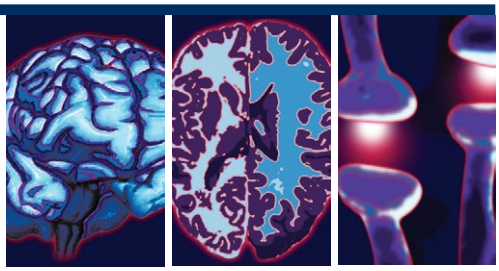
Review



Mechanisms of Vagus Nerve Stimulation for Epilepsy and Associated Comorbidities

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Abstract

While the efficacy of vagus nerve stimulation (VNS) to reduce seizures and improve comorbidities associated with pharmacoresistant epilepsy including mood as well as quality of life is clinically proven, the exact mechanism of VNS remains unclear. VNS exerts antiepileptic or anti-epileptogenic effect possibly through i) neuromodulation of release of noradrenaline from locus coeruleus; ii) induced profound changes in brain blood flow; iii) immunomodulation or anti-neuroinflammation; iv) change EEG brain functional connectivity; v) modification of the proteome of excitatory synapses of amygdaloid/piriform cortex; vi) modulation of adenosine system and DNA methylation. Beyond epilepsy, VNS is also under investigation for the treatment of epilepsy associated comorbidities including cognitive comorbidities and psychiatric comorbidities. Of importance, progression in VNS clinical efficacy over time suggests an underlying disease-modifying neuromodulation, which is an emerging field in pharmacoresistant epilepsy. With bidirectional potential clinical efficacy of VNS in epilepsy, a prototype neuropsychiatric illness, further research on the solid mechanisms of VNS for epilepsy and associated comorbidities is encouraging.

Keywords

Epilepsy, Comorbidity, Vagus nerve stimulation, Neuromodulation

Introduction

Up to 30 percent of patients with epilepsy is pharmacoresistant [1,2], and apart from those who are candidates for resective surgery, most will continue to have disabling seizures and the poor quality of life with a wide range of cognitive and psychiatric symptoms [3-7]. Epilepsy may be regarded as prototype neuropsychiatric illness with interface of neurology and psychiatry, and treatment of comorbidity is likely to improve the overall course of illness as well as quality of life. Therefore, new therapies aim to modify the progression of epilepsy (disease modification) and concomitant comorbidities through by targeting the disease process. Vagus nerve stimulation (VNS) is a neuromodulatory treatment that is used as a palliative therapy for patients with pharmacoresistant epilepsy who are not suitable candidates for resective brain surgery or for whom surgery has failed [8]. VNS is also a possible treatment option for treatment of epilepsy associated comorbidities including cognitive comorbidities and psychiatric comorbidities. VNS has been proved to be effective in the treatment epileptic seizures, improve quality of life as well as progression in VNS clinical efficacy over time [9,10]. Currently, most of the VNS studies in epilepsy mainly focused on VNS effectiveness in seizure control. Several studies demonstrated that the mechanism of action might be related to neuromodulation of release of noradrenaline from locus coeruleus [11], induced

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hippocampal decreases in glucose metabolism [12] and blood flow [13], immunomodulation or anti-neuroinflammation [14,15], change EEG brain functional connectivity [16,17] as well as modification of neuronal activity and the proteome of excitatory synapses of amygdaloid/piriform cortex [18], and possible modulation of adenosine system and DNA methylation [19-23].

Comorbidities in epilepsy represent a major conceptual and therapeutic challenge. Currently, the bidirectional relation between epilepsy and associated comorbidities has been paid more and more attentions [24-26], and advances on the overlap of psychiatric / cognitive and neurologic symptoms from a pathophysiologic and phenomenologic perspective are becoming a hot topic in epilepsy. Depressive disorders are the most common type of psychiatric comorbidity in patients with epilepsy [7,27,28], especially in individuals suffering from refractory temporal lobe epilepsy. Several mechanisms of primary depressive disorders such as endocrine abnormalities, structural and functional abnormalities of cortical and subcortical structures, neurotransmitter abnormalities and immunological inflammation abnormalities [7], have an effect on cortical hyperexcitability and the epileptogenic process. The mechanism of VNS to treat epilepsy associated comorbidities might be through the mechanisms mentioned above [15,25,29,30]. Of scientific interesting, progression in VNS clinical efficacy over time and chronic VNS clearly induces long-lasting changes in the neuronal network involved in epileptogenesis [31,32], indicating that long term use of VNS modify the progression of epilepsy (disease modification or antiepileptogenesis) [33], and that the earlier this is done, the better the outcome for seizures and associated comorbidities control [34]. In this review, we will focus on the mechanisms of action of VNS for epilepsy and associated comorbidities.

