to increased PCS symptom manifestation again enforcing the possibility of CVR targeting in PCS therapy.

It is established that $PaCO_2$ affects CBF and its regulatory mechanisms (i.e., NVC, CVR, CA). What is less clear, however, is the interaction between NVC, CVR and CA in response to hypercapnia. Maggio et al. [42] investigated CBF velocity, BP, heart rate and end-tital CO_2 in 18 subjects during normocapnia or hypercapnia (5% CO_2 inhalation) while the patients performed simple motor tasks. They used a multivariate autoregressive-moving average model in order to calculate the separate effects on CBF velocity. They found a) impaired NVC and depression of CA during hypercapnia at rest and during stimulation; b) CVR was not influenced by hypercapnia or motor stimulation and c) dynamic CA was not influenced by motor stimulation during normocapnia. It therefore appears that NVC does not have the same dilatory effect when a vessel is exposed to hypercapnia and has already maximally dilated. These novel findings suggest two things. First, it appears that NVC and CVR act as

independent regulatory mechanisms, which has therapeutic implications. Second, if NVC and CVR independently contribute to the pathologic state of PCS, then it stands to reason that concussion causes morphologic damage to intracerebral vessels along with the previously described modes of injury.

In short, disruption of CVR occurs in PCS and contributes to the reduced CBF seen in PCS. Though the mechanisms are unclear, it seems to be related to morphologic damage to cerebral vessels secondary to the original acceleration-deceleration forces applied to the moving brain causing shearing of vascular elements [46]. Also, because it appears that CVR acts independently of NVC, the abnormal BOLD signal observed in PCS could be due to neural inactivation, dysregulated NVC, morphologically damaged vessels (as a part of disrupted CVR) or all three.

Neurovascular coupling

For the relatively small size of the brain compared to the body, it consumes a relatively massive portion of the body's energy production, approximately 20%. Much of that energy is used in reversing ionic fluxes produced as a byproduct of action potentials [47]. To maintain this high level of energy consumption, the brain has evolved a highly efficient and accurate NVC mechanism so neurons can self-direct blood flow allowing them to remain appropriately nourished with metabolic substrate. This response has been termed functional hyperemia [21].

Atwell et al. [14] have played a major role in enhancing our understanding of the underpinning mechanisms of how neuronal activity controls its own vascular supply of O_2 and glucose. This and other recent works [48] provide compelling evidence against the prevailing view of hyperemia which proposed that as neurons activate they use more metabolic substrate (i.e., glucose and O_2) with subsequent increase of CO_2 production. Local rises in CO_2 were then hypothesized to act on surrounding vessels to cause dilation, thereby increasing blood flow and more metabolic substrate to the activated area (i.e., negative feedback hypothesis). A more intricate, complex and evolving theory of hyperemia and NVC has begun to replace this dogma. Recent theoretical developments, with solid physiological support, suggest that neurotransmitter-mediated signaling, particularly glutamate has direct action on surrounding vessels and thereby a role in controlling its own blood supply (i.e., feed-forward hypothesis). Astrocytes, in particular seem to be heavily involved in this process. The glutamate-mediated signaling leads to astrocytes releasing arachidonic acid derivatives and neurons releasing NO. Depending on local O_2 levels, changes in these molecular concentrations can either dilate or constrict surrounding vasculature. Further, contractile cells called pericytes act to change capillary diameter. Damage to these cells appears to contribute to the enduring decreased blood flow occurring after stroke and possibly other brain injuries. In sum, current theories on NVC mechanisms favor a feed-forward anticipatory model of neurogenic vasoaction, rather than a feed-back reactionary model.

It is of such importance that neurons have the ability to stimulate their own O_2 uptake that back-ups have been built into the NVC system. Both a direct neuronal action on the vessels as well as indirect routes through astrocytes and pericytes contribute to the robustness of NVC and the neuronal self-regulation of O_2 uptake.

Describing direct neuronal action on vessels separately from neuronal action on astrocytes will aid in understanding NVC, though both contribute. NO appears to play a prominent role. Glutamate released from pre-synaptic neurons binds to post-synaptic NMDA (N-methyl-D-aspartate) receptors leading to an increase in intracellular Ca^{2+} concentrations. Intracellular Ca^{2+} activates neuronal nitric oxide synthase (nNOS), which increases levels of NO and dilates surrounding vasculature. Artificially inhibiting nNOS during cortical activation has demonstrated a lack of blood flow increase to the area, supporting the theory [49]. The blood flow to the activated areas was restored by the addition of NO donors, helping to create a constant concentration of NO and suggesting that a rise in NO during neural activation does not necessarily mean that direct neuron-to-vessel signaling is occurring [50]. On the other hand, NO donors have been shown to not reverse the functional hyperemia reduction caused by inhibition of nNOS within the cerebellum, suggesting that NO released through nNOS activation is essential for functional hyperaemia as opposed to externally-supplied NO [48,51]. This contraindication may be explained by the fact that NO probably also plays a role in the activation of astrocytes to release vasoactive substances.

NO playing a prominent role in NVC has treatment implications. The downstream activation of nNOS requires the presence of metabolic substrate (i.e., glucose and O_2). However, in the reduced rCBF-state of PCS, less metabolic substrate reaches the damaged neural area, which leads to impair NO production, which leads back to decreased metabolic substrate reaching the area, referred to by Attwell et al. [14] as a "damaging feedback loop". Breaking this cycle will play a role in our treatment theory.

Astrocytes are positioned in an anatomically advantageous position to aid NVC. That is, one part of the cell surrounds synapses and can therefore be activated by neurons and another part encompasses the blood vessel and can act on its smooth muscle controlling lumen diameter. Again, glutamate serves as a key player. Glutamate released from pre-synaptic neurons binds to metabotropic glutamate receptors (mGluRs) increasing intracellular Ca^{2+} with subsequent activation of phospholipase A2. This enzyme is responsible for the mobilization of arachidonic acid from cellular membranes. The subsequent metabolic breakdown products of arachidonic acid, prostaglandins and epoxyeicosatrienoic acids (EETs), dilate nearby vasculature [52]. Paradoxically, the increased levels in intracellular Ca^{2+} may also lead to vessel constriction. If arachidonic acid is converted to 20-hydroxyeicosatetraenoic acid (20-HETE) then subsequent constriction occurs. Whether arachidonic acid is converted to dilatory or constrictive downstream products seem to be determined by pre-existing vessel tone and O_2 concentrations [53,54].

