



Leaky Blood–Brain Barrier: A Double Whammy for the Brain

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Blood–Brain Barrier Dysfunction in Aging Induces Hyperactivation of TGF β Signaling and Chronic Yet Reversible Neural Dysfunction

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A growing body of evidence shows that epileptic activity is frequent but often undiagnosed in patients with Alzheimer disease (AD) and has major therapeutic implications. Here, we analyzed electroencephalogram (EEG) data from patients with AD and found an EEG signature of transient slowing of the cortical network that we termed paroxysmal slow wave events (PSWEs). The occurrence per minute of the PSWEs was correlated with level of cognitive impairment. Interictal (between seizures) PSWEs were also found in patients with epilepsy, localized to cortical regions displaying blood–brain barrier (BBB) dysfunction, and in 3 rodent models with BBB pathology: aged mice, young 5 \times familial AD model, and status epilepticus–induced epilepsy in young rats. To investigate the potential causative role of BBB dysfunction in network modifications underlying PSWEs, we infused the serum protein albumin directly into the cerebral ventricles of naive young rats. Infusion of albumin, but not artificial cerebrospinal fluid control, resulted in high incidence of PSWEs. Our results identify PSWEs as an EEG manifestation of nonconvulsive seizures in patients with AD and suggest BBB pathology as an underlying mechanism and as a promising therapeutic target.

Commentary

The human has more than 600 km of blood vessels that deliver oxygen and nutrients to and remove metabolic wastes from the brain. Providing nearly 12 m² of endothelia cell surface area, brain capillaries are a major site of blood–brain barrier (BBB) that is composed of specialized endothelia cells ensheathed by pericytes, smooth muscle cells, and astrocytic end feet.¹ With tightly sealed cell-to-cell contacts, the BBB strictly controls the entry of blood-borne molecules and therefore establishes a precisely regulated microenvironment that contains uniquely balanced chemical composition, brain-specific growth factors, and signaling molecules.² This brain internal milieu is immune privileged and required for proper synaptic, neuronal, and network activity.

Remarkably, epidemiological studies show vascular pathologies are a major risk factor for dementia and predict low scores in all cognitive domains. Emerging evidences including neuroimaging, postmortem, and biomarker studies have shown disrupted BBB integrity in neurodegenerative diseases.³ Blood–brain barrier breakdown allows neurotoxic blood-borne molecules (eg, albumin and plasmin) and cells (eg, leukocytes) to enter the brain, which subsequently initiates neuroinflammatory as well as innate and adaptive immune

responses through activating microglia and astrocytes. In addition, dysfunctional efflux at the disruptive BBB leads to impaired clearance of metabolic waste and toxic Abeta species from the brain. These observations give rise to the 2-hit vascular hypothesis proposing that BBB dysfunction leads to Abeta-independent neuronal injury (first hit) and acts with genetic and environmental risk factors to promote Abeta accumulation (second hit) due to faulty clearance. On the other hand, recent findings suggest that immune response, glial dysfunction, and persistent inflammation associated with dysfunctional BBB play a critical role in epileptogenesis.⁴ In addition to cytokines produced by invaded immune cells, albumin extravasation has been observed in the brain parenchyma in different epilepsy models. Astrocytes take up albumin via transforming growth factor beta (TGF- β) receptors followed by downregulated expression of potassium channels and glutamate transporters. Transforming growth factor- β activation also gives rise to inflammation, disrupted perineuronal net, and excitatory synaptogenesis.

Indeed, association between dementia and epilepsy has long been observed from epidemiological data. In the 1950s, Sjogren et al noticed a seizure prevalence of 22% in a small sample of pathologically proven patients with late-stage Alzheimer



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disease (AD). More recent studies established an incidence rate of seizures in AD between 4.8 and 11.9/1000 person-years.⁵ Although the widely held view is that epilepsy occurs at the late stage of AD, subclinical seizures and spikes and/or MEG epileptiform discharges could be detected in 42% of patients with AD without a history of seizures.⁶ However, the underlying mechanisms are poorly understood. Although a direct excitatory effect of Abeta and/or Tau on brain networks has been proposed, the role of neuroinflammation may represent the missing link between neurodegeneration and seizures in light of mounting experimental evidences.


