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RESEARCH ARTICLE

Serotonin receptor expression in hippocampus and temporal cortex of temporal lobe epilepsy patients by postictal generalized electroencephalographic suppression duration

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Abstract

Objective: Prolonged postictal generalized electroencephalographic suppression (PGES) is a potential biomarker for sudden unexpected death in epilepsy

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(SUDEP), which may be associated with dysfunctional autonomic responses and serotonin signaling. To better understand molecular mechanisms, PGES duration was correlated to 5HT1A and 5HT2A receptor protein expression and RNAseq from resected hippocampus and temporal cortex of temporal lobe epilepsy patients with seizures recorded in preoperative evaluation.

Methods: Analyses included 36 cases (age = 14–64 years, age at epilepsy onset = 0–51 years, epilepsy duration = 2–53 years, PGES duration = 0–93 s), with 13 cases in all hippocampal analyses. 5HT1A and 5HT2A protein was evaluated by Western blot and histologically in hippocampus (n = 16) and temporal cortex (n = 9). We correlated PGES duration to our previous RNAseq dataset for serotonin receptor expression and signaling pathways, as well as weighted gene correlation network analysis (WGCNA) to identify correlated gene clusters.

Results: In hippocampus, 5HT2A protein by Western blot positively correlated with PGES duration (p = .0024, R^2 = .52), but 5HT1A did not (p = .87, R^2 = .0020). In temporal cortex, 5HT1A and 5HT2A had lower expression and did not correlate with PGES duration. Histologically, PGES duration did not correlate with 5HT1A or 5HT2A expression in hippocampal CA4, dentate gyrus, or temporal cortex. RNAseq identified two serotonin receptors with expression that correlated with PGES duration in an exploratory analysis: *HTR3B* negatively correlated (p = .043, R^2 = .26) and *HTR4* positively correlated (p = .049, R^2 = .25). WGCNA identified four modules correlated with PGES duration, including positive correlation with synaptic transcripts (p = .040, Pearson correlation r = .52), particularly potassium channels (*KCNA4*, *KCNC4*, *KCNH1*, *KCNIP4*, *KCNJ3*, *KCNJ6*, *KCNK1*). No modules were associated with serotonin receptor signaling.

Significance: Higher hippocampal 5HT2A receptor protein and potassium channel transcripts may reflect underlying mechanisms contributing to or resulting from prolonged PGES. Future studies with larger cohorts should assess functional analyses and additional brain regions to elucidate mechanisms underlying PGES and SUDEP risk.

KEYWORDS
hippocampus, PGES, serotonin, SUDEP

1 | INTRODUCTION

Postictal generalized electroencephalographic (EEG) suppression (PGES) may occur after a generalized tonic–clonic seizure (GTCS), and prolonged PGES is associated with impaired arousal, respiration, and other autonomic functions.[1–3] PGES has been suggested as a potential sudden unexpected death in epilepsy (SUDEP) biomarker,[1–5] and occurs with autonomic dysfunction and respiratory arrest in SUDEP animal models.[6,7]

Potential mechanisms underlying PGES and SUDEP include dysfunctional serotonin signaling,[8] as serotonin

Key Points

- The 5HT2A receptor protein positively correlated with PGES duration in the hippocampus of TLE patients by Western blot
- The transcripts *HTR3B* negatively correlated and *HTR4* positively correlated with PGES duration in hippocampus
- Potassium channel transcripts positively correlated with PGES duration in hippocampus

modulates respiration, arousal, and seizures.[9] Serotonergic neurons and serotonergic receptors are present in various regions throughout the brain, and thus serotonin receptor modulators may have opposing effects on seizure threshold related to factors like ligand specificity, dose, and cell type.[10,11] In temporal lobe epilepsy (TLE) patients, magnetic resonance imaging and positron emission tomographic imaging revealed decreased 5HT1A receptor binding.[12,13] In animal models, elevated serotonin reduced seizure frequency, seizures reduced serotonergic firing, low serotonin or serotonin receptor deletion (5HT1A, 5HT2C, 5HT4, 5HT7) promoted seizures, and 5HT1A overexpression resulted in sporadic autonomic dysfunction and death.[14–21] The 5HT2 receptor family generally facilitates excitatory effects[17]; thus, 5HT2A antagonists may provide improvement of epilepsy.[10,22] In high-risk SUDEP patients, hippocampal serotonin transporter (SERT) protein expression was increased.[21] The brainstem contains serotonergic neurons that project to various brain regions,[23,24] and we recently identified altered signaling pathways in the serotonergic dorsal raphe of the midbrain in SUDEP cases compared to controls.[25] Furthermore, an animal model linked PGES to dysfunctional serotonin signaling, and dorsal raphe stimulation reduced PGES duration.[26] With increasing PGES duration, interictal serum serotonin is decreased in epilepsy patients,[27] although serotonin levels have not been studied in brain tissue.

We evaluated whether PGES duration recorded during presurgical evaluation correlated with serotonin receptor expression and RNAseq data in resected hippocampus and temporal cortex of TLE patients.