Dysregulation of NVC is implicated in the pathologic state of PCS [55]. Evidence supports the theory, at least in the pediatric population, that PCS symptoms occurring after an mTBI are at least partially secondary to ongoing dysfunction of the neurovsacular unit as manifested by disrupted CVR reduced CBF and reduced rCBF [17]. More direct evidence for NVC dysfunction in PCS is found in pediatric and adult populations in the task-related fMRI studies discussed above and later in this paper.

In short, recent work has supported the hypothesis that neuronal vascular supply is largely a feed-forward mechanism. That is, neurons directly act on blood vessels or on astrocytes that act on blood vessels via vasoactive molecules. Either route of neuronal activation involves neurotransmitters, glutamate in particular with NO playing a prominent role. Dysregulated NVC is implicated in the pathologic state of PCS and serves as a therapeutic target.

CVR, ANS and NVC influences CBF, rCBF and intracerebral steal

CVR disruptions and ANS/NVC dysregulation contribute to the pathologic state of PCS largely through their influences on CBF, rCBF and intracerebral steal. Considering CBF first, reduced CBF represents one of the most enduring markers of brain injury after trauma in animal models of concussion [56-58]. Other studies investigating CBF in the chronic phase of mTBI's also suggest that hypoperfusion is present [17,59-61]. Perfusion computed tomography (CT) studies exploring CBF in the acute and subacute phases of mTBI also report reduced flow [62]. Reduced global CBF is reported in pediatric patients with mTBI using arterial spin labeling (ASL) [39]. Lastly, some studies have shown that CBF changes in brain injury may play a role as prognostic markers for clinical outcomes [63-65]. The connection between CVR and CBF should be clearly stated. That is, CBF can be thought of as the total sum of CVR in the brain and, as will be discussed later, it plays an important role in increasing CBF. ANS dysregulation, along with CD, acts on the internal carotid and basilar arteries and plays a role in decreasing CBF. Regardless of the mechanism, CBF has been shown to be consistently reduced in PCS and is related to CVR disruption and ANS dysregulation [30].

There are several factors, both clear and unclear, that influence rCBF. However, in the pathologic state of PCS, NVC dysregulation seems to have a vital influence as NVC serves as a more local operator of blood flow. Therefore, the effects of NVC on rCBF are of particular importance both to understanding the pathologic state of PCS but also in the pending discussion concerning PCS treatment theory.

Regionally, in order for the brain to meet increased demands either from activation or disease, there must be an appropriate cerebrovascular response to local, as opposed to global (largely ANS and CVR determined), physiologic stimuli such as neuronal firing. NVC may be influenced by direct-acting signaling messengers derived from neuronal tissue (calcium ions, acetylcholine, vasoactive intestinal polypeptide (VIP), calcitonin gene-related polypeptide, NO, substance P and prostaglandins) or factors that act independently of NVC on the vessels (i.e., CVR) such as intraluminal hydrostatic pressure, pH, $PaCO_2$ and PaO_2 [14].

Several studies show both reduced rCBF in patients with PCS and correspondence of those regions with hypoactivation of BOLD signaling on fMRI [66-68]. One study of interest, Wintermark et al. [69], found that low rCBF 3 months out from TBI was associated with worse clinical outcomes using perfusion CT suggesting clinical significance in rCBF abnormalities in widely distributed brain regions apart from focal abnormalities found in fMRI studies. Most studies investigating rCBF in mTBI and PCS do not use task stimulation suggesting that regions of neural tissue consistently receive less blood flow regardless of neural activation levels. However, these findings are not homogeneous. For example, one study used arterial spin labeling to investigate rCBF in children with mTBI who had varied clinical recovery patterns [70]. They looked at children who were symptomatic at one-month post mTBI, asymptomatic at one month and a healthy control subset. They found increased rCBF in the symptomatic group compared to the healthy control group and decreased rCBF in the asymptomatic group compared to the healthy control group. These differences in study outcomes highlight the multifactorial and complex nature of rCBF and its role in PCS pathology.

As neural functioning is highly dependent upon its oxygen supply, the role regional ischemia plays in the perpetuation of brain injury after initial trauma continues to be of interest [60,71,72]. Studies on rodent models of brain trauma show hypoperfusion not only at the site of injury but also at distant sites [73-77]. These studies, however, have largely investigated the acute phase of injury. On the other hand, one study showed reduced rCBF and associated delayed cognitive deficits in similar rodent models at one year out from injury suggesting the long term effects of regional ischemia in mTBI [78].

In short, NVC largely influences rCBF. How it exerts that influence in the setting of PCS is unclear in the literature. Regardless of the mechanisms, reduced rCBF is observed in patients with PCS and is a key component of the pathologic state of PCS.

Lastly, the intracerebral steal phenomenon perpetuates and compounds the reduction in rCBF. This phenomenon occurs when vessels surrounding a site of injury are in a "state of maximum compensatory vasodilation to maintain cerebral perfusion" [31]. That is, when one part of the brain is damaged the blood vessels in healthy surrounding areas will dilate as a response to what it perceives as a reduction in global blood flow (also seen in hypotension or hypercapnia). This causes those vessels to "steal" blood from the affected areas compounding the issues occurring in that area.

Intracerebral steal has been shown to occur in the deep white matter tracts of healthy subjects, those with steno-occlusive vascular disease, arteriovenous malformations and vasculitis [79-82]. It has been associated with white matter microangiopathic disease, enhanced stroke risk, cognitive impairment and cortical thinning [22,83-86]. The presence of intracerebral steal is highly specific for disruptions in CVR but is not sensitive. That is, the absence of intracerebral steal does not indicate normal CVR. There are a few reasons for this. First, if the stimulated blood flow demand does not exceed supply capacity no steal will occur even if CVR is markedly reduced. Second, if there is a widespread disruption of CVR, there will be no differential vasodilatory effects in adjacent vascular territories and therefore steal will not occur. Lastly, steal may not occur if vessels in damaged neural areas have conserved some vasodilatory capacity. This suggests that ANS and NVC dysregulation play a larger role in the intracerebral steal phenomenon than does CVR.

When intracerebral steal begins to occur in a healthy brain, noradrenergic signaling helps constrict vessels peripheral to the site of activation and decrease the distribution of blood away from the site of target NVC action. However, in the ANS-dysregulated state of PCS, this compensatory system does not occur efficiently and blood is continually shunted away from NVC compromised areas. Therefore, damaged areas of brain in PCS receive a double hit of reduced blood flow. First, they lack normal dilatory mechanisms (i.e., NVC) and second, blood is being shunted away further depleting its already reduced blood supply. This is of utmost importance, as the damaged neurons contributing to the pathologic state of PCS require an adequate and consistent source of blood supply to rehabilitate. The prevention of intracerebral steal will figure prominently into our PCS treatment theory.