Mechanisms of Action of VNS for Epilepsy

■ Serotonin as a mediator of the antiepileptic effects of VNS

Recent study provided convincing evidence for the existence of a strong causal link between increased noradrenergic signaling and the anticonvulsant effect of VNS. Increased in extracellular hippocampal noradrenaline (NE), but not of dopamine, serotonin and GABA, has been indicated to be responsible for its seizure-suppressing effect in a model for limbic seizures, and regarded as a potential biomarker for the efficacy of VNS in temporal lobe epilepsy [11]. Selective a_2-adrenoreceptor antagonism in proximity of the seizure focus abolishes the seizure-suppressing effect of VNS [11]. The locus coeruleus, the most important source of NE in the brain [35], appears to be crucial for the anticonvulsive effects of VNS since seizure-suppressive effects of VNS were prevented by LC lesioning [36,37]. Serotonergic transmission may also play a role since basal firing rates of serotonergic neurons in the dorsal raphe nucleus significantly increased after chronic VNS. However, this effect seems to be NE-dependent since selective lesioning of the locus coeruleus prevented this enhancement of serotonin neuron firing [38,39].

■ Induced profound changes in brain blood flow

Positron-emission tomography and functional magnetic resonance imaging of the effects of VNS in human beings have confirmed the influence the vagus nerve on higher brain structures. Stimulation of VNS causes increases in cerebral blood flow and can alter electroencephalographic patterns. Clinical studies with positron-emission tomography demonstrated that VNS increased blood flow to the right thalamus, the right posterior temporal cortex, the left putamen, and the left inferior cerebellum at interictal stage of seizures [40]; Clinical studies with functional magnetic resonance imaging indicated that the areas of significant activation in response to VNS were the bilateral orbitofrontal and parieto-occipital cortex, the left temporal cortex, and the left amygdala at interictal stage of seizures [41]. Animal study demonstrated that VNS can arrest ongoing seizure activity (ictal stage of seizures) by ultimately decreasing hippocampal blood flow [13].

■ Anti-neuroinflammation or immunomodulation

Extensive experimental and clinical evidence supports a link between inflammation and epilepsy, both in terms of epileptogenesis and the long-term consequences of seizures, which indicates that activation of inflammatory processes in the brain is a common feature of various epileptic disorders [42,43]. With an intact vagal-immune network, VNS can dampen inflammatory response. The vagus nerve is implicated in immunomodulation as efferent vagus nerve fibres systemically inhibit pro-

inflammatory cytokine release [30]. In addition, VNS activates the hypothalamic-pituitary-adrenal axis. Animal research has demonstrated that VNS-induced increased hippocampal expression of corticotrophin releasing factor and increased plasma levels of adrenocorticotropic hormone and corticosteron [14], which support the role of the VNS in immunomodulation or anti-neuroinflammation.

■ Change EEG brain functional connectivity

EEG brain functional connectivity is a way to study brain function through the study of pair-wise correlations, and reflects how different brain areas coordinate their activities. Estimating changes of EEG brain functional connectivity is indicated as a promising tool for predicting response to VNS [16,44]. The effect of VNS on functional connectivity has been studied using scalp EEG demonstrated that functional connectivity tended to be lower in the on period, and that this effect was maximal for responder patients [44]. More recently, study investigated the impact of VNS on brain functional connectivity with stereotactic EEG signals [16]. The results demonstrated that VNS can decrease or increase the functional connectivity changes with variable effect from patient to patient, and clinical responder with decreased functional connectivity [16].

■ Modification the proteome of excitatory synapses of amygdaloid/piriform cortex

The molecular mechanisms underlying VNS for epilepsy are overall unclear. Plasticity of excitatory synapses is thought to contribute to the hyperexcitability of epilepsy [45]. The postsynaptic density (PSD) is a membrane specialization of the postsynaptic component of excitatory synapses in the CNS and the protein composition of the PSD is regulated by neuronal activity [46]. Recent study demonstrated that VNS modifies both neuronal activity in amygdala and hippocampus and the composition of excitatory synapses in the CNS [47], which suggested that activity-dependent formation of excitatory synapses might be molecular targets of VNS for epilepsy.

