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BRIEF COMMUNICATION

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Serum serotonin levels in patients with epileptic seizures

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Summary

Profound cardiovascular and/or respiratory dysfunction is part of the terminal cascade in sudden unexpected death in epilepsy (SUDEP). Central control of ventilation is mediated by brainstem rhythm generators, which are influenced by a variety of inputs, many of which use the modulatory neurotransmitter serotonin to mediate important inputs for breathing. The aim of this study was to investigate epileptic seizure–induced changes in serum serotonin levels and whether there are potential implications for SUDEP. Forty-one epileptic patients were pooled into 2 groups based on seizure type as (1) generalized tonic–clonic seizures (GTCS) of genetic generalized epilepsy and focal to bilateral tonic–clonic seizures (FBTCS; n = 19) and (2) focal seizures (n = 26) based on clinical signs using surface video-electroencephalography. Postictal serotonin levels were statistically significantly higher after GTCS and FBTCS compared to interictal levels (P = .002) but not focal seizures (P = .941). The change in serotonin (postictal-interictal) was inversely associated with a shorter duration of tonic phase of generalized seizures. The interictal serotonin level was inversely associated with a shorter period of postictal generalized electroencephalographic suppression. These data suggest that peripheral serum serotonin levels may play a role in seizure features and earlier postseizure recovery; these findings merit further study.

KEYWORDS
focal, generalized, postictal EEG suppression, sudden unexpected death in epilepsy, tonic

1 | INTRODUCTION

Sudden unexpected death in epilepsy (SUDEP) is the second leading neurological cause of total years of potential life lost after stroke in the United States.[1] The occurrence of SUDEP is usually seizure-related, and SUDEP risk factors include frequent generalized seizures and long-standing epilepsy.[2] The precise pathophysiological mechanisms of death are unclear, but ictal, postictal, or interictal cardiorespiratory dysfunction, with arousal failure, are thought to account for most deaths that have been monitored.[3]

Serotonin (5-hydroxytryptamine [5-HT]) is a major neurotransmitter that is produced by serotonergic raphe neurons in the brainstem, which project throughout the central nervous system.[4] These serotonergic neurons play an important role in cardiovascular control, breathing, and arousal mechanisms, and a "serotonin axis" may comprise the common link between these mechanisms, seizures, and SUDEP.[5] The majority of serotonin (>95%) in the body is found outside the nervous system.[6] Peripheral serotonin is synthesized by enterochromaffin cells in the gut, which release some of it to be taken up by platelets that express serotonin transporter (SERT), but not serotonin-synthesizing proteins. The pool of serotonin in the periphery is largely separate from that in the brain, because serotonin does not easily cross the blood-brain barrier (BBB).[7] However, during intense seizure activity, such as status epilepticus, the permeability of the BBB increases,[8] potentially allowing exchange of 5-HT between peripheral circulation and the central nervous system**.** We set out to examine whether seizures induce changes in peripheral serotonin levels, which have hitherto not been systematically studied in humans.

2 | MATERIALS AND METHODS

We studied patients with intractable epilepsy who were admitted to the Epilepsy Monitoring Unit and consented to participate in a multi-institution, institutional review board–approved, prospective, multicenter SUDEP study as part of the National Institute of Neurological Disorders and Stroke Center for SUDEP Research's Autonomic and Imaging Biomarkers project. Seizures of 41 epileptic patients were monitored by standard surface video-electroencephalographic (EEG) methods using the Nihon-Kohden system (Tokyo, Japan). Peripheral capillary oxygen saturation (SpO_2) was measured using pulse oximetry (Nellcor OxiMax N-600x; Convidien, Dublin, Ireland). Epilepsy phenotypic and electroclinical data were collected, including age, gender, body mass index (BMI), epilepsy syndrome, seizure types, etiology, seizure frequency, seizure duration, seizure phase (tonic, clonic, jittery), duration of postictal generalized EEG suppression (PGES),[9] and medications. Postictal and interictal venous blood samples were collected in serum separator tubes and spun down, and serum was frozen and sent to a reference laboratory (LabCorp, Burlington, NC, USA) for measurement of serotonin levels using high-performance liquid chromatography with electrochemical detection. Because seizure occurrence during admission was not guaranteed, interictal sampling was only carried out if postictal samples were successfully obtained. Interictal samples were obtained at rest and always at least 12 hours after the last recorded clinical seizure. The normal laboratory reference values for serum serotonin are 21-321 ng/mL. Seizures were classified according to the International League Against Epilepsy 2017 seizure classification. Statistical analysis was done using SPSS software version 24.0 (IBM, Armonk, NY, USA). A bivariate Spearman correlation coefficient was used for comparing interictal serum serotonin levels and PGES or duration of the tonic phase. $P < .05$ was considered to be statistically significant.