Normalization of the pathologic state of PCS in EPIC treatment: Overview

EPIC treatment seeks to address the major components of the pathologic state of PCS (Figure 2). Briefly, aspects of EPIC treatment that target the dysregulated ANS include aerobic exercise, breathing exercises and neuromuscular therapy.

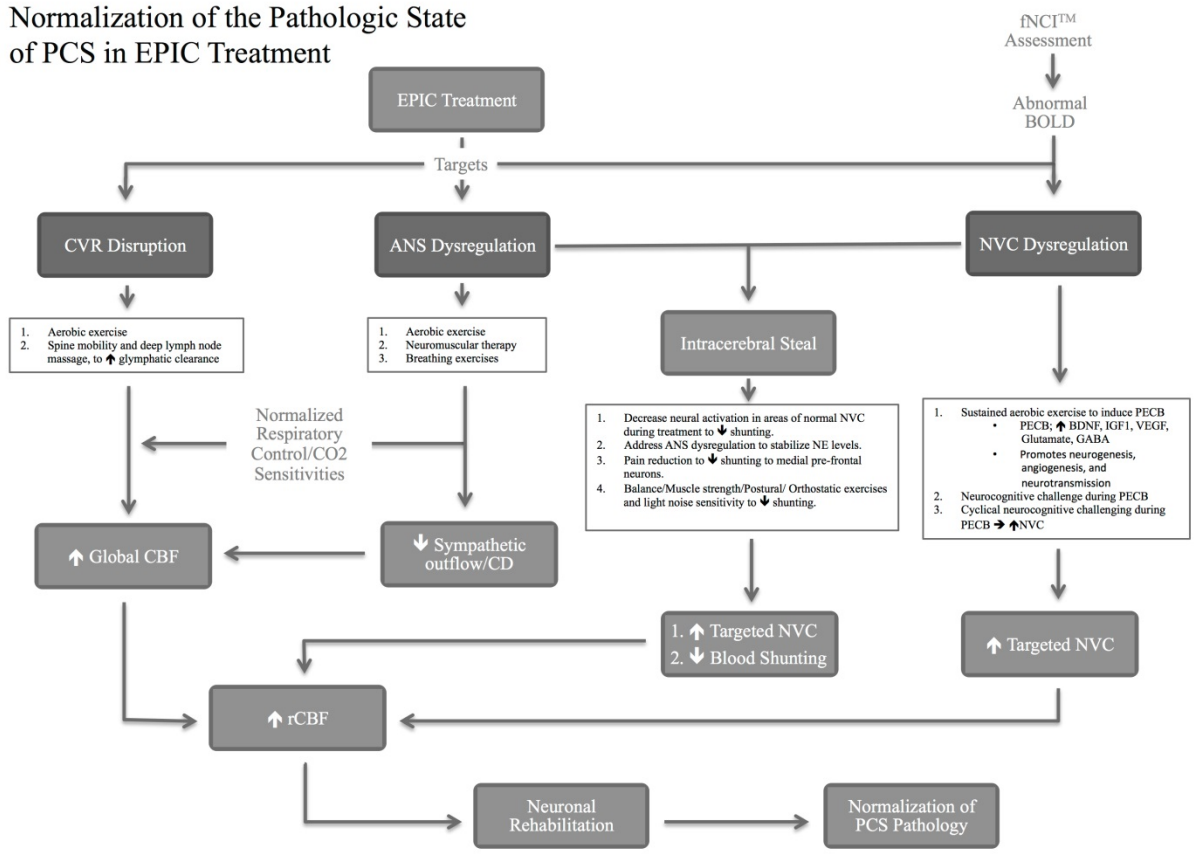

Figure 2: The use of EPIC treatment in normalizing the pathologic state of PCS.

Aspects of EPIC treatment that target dysregulated ANS include aerobic exercise, breathing exercises and neuromuscular therapy. Disruption of CVR is targeted by aerobic exercise, increasing spinal mobility and deep cervical lymph node massage therapy. Dysregulation of NVC is identified using fNCI, which reveals and quantitatively assesses severity of NVC dysregulated regions using BOLD signals. Those regions are then targeted by neurocognitive challenges during the post-exercise cognitive boost, brought on by aerobic exercise. Preventing intracerebral steal may be accomplished by decreasing activation in other parts of the brain by limiting cognitive rehabilitation tasks that draw heavily on areas showing already intact NVC. It also includes pain reduction, improving balance, strength/postural stability, cardiovascular orthostatic control and light-noise sensitivity control. Targeting these aspects of PCS pathology will in turn affect CD, CBF and rCBF leading to neuronal rehabilitation and normalization of the PCS pathologic state. It is the most likely reason for the normalization of BOLD signal observed by Wing et al. [23].

Disruption of CVR is targeted by aerobic exercise, increasing spinal mobility and deep cervical lymph node massage therapy. Dysregulation of NVC is targeted by aerobic exercise and neurocognitive challenges during and post-exercise. Preventing intracerebral steal may be accomplished by decreasing activation in other parts of the brain by limiting cognitive rehabilitation tasks that draw heavily on areas showing already intact NVC, as well as pain reduction, improving balance, strength/postural stability, cardiovascular orthostatic control and light-noise sensitivity control. Targeting these aspects of PCS pathology will in turn affect CD, CBF and rCBF and is the most likely reason for the normalization of BOLD signal observed by Wing et al [23]. It should be noted, however, that the mentioned therapeutic interventions can affect multiple PCS pathological aspects and will often demonstrate crossover beneficial effects. Features of EPIC treatment targeting PCS pathological components will each be discussed in greater detail.

Enhanced performance in cognition (EPIC) treatment

EPIC treatment and related imaging components of it have previously been reported in Wing et al. [23] and Epps et al. [87]. Using functional neurocognitive imaging (fNCI) techniques, areas of deficient neural NVC are targeted for rehabilitation. The development of fNCI, which is a quantitative, biomarker-based form of fMRIand how it is used to reliably and accurately detect neural regions of dysregulated NVC are beyond the scope of the present report but can be found in Wing et al. [23]. Upon identification of neural regions of interest showing deficits in NVC, EPIC treatment integrates three fundamental neurocognitive rehabilitation components: Prepare, activate and rest. Therapeutic activities used in each of these three phases are the result of research, clinical experience, screening and empirical testing [88-93]. The preparatory stage includes aerobic