■ Modulation of adenosine system and DNA methylation

Adenosine is an inhibitory modulator of brain activity, and its anticonvulsant and seizure terminating effects, mediated by both receptor-dependent and receptor–independent pathways, have been illustrated in a wide range of experimental models of epilepsy and clinical studies [20,48-60]. Therapeutic adenosine augmentation is a powerful therapeutic strategy to suppress epileptic seizures and epileptogenesis [20,61-64]. Neurostimulation has been indicated to increase the extracellular adenosine concentration in the brain [21-23] to enhance adenosine signaling and adenosine A1 receptor-dependent activation. On the other hand, increase of adenosine levels in the brain might also exert receptor-independent effects in DNA methylation homeostasis to reduce DNA methylation [20,65]. There is every indication that agents able to increase adenosine availability may have a place in the future treatment of epilepsy via adenosine receptor-dependent pathway and adenosine receptor-independent pathway [66]. How the VNS modulated adenosine system and exert its efficacy in the treatment of epilepsy and modification the progression of epilepsy needs further investigation in the future.

Mechanisms of action of VNS for comorbidities associated with epilepsy

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of this condition [67]. Up to 30 percent of patients with epilepsy is pharmacoresistant [1], and apart from those who are candidates for respective surgery, most will continue to have disabling seizures and the poor quality of life with a wide range of cognitive and psychiatric symptoms [5,7]. Recurrent seizures induced the reorganization of neural circuits and activities in the brain, therefore, patients frequently experience cognitive, psychiatric and mood disorders [68]. On the other hand, the most recent research indicates that some neurocognitive and psychological comorbidities as well as structural brain changes predate the onset of seizures, with the early cognitive compromise being further magnified by the onset of epileptogenesis, and later on, by the chronicity of seizures [69,70]. Epilepsy, being regarded as a prototype of neuropsychiatric /neurocognitive and illness, epilepsy and associated comorbidities are usually frequent and share common underlying mechanisms with epilepsy. Currently, the bidirectional relation between epilepsy and associated comorbidities has been paid much more attentions [25,26,71].

Currently, mechanisms of dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis, and compromised raphe-hippocampal serotonergic transmission are well accepted behind epilepsy and neuropsychiatric disorders [72]. VNS has been proved to increase the basal firing rates of serotonergic neurons in the dorsal raphe nucleus, thus plays a role in both seizures and associated comorbidities.

Recently, adenosine dysfunction has been indicated as the underlying mechanism for comorbidities associated with epilepsy and that therapeutic adenosine augmentation might be effective for the treatment of epilepsy and comorbid symptoms in epilepsy [73]. Clinical as well as experimental data suggest that a triad of synaptotoxicity, astrogliosis, and overexpression of ADK, resulting in a deficiency of homeostatic adenosine can directly cause a wide range of cognitive and psychiatric symptoms commonly seen as comorbidities in epilepsy [73] as follows:

■ Adenosine and epilepsy

As introduced above, extensive experimental and clinical evidence demonstrated that dysfunctional astrocytic adenosine homeostasis as one of the early pathophysiologic mechanisms of epilepsy, and therapeutic adenosine augmentation exerts anticonvulsant and seizure terminating effects, mediated by both receptor-dependent and receptor–independent pathways [20,54,57,61].

■ Adenosine and cognition

Adenosine affects cognitive processes on several mechanistic levels through locally refined neuronal and astroglial A2AR signaling effects and modulation of glutamatergic, dopaminergic, GABAergic, and BDNF-dependent mechanisms [73]. Deletion of adenosine A2A receptors from astrocytes disrupts glutamate homeostasis leading to cognitive impairment [74]. Adenosine augmentation to the hippocampus can improve cognitive function [75]. These findings suggest that therapeutic adenosine augmentation might constitute a promising approach for the treatment of comorbid depression in a wide range of neurological and neuropsychiatric disorders.

■ Adenosine and depression

Recent study demonstrated that astrocytic signaling to adenosine A1 receptor was required for the robust reduction of depressive-like behaviors in mice following 12 h of sleep deprivation [76]. Approaches known to increase adenosine level such as exercise [76,77], sleep deprivation [76,78], acupuncture [22], deep brain stimulation [23], or ketogenic diet [79] have demonstrated antidepressive effects. S-adenosylhomocysteine, a precursor of adenosine, has been used for the treatment of major depression [80]. VNS, the most commonly used neuromodulation for pharmacoresistant epilepsy, might constitute a promising approach for the treatment of epilepsy associated comorbidities as well through adenosine system.