3 | RESULTS

Patient characteristics are shown in Table 1. A total of 41 patients were enrolled in the study (18 males and 23 females) with an average age of 40.6 ± 14 (range = 20-77) years and mean BMI of 29.3 ± 7.6 (range = 19-53). Patients were pooled into 2 groups based on the type of seizures recorded, with 19 seizures in the group with generalized seizures (generalized tonic–clonic seizures of genetic generalized epilepsy and focal to bilateral tonic–clonic seizures) and 26 seizures in the group with focal seizures without secondary generalization. The distribution of gender and race was similar in both groups. No significant differences were seen in age, BMI, or number of antiepileptic drugs used (Table 1). Patients with generalized seizures had a history of epilepsy for a mean duration of 20.5 ± 13.3 years, whereas patients with focal seizures had a mean duration of epilepsy of 16.2 ± 16.3 years ($P = .33$). Preexisting health issues associated with cardiac, pulmonary, sleep, or psychiatric disorders were similar in both groups. The psychiatric disorders were mainly anxiety, depression, or bipolar disorder. However, none of the patients was on selective serotonin reuptake inhibitors (SSRIs). The epileptogenic zone was mainly temporal for focal seizures. Seizure semiologies for both groups are shown in Table 1. No differences were seen for EEG seizure duration and clinical seizure duration (Table 1). SpO_2 nadir (%) significantly differed ($P < .001$) between the 2 seizure groups, with a more pronounced decline in oxygen levels in the generalized seizure group when compared to

TABLE 1 Clinical characteristics of the patients

	Generalized convulsive seizures, n = 19[a]	Focal seizures, n = 26	*P*
Demographics			
Gender			
Male	10 (58%)	8 (36%)	.68
Female	9 (42%)	14 (64%)	
Age, y	41.6 ± 15.5	39.9 ± 13.0	.70
BMI	30.1 ± 7.5	28.6 ± 7.8	.53
History			
Antiepileptic medications, n	2.7 ± 1.1	2.5 ± 1.2	.49
Epilepsy duration, y	20.5 ± 13.3	16.2 ± 16.3	.33
Cardiac disorder	5 (26.3%)	8 (30.8%)	
Pulmonary disorder	1 (5.3%)	4 (15.4%)	
Sleep disorder	—	2 (7.7%)	
Psychiatric disorder[b]	6 (31.6%)	6 (23.1%)	
Type of epilepsy			
Generalized	4 (21.0%)	—	
Focal	15 (79.0%)	26 (100.0%)	
Epileptogenic zone			
Generalized	4 (21.0%)	—	
Temporal	3 (15.8%)	15 (57.7%)	
Frontal	4 (21.0%)	4 (15.3%)	
Parietal	1 (5.3%)	3 (11.5%)	
Unknown	7 (33.3%)	4 (15.3%)	
Seizure semiology[c]			
Generalized onset motor tonic–clonic	4 (21.1%)	—	
FOIA clonic	—	2 (7.7%)	
FOIA nonmotor onset	—	8 (30.8%)	
FOA motor onset automatisms	—	3 (11.5%)	
FOIA motor onset hyperkinetic	—	13 (50%)	
Focal to bilateral tonic–clonic	15 (78.9%)	—	
Seizure parameters			
EEG seizure duration, s	94.8 ± 52.2	166.6 ± 451.6	.43
Clinical seizure duration, s	87.0 ± 51.9	167.0 ± 471.0 (n = 24)	.42
Seizures with apnea	5 (26.3%)	—	
Seizures with hypoxemia	5 (26.3%)	—	
SpO_2 nadir, %	63.6 ± 16.3 (n = 14)	92.8 ± 3.6 (n = 18)	<.001
Tonic phase duration, s	6.0 ± 2.7 (n = 12)	—	
Jittery phase duration, s	12.9 ± 10.6 (n = 13)	—	
Clonic phase duration, s	27.3 ± 15.5 (n = 18)	—	
PGES duration, s	28.0 ± 19.2 (n = 11)	—	
Postictal EEG burst suppression, s	99.8 ± 142.8 (n = 4)	—	
Postictal EEG continuous slow, s	723.3 ± 519.9 (n = 8)	—	