exercise and neuromuscular therapy, titrated to patients exercise tolerance and lasts 50 min. Following the preparatory stage is the activation stage, which includes 50 min of "complex multistep problem solving, logic puzzles, functional and short-term memory challenges, digital therapeutic games, visual exercises, motor skill retraining and psychosocial therapy", all of which is tailored to the neural regions that exhibited NVC deficits on fNCI [23]. If any visual spatial deficits were identified in the pre-treatment scan, visual spatial and sensorimotor therapeutic programs, including Dynavision^{TM} and other commercial and in-house technologies are incorporated. The activation stage is followed by the rest phase, which includes an auditory binaural beats brainwave entrainment program to reduce stress while concurrently promoting cortico-thalamic synchrony of post-synaptic activity at frequency ranges that are often disrupted in PCS. The Preparation, Activation and Rest phases are then repeated in cyclical fashion for 6-8 h per day for 4 contiguous days and can be fine-tuned based upon symptom severity. It is important to note that the timing and order of these interventions are just as critical to successful treatment as are the interventions themselves. That is, careful and deliberate rotation between the aerobic challenge (preparation phase) and the cognitive challenge (activation phase) is a fundamental aspect in pairing rCBF and neuronal firing. The precision of this pairing requires a multidisciplinary team including athletic trainers, neuromuscular therapists, neurocognitive therapists, neurological occupational therapists and clinical neuropsychologists. Lastly, post-EPIC fNCI is performed and the degree of normalization of NVC is identified. Further treatment targeting neural areas of persistent NVC dysregulation may be performed.

Wing et al. [23] report the use of the EPIC protocol in 270 patients with PCS. The outcomes reported include the severity index score (SIS) and the post-concussion symptom scale (PCSS). The SIS, which is computed from the initial pre-treatment fNCI brain scan, was developed to provide a single objective measurement that represents the overall presence and severity level of each PCS biomarker. The development and calculation formulation can be found in Wing et al. [23]. The PCSS is an existing assessment standard for neurocognitive assessment in patients with concussion. It is a self-reported survey that provides a subjective assessment of clinical concussion symptoms. The SIS was calculated pre- and post-treatment. The PCSS was administered before, during and after treatment.

Despite the wide range in patient profile with regards to age, sex, time from injury and mode of injury, universal improvement was noted in both the quantitative and qualitative measures. Among all patients, the average percent improvement in SIS outcomes was 75.3% and 62.2% in PCSS outcomes. These outcomes were compared to a control group consisting of concussed patients who chose to delay EPIC treatment. There were no similar improvements found in this group compared to the treatment group. Test-retest measures on a healthy control group helped to support the reliability of the improvement measures found in the treatment group. Further, SIS outcomes performed at an average of 8.8 months post-EPIC demonstrate maintenance of initial improvement.

Since the time Wing et al. [23] reported these findings, unpublished data from 230 more PCS patients that have undergone fNCI with subsequent EPIC treatment appear to show consistently significant improvements, with evidence for stable brain activation patterns measured from follow-up fNCI scans 2-years post-treatment.

We propose that EPIC treatment helps to normalize the pathologic state of PCS by re-regulating the ANS, restoring CVR and promoting NVC. This, in turn, leads to restored CBF, rCBF, CA and less intracerebral steal. How EPIC (or EPIC-style) treatment affects each of these PCS features will be considered individually.

Re-regulation of ANS and CD

As previously discussed, typical PCS patients experience CD from dysregulated ANS functioning with over biased sympathetic activation leading to decreased CBF. Decreased CBF then hinders the rehabilitative process of neurons responsible for ANS entering the PCS brain into a brutal cycle. EPIC treatment features that aid in the re-regulation of the ANS and thereby addressing CD include aerobic exercise, neuromuscular therapy, lymphatic massage and breathing exercises during the Preparation phase.

Aerobic exercise plays a pivotal role in the EPIC treatment as it serves a function in the re-regulation of the ANS, restoration of CVR and promotion of NVC all of which work to restore CBF and rCBF. As such, further discussion of aerobic exercise in the treatment of PCS will be found in the corresponding sections. Concerning aerobic exercise in the regulation of the ANS, Carter et al. [94] discuss the effects of long-term endurance exercise on the ANS. In brief, endurance training increases parasympathetic activity and decreases sympathetic activity. A practical example often seen in sports medicine clinics is that of a highly trained long-distance runner with a low resting heart rate. Using microneurography, muscular sympathetic nerve activity (MSNA) can be measured to assess sympathetic activity. Miruma et al. [95] found a reduction in MSNA in a group of patients who underwent a four-week aerobic exercise program compared to no reduction in MSNA seen in the control group. Further, Notarius et al. [96] performed a meta-analysis investigating muscle sympathetic activity in resting and exercising people and concluded, "Thus, endurance exercise training in healthy subjects lowers MSNA during exercise". Of particular interest to PCS patients, as these patients have biased sympathetic activation, it was demonstrated that regular aerobic exercise could normalize basal over-activation of sympathetic nerves [97]. These studies were performed in congestive heart failure patients, as an over activated sympathetic system have also been implicated in this disease's pathophysiology. The aerobic exercise program these patients completed included three 60 min exercise sessions per week, including both cycling and strength training for 4-6 months. MSNA values pre- and post-exercise programs were measured. Baseline MSNA values were reduced significantly, from 45 bursts/min (considered abnormally over-activated) to 30 bursts/min (normal values relative to healthy controls) with no change in MSNA in the untrained group. These studies suggest that aerobic exercise increases parasympathetic activity. They also suggest that it helps to normalize the ANS that is, at baseline, biased to sympathetic activation.

However, as vital as aerobic exercise is to normalizing the pathologic PCS state, it also presents a therapeutic challenge. Many concussion patients experience symptom exacerbation during physical exertion. In fact, for athletes who have suffered a concussion, the return-to-play protocol utilizes a staged exercise evaluation in which graduated levels of exercise intensity are performed and the athlete may only advance if they complete the level of exercise intensity without symptom exacerbation [98]. These protocols are directed at the acute concussion recovery pattern, typically lasting 7 to 10 days and though it has been used in patients with PCS, little evidence supports its use in these patients. Of note, symptom exacerbation during exercise in concussion is thought to be related to an uncoupling of the ANS and cardiovascular systems [41]. Though this proposed mechanism has

only been studied in acute concussion it may also play a role in PCS (Autonomic Nervous System). The challenge presented in PCS is that aerobic exercise is a vital element in normalizing the pathologic state of PCS specifically re-regulating the ANS, yet many patients may be exercise intolerant due to the dysregulation of their ANS. Therefore, it is of utmost important to help PCS patients become exercised tolerant for the EPIC treatment to be successful. Further, elderly patients or patients with co-morbidities may have inherent difficulties performing aerobic exercise. Physical therapists and athletic trainers may adjust the types of exercise assigned to meet the needs of this particular patient population.

