Serotonin Receptor Expression in Hippocampus and Temporal Cortex of TLE Patients by PGES Duration

Dominique F. Leitner[1,2,3], Sasha Devore[1,3], Juliana Laze[1,3], Daniel Friedman[1,3], James D. Mills[4], Yan Liu[5], Michael Janitz[6], Jasper J. Anink[4], Johannes C. Baayen[7], Sander Idema[7], Erwin A. van Vliet[4,8], Beate Diehl[9], Catherine Scott[9], Roland Thijs[10], Maromi Nei[11], Manor Askenazi[12,13], Thomas Wisniewski[2,3,14,15], Maria Thom[9], Eleonora Aronica[4,10], Maura Boldrini[5], Orrin Devinsky[1,3]

Affiliations:
[1] Comprehensive Epilepsy Center, NYU Grossman School of Medicine, New York, NY, USA.
[2] Center for Cognitive Neurology, NYU Grossman School of Medicine, New York, NY, USA.
[3] Department of Neurology, NYU Grossman School of Medicine, New York, NY, USA.
[4] Department of (Neuro)Pathology, Amsterdam UMC, University of Amsterdam, Amsterdam Neuroscience, Meibergdreef 9, Amsterdam, the Netherlands.
[5] Department of Psychiatry, Columbia Medical College, New York, NY, USA.
[6] School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, New South Wales, Australia.
[7] Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Neurosurgery, Amsterdam Neuroscience, De Boelelaan 1117, Amsterdam, the Netherlands.
[8] Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, Amsterdam, the Netherlands.
[9] Department of Clinical and Experimental Epilepsy, University College London Institute of Neurology, London, UK.
[10] Stichting Epilepsie Instellingen Nederland (SEIN), Heemstede, the Netherlands.
[11] Department of Neurology, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA, USA.
[12] Biomedical Hosting LLC, Arlington, MA, USA.
[13] Department of Biochemistry and Molecular Pharmacology, NYU Grossman School of Medicine, New York, NY, USA.
[14] Department of Pathology, NYU Grossman School of Medicine, New York, NY, USA.
[15] Department of Psychiatry, NYU Grossman School of Medicine, New York, NY, USA.

Author information:
Dominique F. Leitner: Dominique.leitner@nyulangone.org; ORCID: 0000-0002-1371-9861
Sasha Devore: sasha.devore@nyulangone.org
Juliana Laze: Juliana.laze@nyulangone.org
Daniel Friedman: daniel.friedman@nyulangone.org
James D. Mills: j.d.mills@amsterdamumc.nl
Yan Liu: ylbrains@gmail.com
Michael Janitz: m.janitz@unsw.edu.au
Jasper J. Anink: j.j.anink@amsterdamumc.nl
Johannes C. Baayen: jc.baaijen@amsterdamumc.nl
Sander Idema: s.idema@vumc.nl
Erwin A. van Vliet: e.a.vanvliet@uva.nl
Beate Diehl: b.diehl@ucl.ac.uk
Catherine Scott: catherine.scott21@nhs.net
Roland Thijs: r.d.thijs@lumc.nl
Maromi Nei: maromi.nei@jefferson.edu
Manor Askenazi: manor@biomedical.hosting
Thomas Wisniewski: Thomas.Wisniewski@nyulangone.org
Maria Thom: m.thom@ucl.ac.uk
Eleonora Aronica: e.aronica@amsterdamumc.nl
Maura Boldrini: mb928@cumc.columbia.edu
Orrin Devinsky: od4@nyu.edu; ORCID: 0000-0003-0044-4632

Corresponding author:
Orrin Devinsky
223 East 34th Street
New York, NY 10016
Email: Orrin.Devinsky@nyulangone.org

Abstract

Objective

Evaluate whether postictal generalized EEG suppression (PGES) duration correlates to 5HT1A and 5HT2A receptor protein expression and RNAseq data from resected hippocampus and temporal cortex of temporal lobe epilepsy (TLE) patients who had seizures recorded as part of their preoperative evaluation.