2 | MATERIALS AND METHODS

2.1 | Human brain tissue

Surgical brain tissue was obtained with approval by the New York University (NYU) School of Medicine Institutional Review Board (#17-00398). Frozen brain tissue and formalin-fixed paraffin-embedded (FFPE) tissue for protein analyses were available from TLE patients undergoing surgical resection at the NYU Epilepsy Brain Bank, Amsterdam University Medical Center, Royal Melbourne Hospital, University College of London, and Thomas Jefferson University. Informed consent was provided by each patient, and patients were enrolled in the brain tissue repositories from 2003 to 2019. Patients were considered for analyses who had a GTCS recorded by EEG prior to surgical resection. PGES was quantified at each center blinded to the molecular analysis results through expert visual review by an experienced clinical neurophysiologist (D.F., B.D., R.T., M.N., T.O.). PGES was defined as suppression of the EEG activity to ≤10 μV across all head regions on scalp EEG following a seizure, and termination was defined as the return of any cerebral activity of <10 μV on any electrode.[3,28] Review of video was used to determine whether observed signals were related to obvious artifacts. When artifact precluded the determination of the offset PGES, PGES duration was defined as the duration of the interpretable portion of the postictal EEG. When multiple PGES readings were available from one patient, the longest PGES duration was evaluated in analyses. Patients were selected to provide coverage of the PGES spectrum, including no PGES (0 s), PGES of <50 s associated with low-risk SUDEP, and PGES of ≥50 s associated with high-risk SUDEP.[3] Case history is summarized in Table 1 and detailed in Table S1, with 13 cases overlapping in hippocampus for protein, histology, and RNAseq analyses.

2.2 | Western blot

Protein was isolated from frozen brain tissue (40 mg/sample) at 20% weight/volume in Tris-NaCl buffer (20 mmol·L^{-1} Tris base, 150 mmol·L^{-1} NaCl, .1% Triton-X 100, protease and phosphatase inhibitors at pH 7.5) using a handheld homogenizer equipped with a pestle on ice. Samples were incubated on ice for 15 min and centrifuged for 15 min at 14 000 × *g* at 4°C, and supernatant was isolated. Protein concentration was determined by bicinchoninic acid assay according to the manufacturer's protocol (Pierce). Hippocampal (30 μg/lane) and temporal cortex (40 μg/lane) lysates were boiled in Bolt LDS Sample Buffer and dithiothreitol. For the hippocampus, one sample was included on both gels to allow for normalization across blots for all samples. Proteins were resolved on a 4%–12% Bis-Tris gel (Invitrogen) and transferred onto nitrocellulose membranes. After blocking in 5% milk Tris-buffered saline with Tween (TBST), blots were probed for 5HT1A (1:500, Abcam, ab227165), 5HT2A (1:500, Santa Cruz Biotechnology, sc-166 775), or actin (1:3000, Sigma, A5441) in 5% milk TBST overnight at 4°C. Blots were incubated with corresponding horseradish peroxidase-conjugated secondary antibodies (1:3000, GE Healthcare) for 1 h at room temperature. Bands were visualized after enhanced chemiluminescence (Pierce) on a Bio-Rad Laboratories ChemiDoc with the NYU Small Instrument Fleet. Blot images were analyzed in Fiji ImageJ for quantification, with intensity normalized to actin.

2.3 | Immunohistochemistry

Subregional protein expression was assessed by immunohistochemistry as described.[29] FFPE blocks were sectioned (8 μm) by the NYU Center for Biospecimen Research and Development. Sections were deparaffinized and rehydrated

TABLE 1 Case history summary

	PGES	Cases, *n*	Sex, *n*	PGES, s	Age at surgery, years	Epilepsy onset age, years	Epilepsy duration, years
Western blot							
Hippocampus	<50 s	9	2 M / 7 F	13.3 ± 20.1	37.9 ± 14.1	18.3 ± 18.0	20.1 ± 11.0
	≥50 s	7	3 M / 4 F	58.0 ± 8.1	39.6 ± 11.7	14.9 ± 8.2	22.1 ± 16.5
Temporal cortex	<50 s	6	1 M / 5 F	21.2 ± 15.1	34.8 ± 14.5	20.8 ± 19.0	14.0 ± 9.2
	≥50 s	3	2 M / 1 F	64.3 ± 13.6	30.0 ± 11.4	19.0 ± 20.3	11.0 ± 11.5
Histology							
Hippocampus	<50 s	9	2 M / 7 F	13.3 ± 20.1	37.9 ± 14.1	18.3 ± 18.0	20.1 ± 11.0
	≥50 s	7	3 M / 4 F	58.0 ± 8.1	39.6 ± 11.7	14.9 ± 8.2	22.1 ± 16.5
Temporal cortex	<50 s	6	3 M / 3 F	19.5 ± 13.3	28.5 ± 15.4	14.7 ± 14.3	13.8 ± 9.7
	≥50 s	3	2 M / 1 F	64.3 ± 24.8	36.0 ± 16.1	17.3 ± 15.3	18.7 ± 3.5
RNAseq							
Hippocampus	<50 s	8	2 M / 6 F	14.8 ± 21.0	43.9 ± 14.0	24.4 ± 21.4	20.6 ± 12.2
	≥50 s	8	4 M / 4 F	57.1 ± 7.9	37.8 ± 12.0	15.3 ± 7.7	20.3 ± 16.2

Note: The same hippocampal cases were used in protein and histology analyses. There are 13 cases that overlap in hippocampus for protein, histology, and RNAseq analyses. Mean ± SD is indicated.