Leddy et al. have played a major role in investigating exercise in patients with PCS [24,25,89-91,93,99-103]. They demonstrated the safety and efficacy of a graduated treadmill exercise test in patients with PCS. Therefore, helping PCS patients become exercise tolerant is safe and is vital for the EPIC treatment to be successful in exercise-intolerant PCS patients.

Another component of the EPIC treatment that seeks to address the dysregulated ANS is Functional Neuromuscular Therapy (FMNT), which is a performance-based soft tissue modality that (among other things) targets pliability and strength and stimulates nerve pathways and clusters, lymphatic structures and peripheral blood vessels. While specific studies on this specialized form of neuromuscular therapy are forthcoming, existing research on massage therapy in general is informative. One study applied massage therapy to 139 volunteers for 40 min per day, 5 days per week for 2 weeks and measured HRV, sympathetic skin response and serum cortisol and norepinephrine levels before and after the treatment [104]. They found that serum cortisol levels and norepinephrine were significantly decreased, HRV increased and the sympathetic skin response latencies increased, all indications of a reduced sympathetic outflow. On the other hand, this study applied heat with massage and may serve as a potential confounder. However, a meta-analysis reviewing the physiological adjustments to stress measures following massage therapy concludes that massage therapy has shown some effects on the ANS but "the general research body on this topic lacks the necessary scientific rigor to provide a definitive understanding of the effect massage therapy has on many physiological variables associated with stress" [105]. In short, though research has not clearly linked massage therapy and ANS re-regulation, it does appear to have some effect and the EPIC treatment seeks to exploit its benefits on the ANS.

Lastly, breathing exercises may also help to decrease sympathetic output and re-regulate the ANS. Pal et al. [106] studied the effect of breathing exercises on autonomic functions in a group of young, healthy volunteers. 60 volunteers were randomized into a slow breathing group practicing slow breathing exercises and a fast breathing group practicing fast breathing exercises. Breathing exercises were performed for three months and autonomic function testing was performed before and after treatment. They found increased parasympathetic activity and decreased sympathetic activity in the slow breathing group and no significant changes in autonomic functions in the fast breathing group. Though this study showed supporting evidence for breathing exercises in the re-regulation of the ANS, it has several limitations and very few other studies have been performed investigating the same relationship. In short, breathing exercises may help to re-regulate the ANS and though little evidence exists, the EPIC treatment seeks to maximize any benefit breathing exercises may have on the ANS.

Overall, EPIC treatment utilizes aerobic exercise to help re-regulate the ANS. The use of exercise in PCS patients has shown benefit and the effect aerobic exercise has on increasing parasympathetic outflow and decreasing sympathetic outflow is relatively well documented. Though the evidence for massage therapy and breathing exercises helping to re-regulate the ANS has yet to be systematically studied, the EPIC treatment seeks to maximize the obvious theoretical benefits they should have according to the model presented here.

Restoration of CVR

The importance of helping patients with PCS become exercise tolerant cannot be understated. The restoration of CVR plays a crucial role in restoring global CBF, abnormalities, which account for much of the symptomatic exacerbation experienced by PCS patients in general but also during exercise. Therefore, much of the work done to restore CVR and subsequent CBF will be via aerobic exercise.

As previously mentioned, Leddy et al. [24,25,89-91,93,99-103] have played major roles in investigating aerobic exercise in patients with PCS. One study investigated the use of sub-symptomatic threshold aerobic exercise in normalizing some of the physiologic disturbances seen in PCS, particularly BP, minute ventilation (VE), $PETCO_2$ and CBF velocity (CBFV) measured by transcranial Doppler [101]. They compared these measures in nine female division I athletes with PCS to a reference group of 13 recreational aerobic female athletes in association with aerobic exercise. First, they found that these physiologic parameters were different in the patients with PCS. Specifically, patients with PCS exhibited hypoventilation; increased $PETCO_2$ and increased CBFV compared to the reference group. These differences were seen at similar workout loads; however, premature exercise cessation would occur with the appearance of symptoms. Interestingly, they found that with the use of a sub-symptomatic threshold aerobic exercise, these physiologic parameters normalized compared to the non-PCS group. Further, they observed that the hypoventilation experienced by the PCS group appeared to be a consequence of reduced sensitivity to the vascular effects of CO_2. Of extreme interest to our model of PCS pathologic normalization along with the normalization of VE, $PETCO_2$ and CBFV it appears that the sub-symptomatic threshold aerobic exercise also normalized the reduced CO_2 sensitivity effect. These authors hypothesized that change in CO_2 sensitivity in PCS "reflects altered central (medullary) and/or peripheral (carotid body) respiratory control". Building on the findings of Leddy et al. [100], we propose that in addition to damaged respiratory control, morphologic damage to intracerebral vessels in concussion also contributes to the decrease in CO_2 sensitivity. Further, as it is observed that sub-threshold aerobic exercises normalizes reduced CO_2 sensitivity, it is hypothesized that it also plays a role in the preparation of morphologically damaged vessels, subsequent restoration of CVR and eventual normalization of CBF.

Concerning the relation between symptomatic improvement and physiologic normalization, Leddy et al. [100] observed a statistically significant correlation (P=0.04) between heart rate improvement and standardized symptom improvement (Cohen's d). They also found that the rate of symptomatic improvement in their PCS group was directly related to exercise intensity achieved. This emphasizes the importance of not only helping PCS patients achieve exercise tolerance but also working to have that exercise tolerance be at the highest level of intensity possible.

Again, the ultimate goal of CVR restoration through graded aerobic exercise is to normalize CBF, both during exercise and at rest. Leddy et

al. [103] sought to confirm aerobic exercise's role in normalizing CBF using fMRI, an indirect indicator of changes in CBF in areas of increased neural activation. They compared fMRI activation in PCS patients who had undergone a therapeutic exercise program to two control groups. The first consisted of PCS patients who were given a placebo stretching treatment and the second consisted of healthy patients given no treatment. fMRIs were obtained using a math-rest cognitive challenge pre- and post-treatment for all groups. The combined exercise and stretching PCS groups showed different activation patterns compared to the healthy controls at the pre-treatment scans. At the post-treatment scans, however, the exercise PCS group's fMRI activation pattern did not differ from the healthy control group, whereas the stretching PCS group maintained its differing activation pattern. They concluded, "Controlled exercise treatment may therefore help to restore normal local CBF regulation, at least as reflected by fMRI BOLD activation patterns, during a cognitive task." This was the first study to support the hypothesis that a specific aerobic exercise treatment program could restore hemodynamic response on fMRI during cognitive challenge. However, as previously discussed in "Neurovascular Coupling", we demonstrated that NVC contributes to local blood flow regulation. Therefore, it stands to reason that aerobic exercise also plays a role in re-regulating NVC as will be discussed further.