(Continues)

TABLE 1 (Continued)

	Generalized convulsive seizures, n = 19[a]	Focal seizures, n = 26	*P*
EEG return to baseline duration, s	1363.3 ± 1374.5 (n = 7)	—	
EEG seizure end to blood draw, min	11.2 ± 12.1	7.2 ± 7.6	.21

Values are given as n (%) unless otherwise indicated.

BMI, body mass index; EEG, electroencephalographic; FOA, focal onset aware; FOIA, focal onset impaired awareness; PGES, postictal generalized EEG suppression; SpO_2, peripheral capillary oxygen saturation.

[a]Generalized tonic–clonic seizures and focal to bilateral tonic–clonic seizures.

[b]None of the patients was on selective serotonin reuptake inhibitors.

[c]International League Against Epilepsy classification of seizures.

the focal seizure group. Of the 19 generalized seizure patients, the seizure during which the serotonin samples were taken was the first during the admission in 14 of 19 (74%), the second seizure in 4 of 19 (21%), and the third seizure in 1 of 19 (5%).

Postictal serotonin levels in serum were increased after generalized seizures compared to interictal levels but not after focal seizures. The average time elapsed between postictal blood draw and end of seizure was 11.2 ± 12.1 minutes for generalized seizures and 7.2 ± 7.6 minutes for focal seizures; there was no significant difference between these 2 groups (Table 1). Interictal blood samples were drawn after a 12-hour seizure-free period. Mean postictal serotonin levels after generalized seizure were 173.1 ± 91.8 ng/mL (range = 30-386), and in the interictal state were 119.4 ± 63.3 ng/mL (range = 26-227; Figure 1A). For focal seizures, mean postictal and interictal serotonin levels were similar, at 130.9 ± 95.9 ng/mL (range = 25-353) and 132.2 ± 79.6 ng/mL (range = 31-416; Figure 1A). Increase in postictal serotonin levels compared to interictal levels using a paired sample *t* test was statistically significant for the generalized seizure group ($P = .002$), but not for the focal seizure group ($P = .941$, Figure 1A). The change in serum serotonin levels (postictal-interictal) was also statistically significant ($P = .027$) between the generalized and focal seizure groups. The difference in serotonin level (postictal to interictal) was associated with reduced duration of tonic phase during generalized seizures ($P = .03$, Figure 1B). Higher levels of interictal serotonin were significantly associated with shorter duration of PGES ($P = .04$, Figure 1C). In the generalized seizure group, comparison of patients who had PGES (n = 11) with patients who did not have PGES (n = 8) revealed no significant difference in postictal serotonin levels between the 2 groups. No other associations were observed between serotonin levels and other clinical features of the seizure.

4 | DISCUSSION

Serotonin is synthesized through the actions of 2 different tryptophan hydroxylase isoforms encoded by different genes, *TPH1* and *TPH2*, which are expressed in enterochromaffin cells of the intestine and serotonergic neurons in the brainstem, respectively. The source of almost all serotonin in the brain is a small group of neurons in the midbrain and medullary raphe nuclei.[10] Raphe serotonin neurons project throughout the neuraxis and are important for many brain functions, including mood, sleep/arousal, motor output, thermoregulation, autonomic control, and breathing.[5,10] However, the BBB, which consists of a single layer of endothelial cells throughout the cerebral vasculature connected via tight junctions, covered by a basement membrane and surrounded by astrocytic endfeet, acts as a barrier that prevents many substances from being exchanged between the brain and blood.[7] Serotonin is relatively impermeable across the BBB, so the brain is unlikely to be the source of serotonin that leads to the increase in serum 5-HT seen in our study.