Concluding remarks

Advances on the overlap of psychiatric and neurologic symptoms from a pathophysiologic and phenomenologic perspective are becoming a hot topic in epilepsy. New therapies aim to modify the progression of epilepsy and concomitant comorbidities through by targeting the disease process. VNS has demonstrated its potential in pharmacoresistant epilepsy and comorbidities associated with epilepsy, and enhanced VNS efficacy over time clearly reflects a disease modification effects. It is crucial to identify and validate the biomarkers for the VNS therapy that track with disease progression and comorbidities, and predict therapeutic outcome. In the future, research will focus on how to combine neurocognitive and neuropsychiatric markers, allowing systematic advances in our understanding of the natural history of cognitive and behavioral disturbances in the epilepsies relative to the onset and progression of seizures.

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References

1. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N. Engl. J. Med* 342(5), 314-319 (2000).
2. Sillanpaa M, Schmidt D. Natural history of treated childhood-onset epilepsy: prospective, long-term population-based study. *Brain* 129(3), 617-624 (2006).
3. Salpekar JA, Berl MM, Havens K, *et al.* Psychiatric symptoms in children prior to epilepsy surgery differ according to suspected seizure focus. *Epilepsia* 54(6), 1074-1082 (2013).
4. Salpekar JA, Mishra G, Hauptman AJ. Key issues in addressing the comorbidity of depression and pediatric epilepsy. *Epilepsy. Behav* 46(1), 12-18 (2015).
5. Witt JA, Helmstaedter C. Cognition in epilepsy: current clinical issues of interest. *Curr. Opin. Neurol* 30(2), 174-179 (2017).
6. Hu Y, Jiang Y, Hu P, *et al.* Impaired social cognition in patients with interictal epileptiform discharges in the frontal lobe. *Epilepsy. Behav* 57(A), 46-54 (2016).
7. Kanner AM. Can neurobiological pathogenic mechanisms of depression facilitate the development of seizure disorders? *Lancet. Neurol* 11(12), 1093-1102 (2012).
8. Fisher RS, Velasco AL. Electrical brain stimulation for epilepsy. *Nat. Rev. Neurol* 10(5), 261-270 (2014).
9. The neuropsychological efficacy of vagus nerve stimulation in 56 children with catastrophic epilepsy. *Neuropsychiatry-Lond* 7(4), 640-648 (2017).
10. Orosz I, McCormick D, Zamponi N, *et al.* Vagus nerve stimulation for drug-resistant epilepsy: a European long-term study up to 24 months in 347 children. *Epilepsia* 55(10), 1576-1584 (2014).
11. Raedt R, Clinckers R, Mollet L, *et al.* Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a limbic seizure model. *J. Neurochem* 117(3), 461-469 (2011).
12. Dedeurwaerdere S, Cornelissen B, Van Laere K, *et al.* Small animal positron emission tomography during vagus nerve stimulation in rats: a pilot study. *Epilepsy. Res* 67(3), 133-141 (2005).
13. Hotta H, Watanabe N, Orman R, *et al.* Efferent and afferent vagal actions on cortical blood flow and kainic acid-induced seizure activity in urethane anesthetized rats. *Auton. Neurosci* 156(1-2), 144-148 (2010).
14. De Herdt V, Puimege L, De Waele J, *et al.* Increased rat serum corticosterone suggests immunomodulation by stimulation of the vagal nerve. *J. Neuroimmunol* 212(1-2), 102-105 (2009).
15. Pavlov VA, Parrish WR, Rosas-Ballina M, *et al.* Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. *Brain. Behav. Immun* 23(1), 41-45 (2009).
16. Bartolomei F, Bonini F, Vidal E, *et al.* How does vagal nerve stimulation (VNS) change EEG brain functional connectivity? *Epilepsy. Res* 126(1), 141-146 (2016).
17. Wang K, Chai Q, Qiao H, *et al.* Vagus nerve stimulation balanced disrupted default-mode network and salience network in a postsurgical epileptic patient. *Neuropsychiatr. Dis. Treat* 12(1), 2561-2571 (2016).
18. Alexander GM, Huang YZ, Soderblom EJ, *et al.* Vagal nerve stimulation modifies neuronal activity and the proteome of excitatory synapses of amygdala/piriform cortex. *J. Neurochem* 140(4), 629-644 (2017).