Abbreviations: F, female; M, male; *n*, number of cases; PGES, postictal generalized electroencephalographic suppression.

through a series of xylenes and ethanol dilutions, followed by heat-induced antigen retrieval with 10 mmol·L^{-1} sodium citrate and .05% Triton-x 100 at pH 6. Sections were blocked with 10% normal donkey serum and incubated with 5HT1A (1:100, Abcam, ab227165) or 5HT2A (1:100, Santa Cruz Biotechnology, sc-166 775) primary antibodies overnight at 4°C. Corresponding secondary antibodies were used (donkey antirabbit Alexa Fluor 568, donkey antimouse Alexa Fluor 488; Thermo Fisher Scientific) with 4,6-diamidino-2-phenylindole counterstain, and slides were coverslipped. Whole slide scanning was performed on each section at ×20 magnification on a NanoZoomer HT2 (Hamamatsu) microscope with the NYU Experimental Pathology Research Laboratory (hippocampus) or the Leica Aperio Versa 8 microscope (temporal cortex). We analyzed one image in each hippocampal subregion and three images in the temporal cortex at ×5 magnification in Fiji ImageJ by the same binary threshold for all images to determine the number of positive pixels in each image, reported as percentage of total image area.

2.4 | RNAseq

We analyzed our previous RNAseq dataset[29,30] in the European Genome-Phenome Archive (EGAS00001003922) in hippocampus of TLE patients for whom we could obtain an evaluation of PGES duration. Briefly,[30] RNA was isolated from surgical brain tissue by Qiazol Lysis Reagent with the miRNeasy Mini Kit (Qiagen), RNA quality was determined by Fragment Analyzer (Agilent Technologies), library preparation was done with NEBNext Ultra Directional RNA Library Prep Kit (Illumina), ribosomal RNA was depleted (New England Biolabs), and sequencing was done with the Illumina cBot and HiSeq 4000 paired end with 151 nucleotide read length and depth of 50 million reads. *DESeq2* in the R environment was used for library normalization. Case histories were previously detailed,[29,30] are summarized in Table 1 and detailed in Table S1.

2.5 | Weighted gene correlation network analysis

Weighted gene correlation network analysis was performed on our RNAseq dataset[29,30] to determine whether PGES duration correlated with RNAseq in the R environment with the *WGCNA* package with defaults as described,[31] except as stated. Soft threshold power beta was determined at $R^2 = .8$ (power = 8), minModuleSize = 150, and deep Split = 4. Gene ontology (GO) annotations for modules were determined following weighted gene correlation network analysis (WGCNA) with the *anRichment* package in the R environment with Entrez IDs against the human GOcollection (Table S2). GO annotations were considered with a false discovery rate < 5%.

2.6 | Statistical analyses

Statistical analyses used GraphPad Prism (version 9) and the R environment (http://www.r-project.org/). Western blot and histology correlation analyses were calculated

by a Pearson correlation. A p-value $< .05$ was considered significant.

3 | RESULTS

3.1 | Case history

Clinical history is summarized in Table 1 and detailed in Table S1 for each analysis, including cases with coverage of the PGES duration spectrum (PGES = 0–93 s): no PGES (0 s), PGES < 50 s associated with a lower risk of SUDEP, and PGES ≥ 50 s associated with higher risk of SUDEP. There were a total of 36 cases (age at surgery = 14–64 years, age at epilepsy onset = 0–51 years, epilepsy duration = 2–53 years) evaluated across all analyses, and 13 of the same cases with hippocampal tissue were evaluated in protein, histology, and RNAseq analyses.

3.2 | 5HT1A and 5HT2A protein expression in whole brain homogenate

Western blot of whole brain homogenate from resected hippocampus (containing combined dentate gyrus and CA1-4, $n = 16$; Figure 1A) and temporal cortex ($n = 9$; Figure 1B) revealed variable 5HT1A (55 kDa) expression among TLE patients. In the hippocampus, 5HT1A did not correlate with PGES duration ($p = .87$, $R^2 = .0020$; Figure 1C). In temporal cortex, 5HT1A was expressed at a lower level than in hippocampus and did not correlate with PGES duration ($p = .45$, $R^2 = .085$; Figure 1D). Hippocampal 5HT2A (55 kDa) positively correlated with PGES duration ($p = .0024$, $R^2 = .52$; Figure 1E). Temporal cortex 5HT2A was expressed at lower levels than in hippocampus, and there was no correlation with PGES duration ($p = .20$, $R^2 = .22$; Figure 1F).