It has been reported that during intensive acute exercise, serum serotonin levels increase when compared to controls.[11] Moreover, moderate exercise in horses (a 450-m run) causes both free plasma 5-HT and whole blood 5-HT to increase by greater than threefold.[12] The authors concluded that the source of 5-HT was enterochromaffin cells that are thought to release 5-HT in response to exercise. Thus, the exertion associated with generalized seizures may lead to alterations similar to other forms of exercise. 5-HT release induced by muscular activity during a seizure would be consistent with our observation that 5-HT rose with generalized seizures, but not focal seizures. This finding further suggests that seizure activity within the brain during seizures causes an indirect rather than a direct rise in 5-HT.

Our data do not exclude an alternative possibility that seizures cause release of 5-HT from platelets, because we measured serum 5-HT and not whole blood 5-HT. In the periphery, platelet SERT is required for serotonin uptake from the plasma, which does not synthesize 5-HT; platelets regulate serotonin levels to prevent vasoconstriction and thereby keep blood flow stable.[13] A reduction in density of platelet membrane SERT in patients having epileptic seizures compared to normal controls or patients with

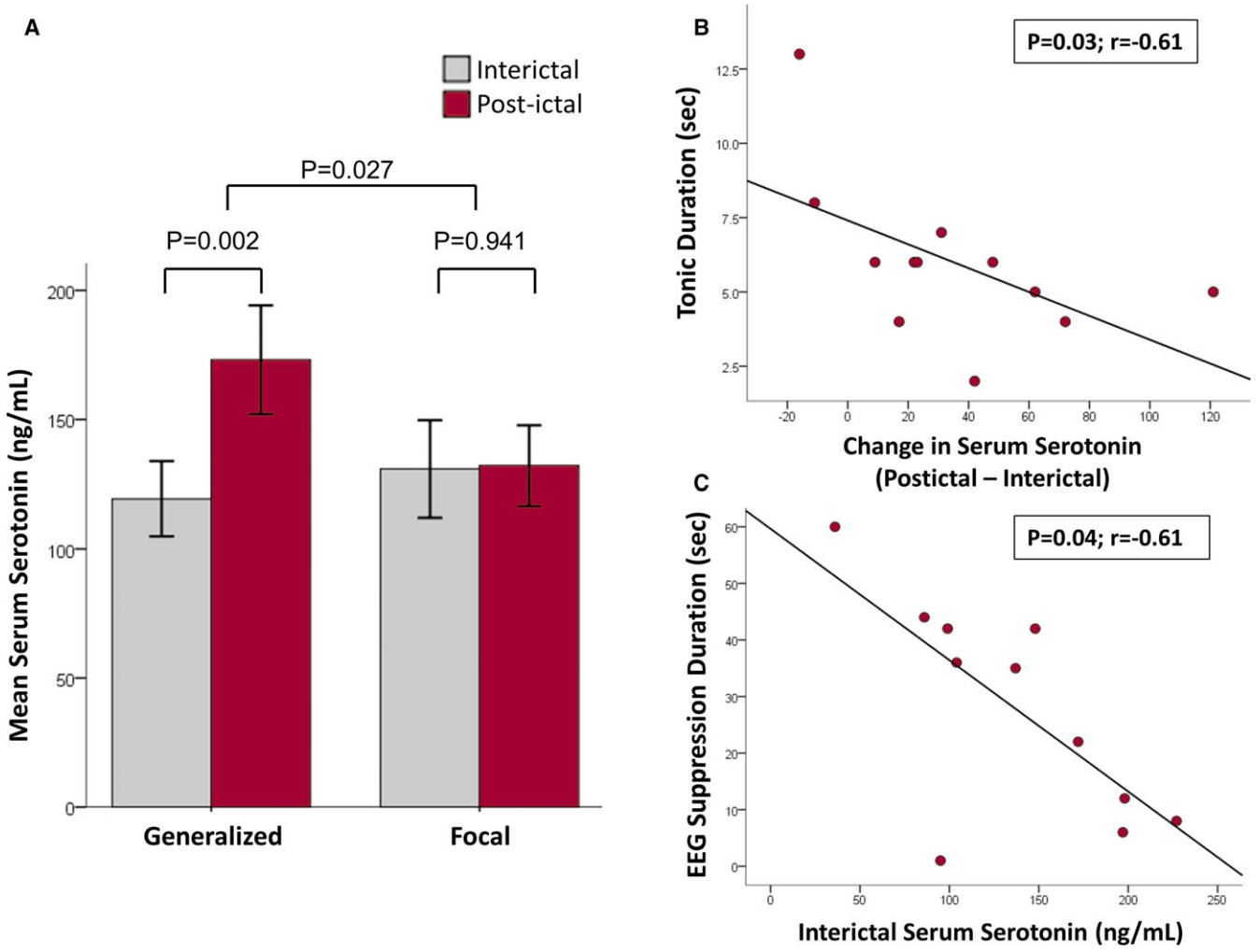

FIGURE 1 A, Serum serotonin levels increase after seizures. The mean serum postictal serotonin levels (in ng/mL) are shown in red bars and interictal serotonin levels are shown in gray bars for the 2 seizure groups: generalized (n = 19) and focal (n = 26). Elevated levels of postictal serum serotonin after generalized seizures were statistically significant when compared to interictal levels (P = .002), but not after focal seizures (P = .941, paired sample t test). Also, the change in serum serotonin level (postictal-interictal) was statistically significant (P = .027, independent 2-sample t test) between the generalized and focal seizure groups. B, Association of tonic duration with serum serotonin. The differences between postictal and interictal serum serotonin levels were plotted against the tonic duration of the seizure. Increased levels of serotonin were significantly associated with reduced duration of tonic phase during generalized seizures (n = 12). C, Association of postictal generalized electroencephalographic (EEG) suppression (PGES) duration with serum serotonin. The interictal serum serotonin levels were plotted against the PGES duration of the seizure. Higher interictal serotonin was significantly associated with shorter period of EEG suppression during generalized seizures (n = 11)