The recent study by Senatorov et al provides impressive evidence uncovering fundamental mechanisms linking BBB breakdown to both age-related cognitive decline and seizures.⁷ In the back-to-back accompanying paper, the author first identified paroxysmal slow wave events (PSWEs, a median power frequency of less than 6 Hz over 5 consecutive seconds) as the electroencephalogram (EEG) signature of patients with AD and showed that the occurrence per minute of the PSWEs was correlated with the level of cognitive impairment. Intriguingly, interictal PSWEs were also found in patients with epilepsy and could be specifically localized to cortical regions displaying BBB dysfunction in the aged mice, young transgenic AD mice, and young epileptic rats induced by status epilepticus.⁸ The authors therefore concluded that PSWEs constitute distinct subclinical paroxysmal events associated with hyperexcitability. Using serum albumin extravasation or the fluorescent tracer, Evans blue, as the marker of BBB permeability, the authors then showed albumin was absent from the hippocampus in young mice but was detectable in the aging hippocampus as early as 12 months (middle age) and consistently increased toward the end-of-life span. Based on the finding that albumin-positive cells were predominately astrocytes, the authors suggested that albumin uptake is cell-type specific. Concordant with previous work, the authors confirmed the relationship between albumin uptake by astrocytes and TGF β activation by quantifying immunolabeled phosphorylated Smad2 protein (pSmad2). Then, the authors used pentylenetetrazole (PTZ) seizure induction assays to test the hypothesis that hyperexcitability may be triggered by BBB decline. They showed an increased seizure severity started at 12 months of age and older mice became highly vulnerable. Although no spontaneous seizures were detected in aged mice, the author reported that they displayed increased aberrant electrographic signals characterized by discrete bursts of PSWEs. In conjunction with data in the accompanying paper, these studies showed that BBB dysfunction and TGF β signaling are early indicators of aging in both rodents and humans and are associated with symptomatic hyperexcitability in rodents.

Next, the author used gain-of-function and loss-of-function experiments to determine the causal link between BBB breakdown and age-related neural dysfunction and cognitive impairment. First, the author infused albumin (iAlb) into the ventricles of healthy young adult animals via an osmotic mini-pump, which robustly triggered astrocytic TGF β signaling within 48 hours of infusion. Pentylenetetrazole-induced

seizure severity and mortality were significantly increased in iAlb mice compared to controls. Electroencephalogram recording in young iAlb rats showed an increased PSWE count that was observed only from the ipsilateral hemisphere receiving iAlb infusion but not in the contralateral hemisphere, indicating specificity of the aberrant neural activity to the tissue affected by iAlb. Morris water maze spatial memory tasks also confirmed that iAlb mice had impaired memory performance. Secondly, the authors generated an astrocytic-specific TGF β signaling knockdown model (TGF β R2/KD) using a floxed TGF β receptor mouse line and an inducible Cre line under glial high-affinity glutamate transporter promoter. Mice with homozygous-induced KD had low vulnerability to PTZ-induced seizures and mortality in early aging (12-16 months) as well as late aging (17- to 21-month-old) mice and made more correct choices in the T-maze task, indicating improved working memory. Thirdly, the authors tested the efficacy of a small-molecule TGF β R1 inhibitor called IPW, and showed the inhibitor reduced pSmad2 amounts and blocked iAlb-induced seizure vulnerability in the young brain. Lastly, the authors gave 5 days of IPW treatment in mice aged to 2 years old, near the end of the life span, and restored TGF β signaling to the extent similar to that of young mice. As in the TGF β R2 KD genetic intervention, IPW showed lower seizure severity and mortality compared to aged control mice treated with vehicle, markedly reduced the number of PSWEs, restored the profile of EEG activity similar to that of young mice, and improved cognitive performance in the T-maze and novel object tasks. Interestingly, the treatment efficacy lasted beyond the treatment phase and persisted through the end of the washout period, indicating that inhibition of TGF β signaling may generate a long-lasting change in the underlying neural circuits.

The authors provided impressive evidence uncovering a foundational mechanism linking BBB integrity to neural dysfunction that leads to age-related hyperexcitability and cognitive decline. Importantly, the authors also tested a new disease-modifying therapy that is mechanistically distinct from other canonical dementia targets. Surprisingly, despite the fact that early BBB dysfunction in aging might generate accumulated irreversible damage, one week of acute treatment reversed the pathological outcomes in aged mice, including elevated TGF β signaling, aberrant EEG activity, seizure vulnerability, and cognitive dysfunction. Limitation of this study includes (1) albumin is not a reliable index of the duration or progression of the BBB disruption because it will continue to diffuse and accumulate once it has entered the extracellular space. A fluorophore-labeled albumin injection can be used to circumvent this problem. (2) Intracerebral ventricle albumin infusion has been shown to induce recurrent and frequent seizures (>1 per day) that might have directly caused cognitive impairment of those tested animals. (3) Other serum signals such as fibrinogen may be implicated in the activation of TGF β pathways.⁹ For future research, one unsolved question is what causes the disruption of BBB in aged mice or patients? In addition, although the back-to-back articles proposed PSWEs as “silent” or “subclinical” epileptiform activities that

appeared to be present in both patients with AD and/or epilepsy, there are clear knowledge gaps between this unique EEG signature, seizure predictability, and underlying neural/network hyperexcitability.

By Yu Wang 

ORCID iD

Yu Wang  <https://orcid.org/0000-0002-2489-1288>

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