Another more theoretical aid in the restoration of CVR involves the exploitation of the glymphatic system. Clearing excess fluids and interstitial solutes plays a crucial role in tissue homeostasis. The lymphatic system includes peripheral vessels designed to collect, interrogate and then recirculate this extracellular fluid. Increased lymphatic vessel density is proportional to the rate of metabolism of any given tissue. Conventional lymphatic vessels are lacking in the central nervous system (CNS). Neural tissue is highly metabolic and as such it stands to reason that there should also be some inherent way to collect, interrogate and recirculate extracellular fluid. The glymphatic system was recently discovered as a means of accomplishing these tasks within the CNS [107]. The glymphatic system primarily drains to the deep cervical lymph nodes. As previously discussed, PCS pathology involves neurometabolic disturbances and exploiting the glymphatic system to help normalize the neurometabolic milieu is included within the EPIC protocol. This includes improving spine mobility to help maintain CSF pressure and perfusion through the brain as well as deep cervical lymph node massage to encourage glymphatic drainage.

Though ANS/CA re-regulation, restoration of CVR and subsequent normalization of CBF is critical, the mechanism of NVC re-regulation is lacking from the theory of PCS rehabilitation. For example, in Leddy et al. [100], daily resting symptoms appeared to diminish over time in correlation with increased exercise capacity; however, symptoms remained highly variable. In fact, some subjects were able to exercise to exhaustion without symptomatic exacerbation yet failed to have resolution of resting symptoms. The authors suggest that this may be due to the fact that there is a large amount of variability in daily PCS symptoms at baseline. They also suggest that their exercise tests and symptom reporting may be assessing different aspects of PCS. Also of interest, for some patients the aerobic exercise therapy helped resolve some, but not all of their symptoms. For example, two non-athlete subjects had persistent cognitive/visual symptoms and migraines though other somatic and affective symptoms resolved.

In short, the re-regulation of ANS/CA and restoration of CVR and CBF may not be enough for complete PCS rehabilitation. We propose that NVC re-regulation and the advantage of rehabilitated ANS and CBF it uses is a vital mechanistic component in the normalization of PCS pathology.

NVC re-regulation

Much of our understanding of ANS/CA re-regulation and restoration of CVR through aerobic exercise has been due to the work performed by Leddy et al. [24,89-91,93,99-103]. EPIC treatment seeks to maximize the benefits of aerobic exercise to re-regulate ANS/CA and to restore CVR. However, little data exists demonstrating the ability to re-regulate NVC at local, neural regional levels. The work done by Wing et al. [23] suggests that NVC can be efficiently normalized in PCS patients. Furthermore, these data suggests that not only can dysregulated NVC be normalized, but that specific regions of dysregulated NVC may be targeted via neuroimaging.

Restoration of NVC should occur in the setting of a re-regulated ANS/CD (decreased sympathetic outflow) and improved CVR leading to appropriate CBF. We propose that the components influencing the re-regulation of NVC during EPIC treatment are aerobic exercise and strength training, neurocognitive challenge during the post-exercise cognitive boost (involving increases in glutamate, gamma-amino butyric acid (GABA), insulin-like growth factor 1 (IGF-1), brain derived neurotropic factor (BDNF) and vascular endothelial growth factor (VEGF)) and the avoidance of intracerebral steal. These influences occurring cyclically (6-8 h per day for 4 contiguous days) serve to coax an abnormally regulated NVC system back into its pre-dysregulated state. Each component of the theory will be discussed further.

First, a basic tenant of NVC rehabilitation in EPIC treatment is that the targeted region of dysfunctional NVC must be activated via neurocognitive challenging (brain games, etc.). However, as Leddy et al. [103] described, PCS patients exposed to prolonged cognitive tasks (memory tasks in their studies) experience fatigue, difficulty concentrated and ultimately struggle in maintaining the cognitive energies needed to complete the tasks. If PCS patients easily fatigue during one cognitive task, they will most certainly find difficulty in completing the prolonged, repetitive and intense neurocognitive challenges presented during EPIC treatments. In order to address this issue, EPIC treatment takes advantage of what has been term the post-exercise cognitive boost (PECB). Essentially, the PECB provides a window of time after exercise in which cognitive abilities are sharpened. Experience in performing EPIC treatment on over 500 PCS patients demonstrates that patients are able to perform more complex neurocognitive challenges for longer durations than if they had not completed any aerobic exercise. Therefore, taking full advantage of the PECB and activating target regions of dysregulated NVC is a key component to successful EPIC treatment.

The relationship between fitness and neural plasticity has a relatively convincing body of literature supporting it. For example, Erickson et al. [108] compared hippocampal volume on fMRI during spatial memory tasks as a function of their cardiorespiratory fitness assessed via a maximal graded exercise test in 165 healthy, older adults. They found that higher fitness was associated with increased hippocampal volume and that both higher fitness and increased hippocampal volumes were associated with better spatial memory performance. Another study randomized 120 adults into an exercise treatment group or a control group and measured hippocampal volume after treatment [109]. They found that the hippocampal volume had increased by 2% in the exercise treatment group compared to the control group. In short the concept of exercise positively affecting cognition is not new.

However, the precise timing of post-exercise neurocognitive challenges to rehabilitate NVC in PCS patients is novel to this specific form of treatment (Figure 3).