nonepileptic seizures has been reported.[14] This suggests that serotonin may be released from platelets during seizures and reuptake may be reduced by decreased expression of SERT on platelet membranes.

An inverse correlation between interictal serum 5-HT levels and the duration of PGES observation raises the question of what relationship potentially exists between interictal serum serotonin levels and brain serotonin. A correlation between human platelet serotonin transport and brain synaptosomal transport has been demonstrated in human brain in patients undergoing epilepsy surgery,[15] so that differences observed in peripheral blood 5-HT may be paralleled by similar differences in brain 5-HT. It is also possible that differences in 5-HT synthesis or metabolism could lead to higher peripheral 5-HT levels, and that parallel differences occur in the brain. Also, mice lacking 5-HT neurons in the brain have been shown to display a lower threshold for seizures and are more likely to have PGES and death after seizures.[16]

Alternatively, high peripheral 5-HT levels may possibly lead to increased brain 5-HT if seizures caused breakdown of the BBB, as has been demonstrated under some

conditions,[17] allowing transfer of serotonin into the central nervous system. BBB disruption occurs within minutes after induction of bicuculline-induced seizures in rats. Increased micropinocytosis in cerebral capillaries has been reported during seizures, and epilepsy per se may compromise the BBB.[18]

Serotonin within the brain enhances respiration in response to hypercapnia and also contributes to arousal.[16] It is also well known that seizures in animal models cause release of serotonin from brainstem raphe neurons.[19] Such increases in synaptically released serotonin coupled with the peripherally generated serotonin that may pass into the brain due to seizure-induced disruption of the BBB[7] may add to stimulatory effects of serotonin on respiration and arousal. How higher peripheral serum serotonin levels pertain to observed shorter PGES duration (correlated with postictal immobility duration and oxygen desaturation) and tonic phase durations is unclear; both are markers of seizure severity, and thus potentially of SUDEP.[20] It is possible that serotonin plays a role in early recovery by reducing seizure severity, although we have no definitive evidence of this. DBA/1 and DBA/2 mice have reduced serotonin availability and are susceptible to postseizure death if not resuscitated. Pretreatment with SSRIs prevents death. In this small sample, our data suggest that peripheral serum serotonin levels in epileptic patients are inversely correlated with potential biomarkers of SUDEP and merit further study.