Methods

5HT1A and 5HT2A receptor protein was evaluated in whole brain homogenate by western blot and histologically in the hippocampus (n=16) and temporal cortex (n=9) of TLE patients with PGES (range 0 to 93 seconds). We correlated our previous RNAseq dataset for serotonin receptors and signaling pathways by weighted gene correlation network analysis (WGCNA) in these patients by PGES duration.

Results

In hippocampus, 5HT2A protein expression positively correlated with PGES duration ($p=0.0024$, $R^2=0.52$) but 5HT1A did not ($p=0.87$, $R^2=0.0020$) by western blot. In temporal cortex, 5HT1A and 5HT2A were expressed at lower levels than in hippocampus, and they did not correlate with PGES duration. Histologically, PGES duration did not correlate with 5HT1A or 5HT2A in hippocampal CA4, dentate gyrus, and temporal cortex. RNAseq identified two serotonin receptors with expression that correlated to PGES duration in an exploratory analysis: *HTR3B* negatively correlated ($p=0.043$, $R^2=0.26$) and *HTR4* positively correlated ($p=0.049$, $R^2=0.25$). WGCNA identified 4 modules correlated to PGES duration, including positive correlation ($p=0.040$, corr.=0.52) to synaptic transcripts, particularly potassium channels (*KCNA4*, *KCNC4*, *KCNH1*, *KCNIP4*, *KCNJ3*, *KCNJ6*, *KCNK1*). No modules were associated with serotonin receptor signaling.

Significance

Increased 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 help elucidate mechanisms underlying PGES and sudden unexpected death in epilepsy (SUDEP) risk.

Keywords: serotonin, PGES, SUDEP, hippocampus

Key Points:

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

Introduction

Postictal generalized EEG suppression (PGES) may occur after a generalized tonic-clonic seizure (GTCS) and increase risk of sudden unexpected death in epilepsy (SUDEP).[1-3] Prolonged PGES is associated with impaired arousal, respiration, and other autonomic functions.[1] PGES has been suggested as a potential 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 modulates respiration, arousal, and seizures.[9] In temporal lobe epilepsy (TLE) patients, MRI and PET imaging revealed decreased 5HT1A receptor binding.[10,11] In animal models, elevated serotonin reduced seizures, 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.[12-19] The 5HT2 receptor family generally facilitates excitatory effects,[15] thus 5HT2A antagonists may provide improvement of epilepsy.[20,21] The midbrain periaqueductal gray area undergoes atrophy in SUDEP cases,[23] and we recently identified altered signaling pathways in the dorsal raphe of SUDEP compared to controls.[24] In high-risk SUDEP patients, hippocampal SERT protein expression was increased.[19] In the brainstem, an animal model linked PGES to dysfunctional serotonin signaling and dorsal raphe stimulation reduced PGES duration.[22] With increasing PGES duration, interictal serum serotonin is decreased in epilepsy patients,[26] although serotonin levels have not been studied in brain tissue.

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

Methods

Human Brain Tissue. Surgical brain tissue was obtained with approval by the New York University School of Medicine Institutional Review Board (IRB, #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 Medical Center, 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 occurrence and duration was determined by epileptologists (CS, RT, DF) from one video-EEG for each patient based on criteria previously described.[27] Patients were selected to provide coverage of the PGES spectrum, including no PGES (0 seconds), PGES < 50 seconds associated with low-risk SUDEP, and PGES ≥ 50 seconds associated with high-risk SUDEP.[3] Case history is summarized in Table 1 and detailed in Supplemental Table 1, with 13 cases overlapping in hippocampus for protein, histology, and RNAseq analyses.

Western blot. Protein was isolated from frozen brain tissue (40 mg/sample) at 20% weight/volume in Tris-NaCl buffer (20 mM Tris base, 150 mM NaCl, 0.1% Triton-X 100, protease and phosphatase inhibitors at pH 7.5) using a hand held homogenizer equipped with a pestle on ice. Samples were incubated on ice for 15 minutes, centrifuged for 15 minutes at 14,000*g*, 4°C, and supernatant was isolated. Protein concentration was determined by BCA 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 DTT. 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 TBST, blots were probed for 5HT1A (1:500, Abcam ab227165), 5HT2A (1:500, Santa Cruz sc-166775), or actin (1:3000, Sigma A5441) in 5% milk TBST overnight at 4°C. Blots were incubated with corresponding HRP-conjugated secondary antibodies (1:3000, GE Healthcare) for 1 hour at room temperature. Bands were visualized after ECL (Pierce) on a BioRad ChemiDoc with the NYU Small Instrument Fleet. Blot images were analyzed in Fiji ImageJ for quantification with intensity normalized to actin.