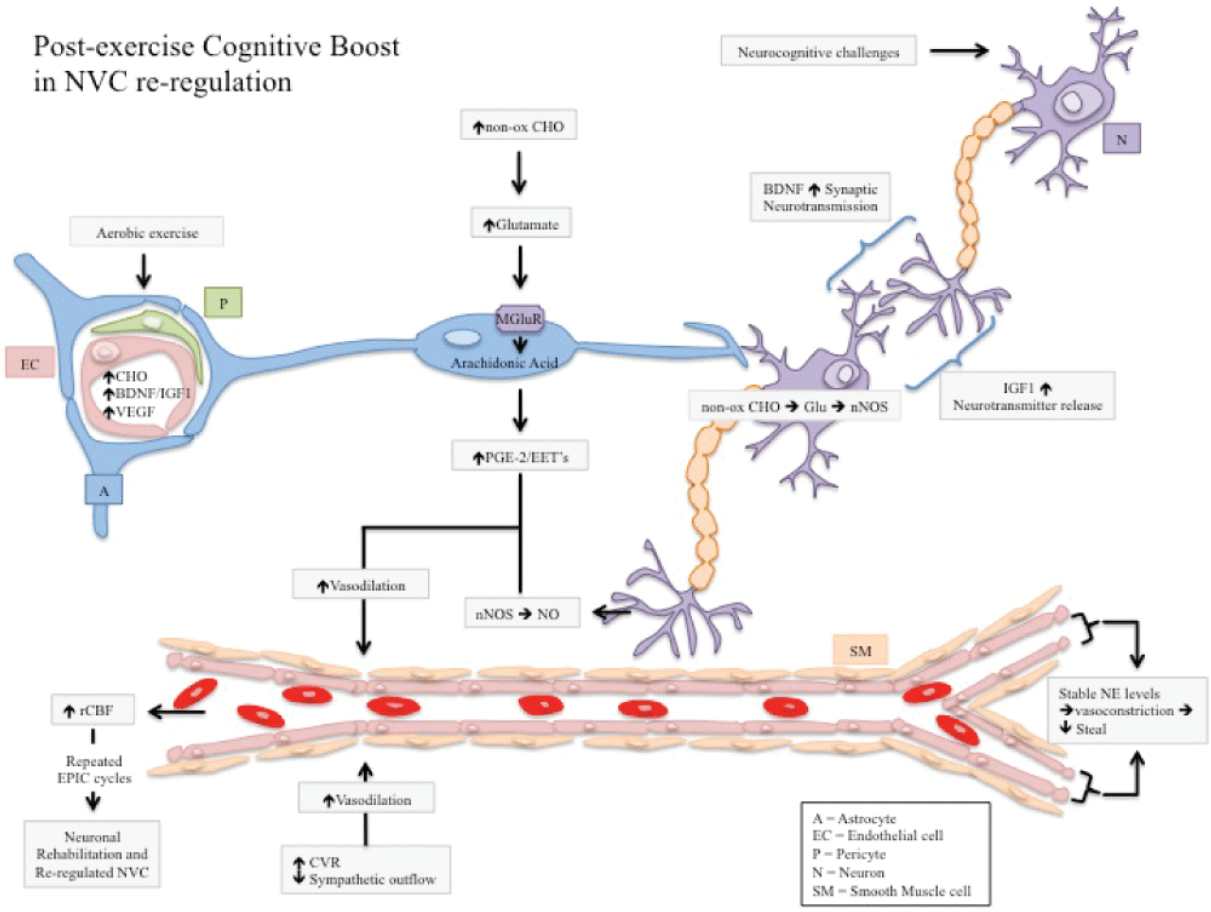

Figure 3: The use of the PECB in the re-regulation of NVC.

A theory detailing the mechanistic processes by which EPIC treatment re-regulates NVC. The prerequisite for NVC restoration is rehabilitated CVR and re-regulated ANS/CD. This provides the ideal setting for NVC re-regulation, including increased global blood flow, more pliable, neural-activity accommodating blood vessels and a stabilized cerebral noradrenergic system. Second, regions of neural NVC dysregulation (targeted by fNCI) must be activated by neurocognitive challenges. This activation must be focused on the regions of interest, intense, prolonged and cyclical. However, because many PCS patients fatigue too quickly upon initiation of these neurocognitive challenges, NVC re-regulation relies heavily on the PECB. The PECB provides a window of time post-exercise where cognitive abilities are enhanced. Aerobic exercise brings on increases in non-oxidized CHO, BDNF, glutamate, GABA, IGF-1and VEGF, which serve to promote neurogenesis, angiogenesis, synaptic activity and neurotransmission, all of which encourages proper NVC. A surplus of CHO is delivered to the brain. Non-oxidized CHO promotes glutamate synthesis. Increases in glutamate have several benefits on rCBF and producing the PECB. First, it binds to mGluR on astrocytes, leading to the mobilization of AA to produce PGE-2 and EET's, both vasodilators. Second, it activates nNOS producing increases in NO, which also acts as a vasodilator. Lastly, it serves as an excitatory neurotransmitter facilitating neurotransmission. Increases in BDNF and IGF1 play vital roles in the promotion of neurogenesis and synaptogenesis. Increases in VEGF leads to angiogenesis, facilitating increases in blood flow during future EPIC cycles. NVC re-regulation is further aided by the avoidance of intracerebral steal. This process, occurring cyclically (6-8 h/day for 4 days), serves to coax an abnormally regulated NVC system back into its pre-dysregulated state.

Increase in post-exercise BDNF has largely been implicated to be at the interface of synaptic plasticity and metabolism and as such plays a crucial role in the PECB. Increased levels of serum BDNF in humans have been associated with both increased hippocampal volumes and higher levels of aerobic fitness [110]. BDNF is a neurotrophin protein involved in the differentiation, development and survival of neurons. An increase in BDNF is proposed to be associated with increased neurogenesis and synaptogenesis in adults, prevention of neuronal loss and enhanced cognitive performance [111,112]. Knaepen et al. [113] performed a systematic review investigating exercise and its effects on BDNF. They note that there is a transient increase in BDNF after acute exercise but did not find the same increase after strength training. Another review by Zoladz et al. [114] found consistent results

regarding acute exercise but found the relationship between resting BDNF increases and chronic exercise less consistent. Further, a review done by Huang et al. [115] found increases in peripheral BDNF concentrations in relation to both acute and chronic aerobic exercise. In short and of great importance to the EPIC treatment, there is a consistent increase in BDNF both promoting neuro- and synaptogenesis and cognitive enhancement after a single acute bout of aerobic exercise whereas the effects of chronic aerobic exercise training on BDNF levels is less clear.

Interestingly, there has been no similar increase in serum BDNF after aerobic exercise if performed within one week of the concussion [116,117]. Also, pre-mature (within one week of injury) voluntary exercise has been associated with poor performance on cognitive tasks [116,117]. Though this does not include the PCS population, it may be a factor to consider if the use of EPIC treatment in acute concussion patients is further investigated.

BDNF is a key player in PECB. However, increased levels of glutamate and GABA also seem to play a role. Glutamate is the principal neuro-excitatory messenger molecule in the brain and its presence is key for proper synaptic functioning and, highly relevant to the present work here, it is the key initiator of NVC signaling. GABA, synthesized from glutamate, is the primary inhibitory neurotransmitter and plays an important role in regulating nervous system excitability. Both are needed for a concerted, appropriate level of excitatory synaptic activity. Both neurotransmitters are synthesized from carbon skeletons derived from carbohydrate (CHO), given a source of amino groups is available. Studies investigating metabolites in the brain both at rest and during exercise have consistently found that physical activity leads to abnormally high levels of CHO in the brain [118,119]. Interestingly, a relatively large portion of that CHO taken up by the brain does not become oxidized [118,119]. What becomes of the surplus non-oxidized CHO is not yet fully understood. However and of interest to the EPIC treatment, it has been proposed that the non-oxidized CHO is utilized for the *de novo* synthesis of glutamate and GABA [118,120]. In a recent human study, Maddock et al. [121] report a consistent increase in cortical glutamate and GABA levels following a single bout of exercise in young, healthy, physically active volunteers. Therefore, along with BDNF's effect, glutamate and GABA increases after aerobic exercise also appear to contribute to the PECB.