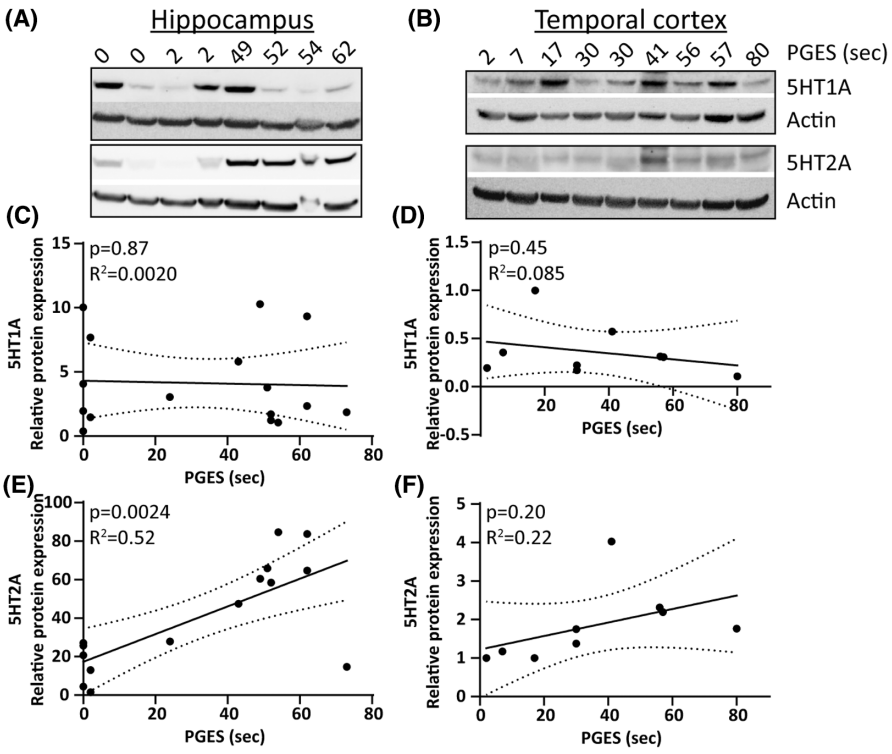

FIGURE 1 5HT1A and 5HT2A protein expression in whole homogenate from the hippocampus and temporal cortex by postictal generalized electroencephalographic suppression (PGES) duration. (A) Representative Western blot in hippocampus of 5HT1A (55 kDa), 5HT2A (55 kDa), and actin. (B) Representative Western blot in temporal cortex of 5HT1A (55 kDa), 5HT2A (55 kDa), and actin. (C) Quantification of 5HT1A relative to actin in hippocampus ($n = 16$) indicates no correlation with PGES duration. (D) Quantification of 5HT1A relative to actin in temporal cortex ($n = 9$) indicates no correlation with PGES duration. (E) Quantification of 5HT2A relative to actin in hippocampus ($n = 16$) indicates a positive correlation with PGES duration. (F) Quantification of 5HT2A relative to actin in temporal cortex ($n = 9$) indicates no correlation with PGES duration

3.3 | 5HT1A and 5HT2A protein expression histologically

5HT1A and 5HT2A expression was evaluated histologically in hippocampus ($n = 16$, dentate gyrus and CA4 subsector) and temporal cortex ($n = 9$) by PGES duration. In dentate gyrus, CA4, and temporal cortex (Figure 2A–F), 5HT1A expression was not correlated with PGES duration (Figure 2G–I). In dentate gyrus, CA4, and temporal cortex (Figure 3A–F), there was no correlation of 5HT2A expression and PGES duration (Figure 3G–I).

3.4 | RNAseq in hippocampus

To determine whether 5HT1A and 5HT2A (encoded by *HTR1A* and *HTR2A*) as well as other serotonin receptors correlated with PGES duration, we analyzed our previous RNAseq dataset[29,30] ($n = 16$), overlapping with 13 patients with hippocampal protein data (Table 1, Figure S1A). There were 14 serotonin receptors detected in the hippocampus (Figure 4A), with an exploratory analysis identifying two receptors significantly correlated with PGES duration: *HTR3B* (also known as 5HT3B), with a negative correlation ($p = .043$, $R^2 = .26$); and *HTR4* (also known as 5HT4), with a positive correlation ($p = .049$, $R^2 = .25$; Figure 4B,C).

To assess enriched serotonin signaling pathway transcripts associated with PGES duration, WGCNA was performed on our hippocampal dataset[29,30]; it revealed no enrichment in this brain region (Tables S2 and S3).

Weighted gene correlation network analysis identified additional transcripts that correlated with PGES duration (Figure S1). There were 2597 of 42 753 transcripts that correlated with PGES ($p = .05$), distributed among all 27 modules (Table S2). The top two transcripts correlated negatively: *PPP1R17* (protein primarily expressed in cerebellum, $p = 1.16 \times 10^{-5}$, $R^2 = .76$; M-white module; Figure S1B) and *DHRS7* ($p = 1.48 \times 10^{-5}$, $R^2 = .75$; M-ivory module; Figure S1C). Among all 27 modules, four significantly correlated with PGES (Figure S1D). PGES duration positively correlated with synapse transcripts ($p = .040$, Pearson correlation $r = .52$; included seven potassium channels: *KCNA4*, *KCNC4*, *KCNH1*, *KCNIP4*, *KCNJ3*, *KCNJ6*, *KCNK1*; Figure S2; Table S3) and stimulus detection (taste receptors, $p = .044$, $r = .51$). PGES negatively