Immunohistochemistry. Subregional protein expression was assessed by immunohistochemistry as described.[28] FFPE blocks were sectioned (8 µm) by the NYU Center for Biospecimen Research and Development (CBRD). Sections were deparaffinized and rehydrated through a series of xylenes and ethanol dilutions, followed by heat-induced antigen retrieval with 10 mM sodium citrate and 0.05% 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 sc-166775) primary antibodies overnight at 4°C. Corresponding secondary antibodies were used (donkey anti-rabbit Alexa-Fluor 568, donkey anti-mouse Alexa-Fluor 488; Thermofisher) with DAPI counterstain and slides were coverslipped. Whole slide scanning was performed on each section at

20X 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 5X 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.

RNAseq. We also analyzed our RNAseq dataset[28,29] in the European Genome-phenome Archive (EGAS00001003922) in hippocampus of TLE patients for whom we could obtain an evaluation of PGES duration. Case histories were previously detailed[28,29] and are summarized in Table 1.

Weighted Gene Correlation Network Analysis (WGCNA). WGCNA was performed on our RNAseq dataset[28,29] to determine whether PGES duration correlated to RNAseq in the R environment with the *WGCNA* package with defaults as described,[30] except as stated. Soft threshold power beta was determined at $R^2 = 0.8$ (power = 8), minModuleSize = 150, and deepSplit = 4. Gene ontology (GO) annotations for modules were determined following WGCNA with the *anRichment* package in the R environment with Entrez IDs against the human GOcollection (Supplemental Table 2). GO annotations were considered with a false discovery rate (FDR) < 5%.

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 < 0.05 was considered significant.

Results

Case History

Clinical history is summarized in Table 1 and detailed in Supplemental Table 1 for each analysis, including cases with coverage of the PGES duration spectrum (range PGES 0 – 93 seconds): no PGES (0 seconds), PGES < 50 seconds associated with low-risk SUDEP, and PGES ≥ 50 seconds associated with high-risk SUDEP. There were a total of 36 cases (range: age at surgery 14 – 64, epilepsy onset 0 – 51, epilepsy

duration 2 – 53 years of age) evaluated across all analyses, and 13 of the same cases with hippocampal tissue were evaluated in protein, histology, and RNAseq analyses.

5HT1A and 5HT2A Protein Expression in Whole Brain Homogenate

Western blot of whole brain homogenate from resected hippocampus (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 ($p = 0.87$, $R^2 = 0.0020$; Figure 1C). In temporal cortex, 5HT1A was expressed at a lower level than in hippocampus and did not correlate with PGES duration ($p = 0.45$, $R^2 = 0.085$; Figure 1D). Hippocampal 5HT2A (55 kDa) positively correlated with PGES duration ($p = 0.0024$, $R^2 = 0.52$; Figure 1E). Temporal cortex 5HT2A was expressed at lower levels than in hippocampus and there was no correlation with PGES duration ($p = 0.20$, $R^2 = 0.22$; Figure 1F).

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).

RNAseq in Hippocampus

To determine whether 5HT1A and 5HT2A (encoded by *HTR1A* and *HTR2A*) as well as other serotonin receptors correlated to PGES duration, we analyzed our previous RNAseq dataset[28,29] (n = 16), overlapping with 13 patients with hippocampal protein data (Table 1, Supplemental Figure 1A). There were 14 serotonin receptors detected in the hippocampus (Figure 4A), with two receptors significantly correlated to PGES duration: *HTR3B* with a negative correlation ($p = 0.043$, $R^2 = 0.26$) and *HTR4* with a positive correlation ($p = 0.049$, $R^2 = 0.25$; Figure 4B-C).