DISCLOSURE OF CONFLICT OF INTEREST

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REFERENCES

1. Devinsky O, Hesdorffer DC, Thurman DJ, et al. Sudden unexpected death in epilepsy: epidemiology, mechanisms, and prevention. Lancet Neurol. 2016;15:1075–88.
2. Lhatoo SD, Nashef L, Tomson T, et al. Association of prone position with sudden unexpected death in epilepsy. Neurology. 2015;85:1185.
3. Ryvlin P, Nashef L, Lhatoo SD, et al. Incidence and mechanisms of cardiorespiratory arrests in epilepsy monitoring units (MORTEMUS): a retrospective study. Lancet Neurol. 2013;12:966–77.
4. Buchanan GF, Richerson GB. Central serotonin neurons are required for arousal to CO_2. Proc Natl Acad Sci U S A. 2010;107:16354–9.
5. Richerson GB, Buchanan GF. The serotonin axis: shared mechanisms in seizures, depression, and SUDEP. Epilepsia. 2011;52 (Suppl 1):28–38.
6. Tyce GM. Origin and metabolism of serotonin. J Cardiovasc Pharmacol. 1990;16(Suppl 3):S1–7.
7. Daneman R, Prat A. The blood–brain barrier. Cold Spring Harb Perspect Biol. 2015;7:a020412.
8. van Vliet EA, Aronica E, Gorter JA. Blood–brain barrier dysfunction, seizures and epilepsy. Semin Cell Dev Biol. 2015;38:26–34.
9. Lhatoo SD, Faulkner HJ, Dembny K, et al. An electroclinical case–control study of sudden unexpected death in epilepsy. Ann Neurol. 2010;68:787–96.
10. Pilowsky PM. Peptides, serotonin, and breathing: the role of the raphe in the control of respiration. Prog Brain Res. 2014;209:169–89.
11. Zimmer P, Stritt C, Bloch W, et al. The effects of different aerobic exercise intensities on serum serotonin concentrations and their association with Stroop task performance: a randomized controlled trial. Eur J Appl Physiol. 2016;116:2025–34.
12. Alberghina D, Giannetto C, Piccione G. Peripheral serotoninergic response to physical exercise in athletic horses. J Vet Sci. 2010;11:285–9.
13. Mercado CP, Kilic F. Molecular mechanisms of SERT in platelets: regulation of plasma serotonin levels. Mol Interv. 2010;10:231–41.
14. Cupello A, Audenino D, Scarrone S, et al. Epileptic seizures but not pseudoseizures are associated with decreased density of the serotonin transporter in blood platelet membranes. Neurochem Res. 2008;33:2263–8.
15. Rausch JL, Johnson ME, Li J, et al. Serotonin transport kinetics correlated between human platelets and brain synaptosomes. Psychopharmacology. 2005;180:391–8.
16. Buchanan GF, Murray NM, Hajek MA, et al. Serotonin neurones have anti-convulsant effects and reduce seizure-induced mortality. J Physiol. 2014;592:4395–410.
17. Janigro D. Blood–brain barrier, ion homeostatis and epilepsy: possible implications towards the understanding of ketogenic diet mechanisms. Epilepsy Res. 1999;37:223–32.

18. van Vliet EA, Aronica E, Gorter JA. Role of blood–brain barrier in temporal lobe epilepsy and pharmacoresistance. Neuroscience. 2014;277:455–73.
19. Lin WH, Huang HP, Lin MX, et al. Seizure-induced 5-HT release and chronic impairment of serotonergic function in rats. Neurosci Lett. 2013;534:1–6.
20. Tao JX, Yung I, Lee A, et al. Tonic phase of a generalized convulsive seizure is an independent predictor of postictal generalized EEG suppression. Epilepsia. 2013;54:858–65.

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