In conjunction with BDNF and amino acid neurotransmitters, IGF-1 may also contribute to the PECB. Recent studies have implicated a role for IGF-1 in several PECB components including synaptic plasticity, neurotransmitter synthesis and release and enhanced cognitive functioning, including learning and memory [122-125]. Further, IGF-1 is produced in peripheral tissue in response to exercise. Though the literature supporting IGF-1 increases in acute exercise is not as convincing as BDNF, it may contribute to the PECB.

However, the fact that BDNF, IGF-1 and amino acid transmitter increases help in facilitating synaptic functioning and other features of neuronal plasticity makes it less likely that neurogenesis is the single variable explaining the effects of exercise on enhanced cognition. Angiogenesis is another variable to be considered. The effect exercise has on brain angiogenesis is relatively well known as compared to the more recent findings of neurovascular adaptions in the hippocampus associated with enhanced cognitive functioning [126,127]. The presence of VEGF is considered essential for the angiogenic effect exercise has on the brain. Endothelial cells within the brain have been shown to proliferate after exercise [128]. Also, serum levels of both IGF and VEGF have been shown to increase in response to exercise, as well as hippocampal IGF gene expression [129-131]. Therefore, the neurogenic effects of BDNF and IGF-1 combine with the angiogenic effects of VEGF to enhance NVC and improve cognitive abilities post-exercise.

The neurocognitive challenge occurring during the PECB period is a crucial feature of NVC re-regulation in the EPIC treatment. However, the intracerebral steal phenomenon presents a barrier to this process. As discussed previously, regions of dysregulated NVC receive a "double hit" of decreased blood flow needed for their rehabilitation. First, the NVC dysregulated regions, by definition, cannot trigger their own blood flow appropriately. Second, noradrenergic systems in the ANS-dysregulated state of PCS are unstable and do not effectively constrict vessels surrounding the activated neural region, leading to blood being shunted away from activated regions. Therefore, avoiding intracerebral steal phenomenon is also a key aspect in NVC re-regulation. First, ANS/CD re-regulation as discussed in previous sections plays a role in stabilizing the cerebral noradrenergic systems, allowing more efficient constriction of vessels surrounding activated neural regions. Second, it is important that during neurocognitive challenge in the PECB period, neural activation be limited to those regions of targeted NVC dysregulation. That is, other neural activity should be minimized as much as possible. Pain serves as one such neural distractor. In fact, the medial prefontal cortex, related to the transmission of pain signals, is one region of interest in the fNCI used to target regions of dysregulated NVC as well as a target of several neurocognitive challenges. Neuromuscular therapists play a vital role in EPIC treatment and help to minimize chronic pain in patients prior to treatment. Also, with the help of the patient's primary care physicians, medical therapy for other pain causing disorders (migraines, etc.) is maximized. Other neural distractors include light and noise stimulation, balance and posturing and cardiovascular orthostatic control. Occupational therapist work to improve balance and posturing and light/noise stimulation is tightly controlled during the neurocognitive challenges. In short, avoiding intracerebral steal is a key component of NVC re-regulation and involves addressing ANS/CD and minimizing other factors that may draw on the brains pool of operative resources.

In summary, Wing et al. [23] have demonstrated initial success in the normalization of NVC in patients with PCS using an NVC-targeting treatment. The present report is the first to propose a novel theory laying out the mechanistic processes by which this form of treatment re-regulates NVC. First, NVC re-regulation should occur in the setting of restored CVR and re-regulated ANS/CD. This provides the ideal setting for NVC re-regulation, including increased global blood flow, more pliable, neural-activity accommodating blood vessels and a stabilized cerebral noradrenergic system. Second, regions of neural NVC dysregulation (targeted by fNCI) must be activated by neurocognitive challenges. The neuronal activation must be a) focused on the regions of interest, b) intense, c) prolongedand d) cyclical. However, because many PCS patients fatigue too quickly upon initiation of these neurocognitive challenges, NVC re-regulation relies heavily on the PECB. The PECB provides a window of time post-exercise where cognitive abilities are enhanced. It involves increases in BDNF, glutamate, GABA, IGF-1 and VEGF, which serve to promote neurogenesis, synaptic activity, neurotransmission and angiogenesis, all of which encourage proper NVC. NVC re-regulation is further aided by the avoidance of intracerebral steal. This process, occurring cyclically (6-8 h per day for 4 contiguous days), serves to coax an abnormally regulated NVC system back into its pre-dysregulated state.

Conclusion

Compared to the pathophysiologic mechanisms occurring in acute concussion, the underlying neuropathophysiology of chronic concussive sequelaeor PCS is less understood, though becoming clearer with emerging research. The present report seeks to provide a theory of the pathologic state of PCS and the repair of that pathology that both unifies the findings of current research and explains the recent therapeutic success seen in PCS patient using NVC-targeting treatment [23]. Briefly, global disturbances in CBF from CVR disruption and ANS dysregulation provide significant contributions to the pathologic state of PCS. EPIC treatment addresses these two characteristics, largely through aerobic exercise. The work of Leddy et al. [24,89-91,93,99-103] provide support to our proposed theory concerning CVR restoration and ANS re-regulation. Concerning local blood flow regulation, NVC dysregulation significantly contributes to PCS pathology. EPIC treatment has successfully normalized dysregulated NVC in over 500 patients with PCS. We propose a novel theory concerning the mechanisms by which NVC dysregulation is normalized. This includes focused, intense and repetitive neurocognitive challenges during PECB and the avoidance of intracerebral steal in the setting of restored and re-regulated CVR and ANS, respectively. The goal of this theoretical description is to make mechanisms and treatment approaches explicit enough that they lead to clear, falsifiable predictions for further research and clinical innovation. Future investigations should include a) gradation of NVC changes after single, isolated rounds of neurocognitive challenging in the post-exercise cognitive boost period, b) effects of NVC re-regulation on patients categorized by symptomatology (i.e., patients with predominate somatic or affective symptoms) and c) NVC re-regulation in the setting of PCS secondary to multiple, repeat mTBI's.

Conflicts of Interest

MDA is a co-owner of Cognitive FX and Notus Neuropsychological Imaging, a privately owned neurocognitive-imaging and treatment center that provides treatment described in this work. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Acknowledgement

Dr. Allen is a co-owner of Cognitive FX, a privately owned and operated neurocognitive-imaging center. This research did not receive any funding or support from agencies in the public, commercial or not-for-profit sectors.

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