19. Pajski ML, Venton BJ. The mechanism of electrically stimulated adenosine release varies by brain region. *Purinergic. Signal* 9(2), 167-174 (2013).
20. Williams-Karnesky RL, Sandau US, Lusardi TA, *et al.* Epigenetic changes induced by adenosine augmentation therapy prevent epileptogenesis. *J. Clin. Invest* 123(8), 3552-3563 (2013).
21. Ross AE, Nguyen MD, Privman E, *et al.* Mechanical stimulation evokes rapid increases in extracellular adenosine concentration in the prefrontal cortex. *J. Neurochem* 130(1), 50-60 (2014).
22. Goldman N, Chen M, Fujita T, *et al.* Adenosine A1 receptors mediate local anti-nociceptive effects of acupuncture. *Nat. Neurosci* 13(7), 883-888 (2010).
23. Miranda MF, Hamani C, de Almeida AC, *et al.* Role of adenosine in the antiepileptic effects of deep brain stimulation. *Front. Cell. Neurosci* 8(1), 312 (2014).
24. Wasade VS, Schultz L, Mohanarangan K, *et al.* Long-term seizure and psychosocial outcomes of vagus nerve stimulation for intractable epilepsy. *Epilepsy. Behav* 53(), 31-36 (2015).
25. Kumar U, Medel-Matus JS, Redwine HM, *et al.* Effects of selective serotonin and norepinephrine reuptake inhibitors on depressive- and impulsive-like behaviors and on monoamine transmission in experimental temporal lobe epilepsy. *Epilepsia* 57(3), 506-515 (2016).
26. Wilson SJ, Baxendale S. Reprint of: The new approach to classification: Rethinking cognition and behavior in epilepsy. *Epilepsy. Behav* 64(B), 300-303 (2016).
27. Tellez-Zenteno JF, Patten SB, Jette N, *et al.* Psychiatric comorbidity in epilepsy: a population-based analysis. *Epilepsia* 48(12), 2336-2344 (2007).
28. Kanner AM, Balabanov A. Depression and epilepsy: how closely related are they? *Neurology* 58(8), S27-39 (2002).
29. Grimonprez A, Raedt R, Dauwe I, *et al.* Vagus nerve stimulation has antidepressant effects in the kainic acid model for temporal lobe epilepsy. *Brain. Stimul* 8(1), 13-20 (2015).
30. Pavlov VA, Tracey KJ. The cholinergic anti-inflammatory pathway. *Brain. Behav. Immun* 19(5-8), 493-499 (2005).
31. Bari AA, Pouratian N. Brain imaging correlates of peripheral nerve stimulation. *Surg. Neurol. Int* 3(4), S260-8 (2012).
32. Nune G, DeGiorgio C, Heck C. Neuromodulation in the Treatment of Epilepsy. *Curr. Treat. Options. Neurol* 17(10), 375 (2015).
33. Yuan H, Silberstein SD. Vagus Nerve and Vagus Nerve Stimulation, a Comprehensive Review: Part III. *Headache* 56(3), 479-490 (2016).
34. Lagae L, Verstrepen A, Nada A, *et al.* Vagus nerve stimulation in children with drug-resistant epilepsy: age at implantation and shorter duration of epilepsy as predictors of better efficacy? *Epileptic. Disord* 17(3), 308-314 (2015).
35. Naritoku DK, Terry WJ, Helfert RH. Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy. Res* 22(1), 53-62 (1995).
36. Krahl SE, Clark KB, Smith DC, *et al.* Locus coeruleus lesions suppress the seizure-attenuating effects of vagus nerve stimulation. *Epilepsia* 39(7), 709-714 (1998).
37. Aalbers M, Vles J, Klinkenberg S, *et al.* Animal models for vagus nerve stimulation in epilepsy. *Exp. Neurol* 230(2), 167-175 (2011).
38. Manta S, Dong J, Debonnel G, *et al.* Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *J. Psychiatry. Neurosci* 34(4), 272-280 (2009).
39. Manta S, Dong J, Debonnel G, *et al.* Optimization of vagus nerve stimulation parameters using the firing activity of serotonin neurons in the rat dorsal raphe. *Eur. Neuropsychopharmacol* 19(4), 250-255 (2009).
40. Ko D, Heck C, Grafton S, *et al.* Vagus nerve stimulation activates central nervous system structures in epileptic patients during PET H2(15)O blood flow imaging. *Neurosurgery* 39(2), 430-431 (1996).
41. Bohning DE, Lomarev MP, Denslow S, *et al.* Feasibility of vagus nerve stimulation-synchronized blood oxygenation level-dependent functional MRI. *Invest. Radiol* 36(8), 470-479 (2001).
42. Vezzani A, French J, Bartfai T, *et al.* The role of inflammation in epilepsy. *Nat. Rev. Neurol* 7(1), 31-40 (2011).