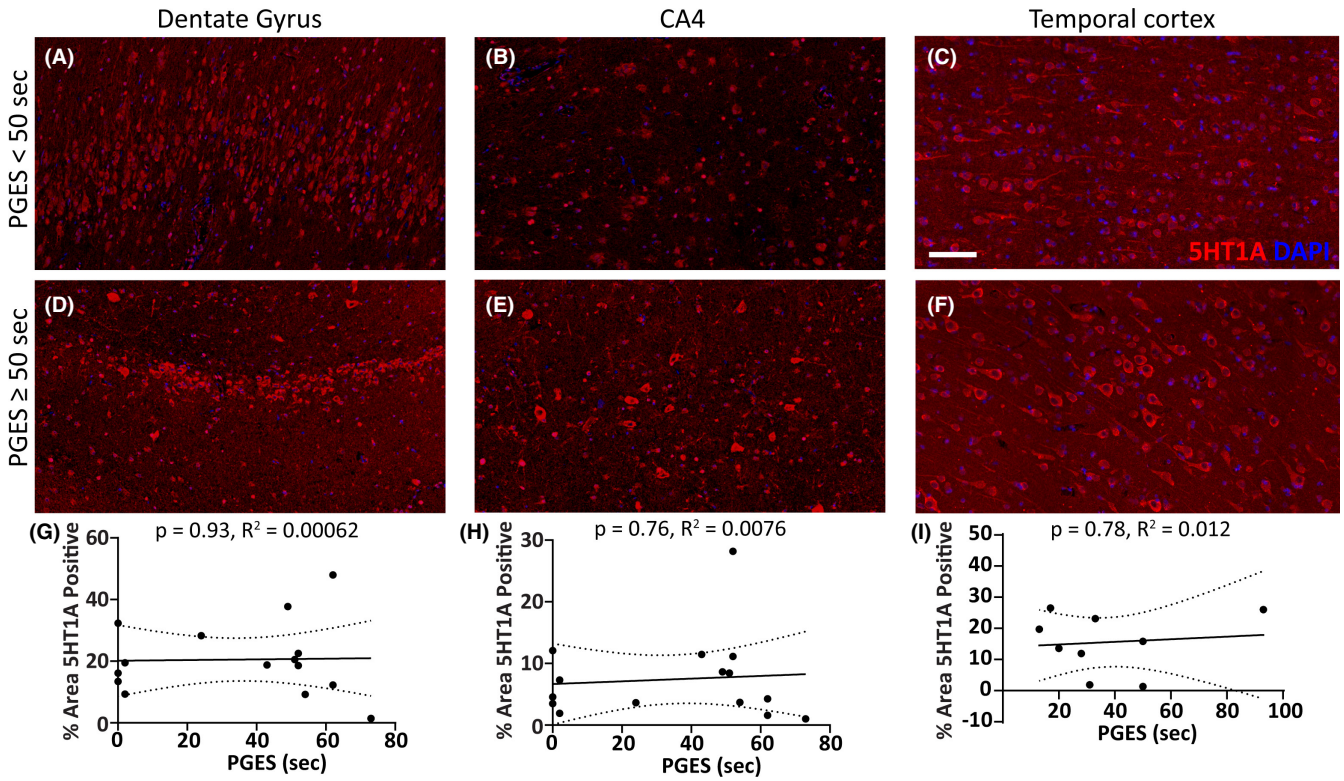

FIGURE 2 5HT1A protein expression in subregions of the hippocampus and temporal cortex by postictal generalized electroencephalographic suppression (PGES) duration. Representative images show 5HT1A expression (red) in surgical brain tissue from epilepsy cases in the hippocampal dentate gyrus, CA4 subsector, and temporal cortex (A–C) with PGES < 50 s and (D–F) PGES ≥ 50 s. (G–I) Semiquantification of 5HT1A expression in subregions across the PGES duration spectrum indicates no correlation in the hippocampus ($n = 16$) or temporal cortex ($n = 9$). Scale bar represents 100 μm for all histology panels