43. Li T, Gao Q, Luan G. HMGB1-TLR Signaling in Rasmussens Encephalitis. *J. Neuroinfectious Diseases* 7(1) (2016).

44. Bodin C, Aubert S, Daquin G, *et al.* Responders to vagus nerve stimulation (VNS) in refractory epilepsy have reduced interictal cortical synchronicity on scalp EEG. *Epilepsy. Res* 113(1), 98-103 (2015).

45. Goussakov IV, Fink K, Elger CE, *et al.* Metaplasticity of mossy fiber synaptic transmission involves altered release probability. *J. Neurosci* 20(9), 3434-3441 (2000).

46. Trinidad JC, Thalhammer A, Burlingame AL, *et al.* Activity-dependent protein dynamics define interconnected cores of co-regulated postsynaptic proteins. *Mol. Cell. Proteomics* 12(1), 29-41 (2013).

47. Alexander GM, Huang YZ, Soderblom EJ, *et al.* Vagal nerve stimulation modifies neuronal activity and the proteome of excitatory synapses of amygdala/piriform cortex. *J. Neurochem* 140(4), 629-644 (2017).

48. Boison D. Adenosinergic signaling in epilepsy. *Neuropharmacology* 104(1), 131-139 (2016).

49. Li T, Gao Q, Luan G. Adenosine dysfunction in Rasmussen's encephalitis. *Neuropsychiatry-Lond* 6(5), 280-285 (2016).

50. Li T, Lan JQ, Boison D. Uncoupling of astrogliosis from epileptogenesis in adenosine kinase (ADK) transgenic mice. *Neuron. Glia. Biol* 4(2), 91-99 (2008).

51. Li T, Lytle N, Lan JQ, *et al.* Local disruption of glial adenosine homeostasis in mice associates with focal electrographic seizures: a first step in epileptogenesis? *Glia* 60(1), 83-95 (2012).

52. Li T, Quan LJ, Fredholm BB, *et al.* Adenosine dysfunction in astrogliosis: cause for seizure generation? *Neuron. Glia. Biol* 3(4), 353-366 (2007).

53. Li T, Ren G, Kaplan DL, *et al.* Human mesenchymal stem cell grafts engineered to release adenosine reduce chronic seizures in a mouse model of CA3-selective epileptogenesis. *Epilepsy. Res* 84(2-3), 238-241 (2009).

54. Li T, Ren G, Lusardi T, *et al.* Adenosine kinase is a target for the prediction and prevention of epileptogenesis in mice. *J. Clin. Invest* 118(2), 571-582 (2008).

55. Luan G, Gao Q, Guan Y, *et al.* Upregulation of adenosine kinase in Rasmussen encephalitis. *J. Neuropathol. Exp. Neurol* 72(11), 1000-1008 (2013).

56. Luan G, Gao Q, Zhai F, *et al.* Adenosine kinase expression in cortical dysplasia with balloon cells: analysis of developmental lineage of cell types. *J. Neuropathol. Exp. Neurol* 74(2), 132-147 (2015).

57. Masino SA, Li T, Theofilas P, *et al.* A ketogenic diet suppresses seizures in mice through adenosine A(1) receptors. *J. Clin. Invest* 121(7), 2679-2683 (2011).

58. Aronica E, Zurolo E, Iyer A, *et al.* Upregulation of adenosine kinase in astrocytes in experimental and human temporal lobe epilepsy. *Epilepsia* 52(9), 1645-1655 (2011).

59. Fedele DE, Li T, Lan JQ, *et al.* Adenosine A1 receptors are crucial in keeping an epileptic focus localized. *Exp. Neurol* 200(1), 184-190 (2006).

60. Li T, Gao Q, Luan G. Rasmussen's Encephalitis Clinical Features and Mechanism Advances. *J. Autism. Epilepsy* 2(1), 1007 (2016).