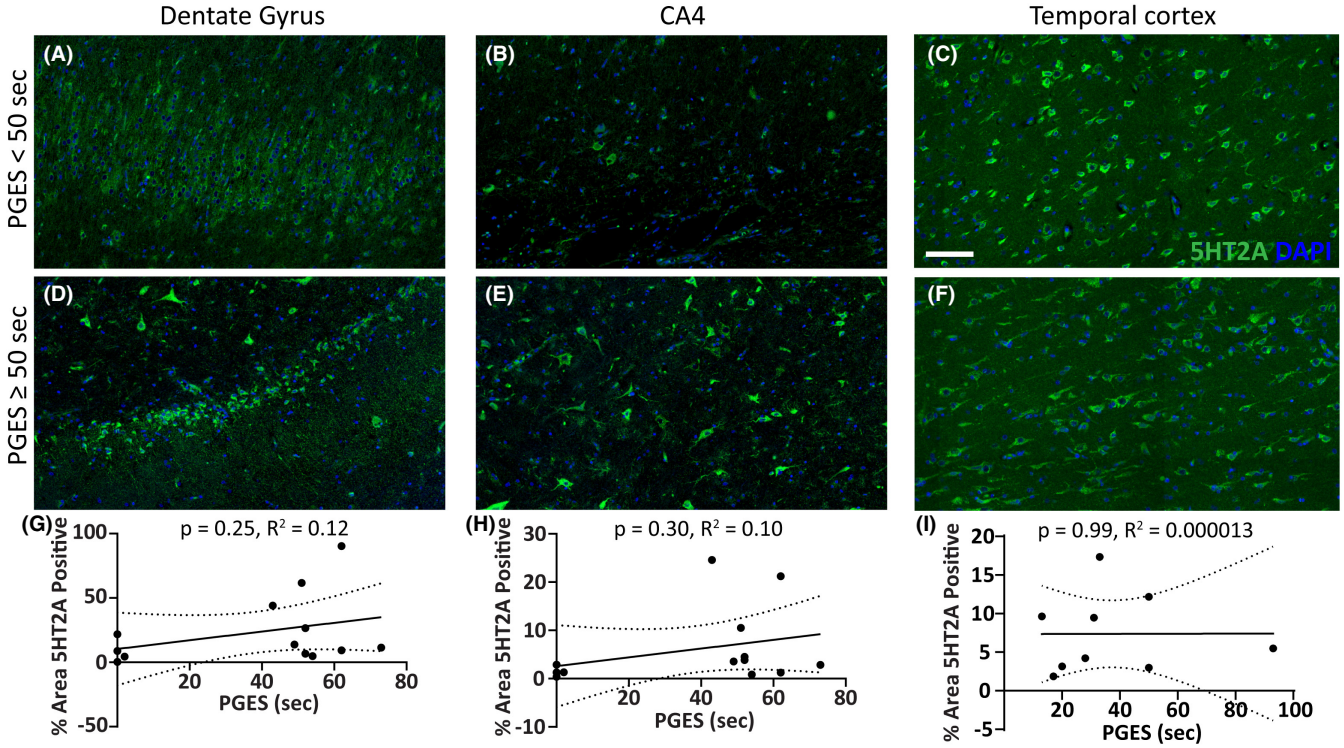

FIGURE 3 5HT2A protein expression in subregions of the hippocampus and temporal cortex by postictal generalized electroencephalographic suppression (PGES) duration. Representative images show 5HT2A expression (green) in surgical brain tissue from epilepsy cases in the hippocampal dentate gyrus, CA4 subsector, and temporal cortex (A–C) with PGES < 50 s and (D–F) PGES ≥ 50 s. (G–I) Semiquantification of 5HT2A expression in subregions across the PGES duration spectrum indicates no correlation in the hippocampus ($n = 16$) or temporal cortex ($n = 9$). Scale bar represents 100 μm for all histology panels

correlated with cellular localization ($p = .0084$, $r = -.63$) and developmental process transcripts ($p = .020$, $r = -.57$).

Five modules significantly correlated with epilepsy onset, duration, age, and sex, regardless of PGES duration, and with no overlap of PGES correlated modules. Epilepsy onset positively correlated with metabolic processes related to ribosomal transcripts ($p = .0075$, $r = .64$), mitochondrial transcripts ($p = .0016$, $r = .72$), and localization related to neuronal projection ($p = .022$, $r = .57$). Epilepsy duration positively correlated with stimulus detection related to olfactory receptors ($p = .010$, $r = .62$). Age positively correlated with metabolic processes related to mitochondrial transcripts ($p = .0026$, $r = .55$). Sex (male gender) positively correlated with a module ($p = 1.09 \times 10^{-7}$, $r = .94$) that did not have a significant GO annotation. There were insufficient cases with temporal cortex samples to perform WGCNA.

4 | DISCUSSION

Postictal generalized EEG suppression duration positively correlated with 5HT2A receptor protein expression in hippocampal homogenate from TLE patients. 5HT1A receptor protein expression did not correlate with PGES duration in hippocampus or temporal lobe. On hippocampal RNAseq, PGES duration negatively correlated with *HTR3B* and positively correlated with *HTR4*, but was not correlated with the serotonin signaling pathway. By WGCNA, PGES duration positively correlated with synaptic transcripts (including potassium channels) and stimulus detection, and negatively correlated with cellular localization and developmental process transcripts.

Serotonin and serotonin receptors are relevant to SUDEP because they modulate seizure activity, arousal, and respiration,[9,10,14] and preclinical models link PGES to serotonergic signaling.[26] Recent clinical studies indicate fenfluramine, active at multiple serotonin receptors, SERT, and potentially at other receptors, modulates seizure activity in Dravet syndrome patients, which is also seen in animal studies along with decreased seizure-associated respiratory arrest.[9] In animal models, serotonin receptors have opposing effects on seizure threshold, partly reflecting ligand specificity and dose, downstream pathways (e.g., 5HT2A couples with G-proteins and GPCRs), seizure etiology, and cell type-specific expression by brain region (e.g., excitatory/inhibitory neurons, astrocytes, oligodendrocytes).[10,32] The 5HT2 receptors facilitate excitation,[17] and 5HT2A antagonists reduce seizures in animal models.[10,22] When activated, 5HT2A can inhibit calcium and

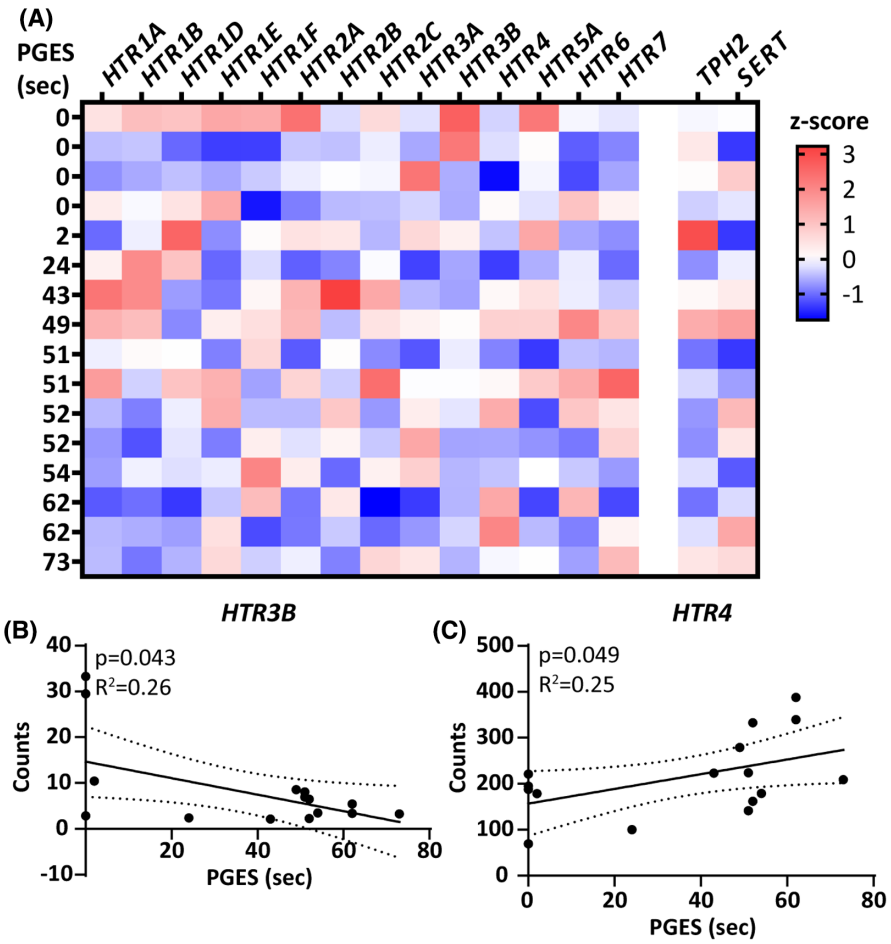

FIGURE 4 Serotonin receptor transcript expression from RNAseq in the hippocampus by postictal generalized electroencephalographic suppression (PGES) duration. (A) Expression of each of the serotonin receptors detected in the hippocampus ($n = 16$) and two related serotonergic transcripts: *TPH2*, the rate limiting enzyme in serotonin synthesis; and *SERT*, the serotonin transporter. PGES duration is indicated on the left, with increasing value from top to bottom. *Z*-score is indicated on the heatmap, with higher values represented in red and lower values in blue. (B) Of the 14 serotonin receptors detected by RNAseq, two correlated with PGES duration. *HTR3B* had a negative correlation with PGES duration ($p = .043$, $R^2 = .26$). (C) *HTR4* had a positive correlation with PGES duration ($p = .049$, $R^2 = .25$)

sodium conductance, release hippocampal arachidonic acid, and influence neuronal morphology and plasticity.[11] Activation of presynaptic autoreceptor and postsynaptic 5HT1A receptors can increase potassium and decrease calcium conductance, decreasing neurotransmitter release.[11] Imaging studies identified decreased 5HT1A binding in TLE compared to controls.[13,33] Among TLE patients, we did not observe 5HT1A changes in this brain region by PGES duration. Our prior proteomics studies comparing SUDEP to non-SUDEP epilepsy cases did not detect serotonin receptors in the frontal cortex, hippocampus, or brainstem nuclei, and thus differences could not be evaluated.[25,29] Future studies should explore mechanistic implications of the association between increased hippocampal 5HT2A and PGES duration, particularly whether 5HT2A expression is increased in other hippocampal subregions like CA1, whether 5HT2A antagonism reduces PGES duration, and whether seizure burden or prolonged PGES upregulates 5HT2A receptor protein expression as a compensatory mechanism or epiphenomenon.

At the RNA level, several transcripts were associated with PGES duration. *HTR3B* decreased with PGES duration. The 5HT3 receptor is a ligand-gated heteromeric ion channel, and activation results in fast depolarization.[34] 5HT3 receptor agonism increases seizure duration, and antagonism decreases severity of convulsions and afterdischarge duration.[14] *HTR4* increased with PGES duration. When activated, 5HT4 inhibits potassium channel conductance that results in longer hyperexcitability, and is implicated in cell survival and spine growth.[11] In epilepsy animal models, 5HT4 brainstem protein expression was decreased,[35] 5HT4 deletion promoted seizures and increased mortality,[18] and 5HT4 agonism decreased seizure-induced respiratory arrest and tonic seizures.[36] *DHRS7* (dehydrogenase/reductase 7) was a lead transcript correlating with PGES duration. Related proteins metabolize prostaglandins, lipids, and steroids[37,38] and interact with cannabinoid receptor 2.[39] The effect that these transcript expression levels may have on PGES and SUDEP risk, and whether they are altered as a result of prolonged PGES or contribute to PGES duration, are unclear; thus, these should be investigated further.