61. Li T, Steinbeck JA, Lusardi T, *et al.* Suppression of kindling epileptogenesis by adenosine releasing stem cell-derived brain implants. *Brain* 130(5), 1276-1288 (2007).

62. Wilz A, Pritchard EM, Li T, *et al.* Silk polymer-based adenosine release: therapeutic potential for epilepsy. *Biomaterials* 29(26), 3609-3616 (2008).

63. Ren G, Li T, Lan JQ, *et al.* Lentiviral RNAi-induced downregulation of adenosine kinase in human mesenchymal stem cell grafts: a novel perspective for seizure control. *Exp. Neurol* 208(1), 26-37 (2007).

64. Szybala C, Pritchard EM, Lusardi TA, *et al.* Antiepileptic effects of silk-polymer based adenosine release in kindled rats. *Exp. Neurol* 219(1), 126-135 (2009).

65. Lusardi TA, Akula KK, Coffman SQ, *et al.* Ketogenic diet prevents epileptogenesis and disease progression in adult mice and rats. *Neuropharmacology* 99(1), 500-509 (2015).

66. Borea PA, Gessi S, Merighi S, *et al.* Adenosine as a Multi-Signalling Guardian Angel in Human Diseases: When, Where and How Does it Exert its Protective Effects? *Trends. Pharmacol. Sci* 37(6), 419-434 (2016).

67. Fisher RS, van Emde BW, Blume W, *et al.* Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 46(1), 470-472 (2005).

68. Jensen FE. Epilepsy as a spectrum disorder: Implications from novel clinical and basic neuroscience. *Epilepsia* 52(1), 1-6 (2011).

69. Hermann BP, Dabbs K, Becker T, *et al.* Brain development in children with new onset epilepsy: a prospective controlled cohort investigation. *Epilepsia* 51(10), 2038-2046 (2010).

70. Hermann BP, Jones JE, Jackson DC, *et al.* Starting at the beginning: the neuropsychological status of children with new-onset epilepsies. *Epileptic. Disord* 14(1), 12-21 (2012).

71. Rao G, Mashkouri S, Aum D, *et al.* Contemplating stem cell therapy for epilepsy-induced neuropsychiatric symptoms. *Neuropsychiatr. Dis. Treat* 13(1), 585-596 (2017).

72. Pineda E, Shin D, Sankar R, et al. Comorbidity between epilepsy and depression: experimental evidence for the involvement of serotonergic, glucocorticoid, and neuroinflammatory mechanisms. *Epilepsia* 51(3), 110-114 (2010).

73. Boison D, Aronica E. Comorbidities in Neurology: Is adenosine the common link? *Neuropharmacology* 97(1), 18-34 (2015).

74. Matos M, Shen HY, Augusto E, *et al.* Deletion of adenosine A2A receptors from astrocytes disrupts glutamate homeostasis leading to psychomotor and cognitive impairment: relevance to schizophrenia. *Biol. Psychiatry* 78(11), 763-774 (2015).

75. Shen HY, Singer P, Lytle N, *et al.* Adenosine augmentation ameliorates psychotic and cognitive endophenotypes of schizophrenia. *J. Clin. Invest* 122(7), 2567-2577 (2012).

76. Hines DJ, Schmitt LI, Hines RM, *et al.* Antidepressant effects of sleep deprivation requires astrocyte-dependent adenosine mediated signaling. *Transl. Psychiatry* 3e, 212 (2013).

77. Dworak M, Diel P, Voss S, *et al.* Intense exercise increases adenosine concentrations in rat brain: implications for a homeostatic sleep drive. *Neuroscience* 150(4), 789-795 (2007).

78. Benington JH, Kodali SK, Heller HC. Stimulation of A1 adenosine receptors mimics the electroencephalographic effects of sleep deprivation. *Brain. Res* 692(1-2), 79-85 (1995).

79. Sussman D, Germann J, Henkelman M. Gestational ketogenic diet programs brain structure and susceptibility to depression & anxiety in the adult mouse offspring. Brain. Behav 5(2), e00300 (2015).

80. De Berardis D, Marini S, Serroni N, *et al.* S-Adenosyl-L-Methionine augmentation in patients with stage II treatment-resistant major depressive disorder: an open label, fixed dose, single-blind study. *Scientific. .World. J* 2013(1), 204649 (2013).