Synaptic transcripts, including potassium channels, were positively correlated with PGES duration (four voltage-gated, two inward rectifiers, one 2-pore domain). Voltage-gated channels facilitate repolarization and modify duration and delay of action potentials, inward rectifiers maintain resting membrane potential, and two-pore domain channels contribute to leak current important for resting membrane potential.[40,41] Potassium channels

are not implicated in PGES, but potassium conductance, expression, and gain- and loss-of-function mutations can cause epilepsy and may increase SUDEP risk.[40,42,43] A comparison of the same TLE cases in this study to controls in another of our studies did not identify an alteration to these seven potassium channels in hippocampus by RNAseq.[30] Our SUDEP proteomics studies[25,29] did not detect the same potassium channels as in the current study. Five different potassium channels were detected but were similar in frontal cortex and hippocampus,[29] and three potassium channels in brainstem nuclei were detected and not altered between SUDEP and non-SUDEP epilepsy.[25] Follow-up studies should investigate whether the altered potassium channel RNA corresponds to functional changes, and whether excitatory or inhibitory neurons are impacted differently, and evaluate additional brain regions like the brainstem. Increased voltage-gated potassium channels may be a compensatory response to hyperexcitability or shift voltage activation to more negative potentials contributing to hyperexcitability, similar to gain-of-function *KCNH1*[43] mutations. Alternatively, they may reflect an overcompensation coupled with dysregulation that suppresses brain activity in PGES.

There were several other correlations of WGCNA-identified modules with clinical history. PGES duration negatively correlated with cellular localization and developmental process transcripts, and positively correlated with stimulus detection related to taste receptors. Neuronal migration defects occur in epilepsy,[44] and taste receptors[45] are expressed in extraoral regions and altered in some disease states. Four other modules with GO annotations were associated with clinical history. Localization related to neuronal projection and metabolic processes positively correlated with epilepsy onset, indicating these transcripts were more elevated at a later age of epilepsy onset than in patients with an earlier onset. Epilepsy onset age is positively related to age at surgery, in which a positive correlation was similarly seen for mitochondrial transcripts. Furthermore, epilepsy duration positively correlated with stimulus detection related to olfactory receptors, indicating these transcripts were more elevated in patients with prolonged epilepsy duration than those with a shorter duration. Hippocampal olfactory receptors have nonsensory functions, ligands include endogenous molecules, and expression is abnormally regulated in neurodegeneration.[46] The meaning of these clinical history associations requires further investigation at the protein and functional levels.

Our study had several limitations. PGES duration can vary in the same patient during different seizures, and the number of convulsive seizures captured on video-EEGs was limited.[1,5,47] 5HT2A and 5HT1A protein levels may vary between Western blot and histology detection techniques due to subregional differences and differences in detection method based on solubility and other factors, as we and others have reported (time to tissue processing/freezing).[48,49] RNAseq did not correlate *HTR1A* or *HTR2A* to PGES. Protein changes may not correspond to transcript changes,[31] although overall pathway analysis may be more comparable. Hippocampal sclerosis was present in most cases for protein analyses (n = 13/16, predominantly International League Against Epilepsy type 1); however, neuronal loss was not associated with decreased serotonin receptors. Patients were not evaluated for pathogenic gene variants. SUDEP cases were not evaluated in the current study, but future studies will be of interest in SUDEP cases with PGES information available to further investigate the association of serotonin receptor and potassium channel expression related to SUDEP risk.

In summary, higher 5HT2A receptor protein by Western blot and potassium channel transcript expression in the hippocampus of TLE patients were associated with prolonged PGES. Future studies should investigate serotonin receptor (5HT1A, 5HT2A, 5HT3B, 5HT4) and potassium channel protein expression in patients with PGES and in SUDEP cases, particularly in implicated brain regions like the hippocampal subregions and brainstem, as well as the mechanistic implications of these expression changes.

AUTHOR CONTRIBUTIONS

Conception and design of study: Orrin Devinsky, Maura Boldrini. Acquisition and analysis of data: Dominique Frances Leitner, Sasha Devore, Juliana Laze, Daniel Friedman, James D. Mills, Yan Liu, Michael Janitz, Jasper J. Anink, Johannes C. Baayen, Sander Idema, Erwin Alexander van Vliet, Beate Diehl, Catherine Scott, Roland Thijs, Maromi Nei, Manor Askenazi, Shobi Sivathamboo, Terence O'Brien, Thomas Wisniewski, Maria Thom, Eleonora Aronica, Maura Boldrini. Drafting significant portion of manuscript or figures: Dominique Frances Leitner, Orrin Devinsky.

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CONFLICT OF INTEREST

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