To assess enriched serotonin signaling pathway transcripts associated with PGES duration, WGCNA was performed on our hippocampal dataset[28,29] and revealed no enrichment in this brain region (Supplemental Tables 2-3).

WGCNA identified additional transcripts that correlated with PGES duration (Supplemental Figure 1). There were 2,597 of 42,753 transcripts that correlated with PGES ($p < 0.05$), distributed among all 27 modules (Supplemental Table 2). The top two transcripts negatively correlated: *PPP1R17* (protein primarily expressed in cerebellum, $p = 1.16 \times 10^{-5}$, $R^2 = 0.76$; M-white module; Supplemental Figure 1B) and *DHRS7* ($p = 1.48 \times 10^{-5}$, $R^2 = 0.75$; M-ivory module; Supplemental Figure 1C). Among all 27 modules, 4 significantly correlated to PGES (Supplemental Figure 1D). PGES duration positively correlated with synapse transcripts ($p = 0.040$, corr. = 0.52; included 7 potassium channels: *KCNA4*, *KCNC4*, *KCNH1*, *KCNIP4*, *KCNJ3*, *KCNJ6*, *KCNK1*; Supplemental Figure 2; Supplemental Table 3) and stimulus detection (taste receptors, $p = 0.044$, corr. = 0.51). PGES negatively correlated with cellular localization ($p = 0.0084$, corr. = -0.63) and developmental process transcripts ($p = 0.020$, corr. = -0.57).

Five modules significantly correlated to 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 ($p = 0.0075$, corr. = 0.64), mitochondrial transcripts ($p = 0.0016$, corr. = 0.72), and localization related to neuronal projection ($p = 0.022$, corr. = 0.57). Epilepsy duration positively correlated with stimulus detection related to olfactory receptors ($p = 0.010$, corr. = 0.62). Age positively correlated with metabolic processes related to mitochondrial transcripts ($p = 0.0026$, corr. = 0.55). Sex (male gender) positively correlated to a module ($p = 1.09 \times 10^{-7}$, corr. = 0.94) that did not have a significant GO annotation. There were insufficient cases with temporal cortex samples to perform WGCNA.

Discussion

PGES duration positively correlated with 5HT2A receptor protein expression in hippocampal homogenate from TLE patients. 5HT1A receptor protein expression did not correlate to PGES duration in hippocampus or temporal lobe. On hippocampal RNAseq, PGES duration negatively correlated with *HTR3B* (also known as 5HT3B) and positively correlated with *HTR4* (also known as 5HT4), 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,[9,12,20] and preclinical models link PGES to serotonergic signaling.[22] 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 in cell type specific expression by brain region; e.g., excitatory/inhibitory neurons, astrocytes, oligodendrocytes.[20,31] The 5HT2 receptors facilitate excitation,[15] and 5HT2A antagonists reduce seizures in animal models.[20,21] When activated, 5HT2A can inhibit calcium and sodium conductance, release hippocampal arachidonic acid, and influence neuronal morphology and plasticity.[32] Activation of presynaptic autoreceptor and postsynaptic 5HT1A receptors can increase potassium and decrease calcium conductance, decreasing neurotransmitter release.[32] Imaging studies identified decreased 5HT1A binding in TLE compared to controls.[11,33] 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 did not detect serotonin receptors in the frontal cortex, hippocampus, or brainstem nuclei.[24,28] Future studies should explore mechanistic implications of the association between increased hippocampal 5HT2A and PGES duration. Does 5HT2A antagonism reduce PGES duration or does seizure burden or prolonged PGES upregulate 5HT2A receptor protein expression as a compensatory mechanism or epiphenomenon?

At the RNA level, several transcripts were associated with PGES duration. *HTR3B* (5HT3B) 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.[12] *HTR4* (5HT4) 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.[32] In epilepsy animal models, 5HT4 brainstem protein expression was decreased,[35] 5HT4 deletion promoted seizures and increased mortality,[16] 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 (CB2).[39] It is unclear the effect that these transcript expression levels may have on PGES and SUDEP risk, whether they are altered as a result of prolonged PGES or contribute to PGES duration, and thus should be investigated further.

Synaptic transcripts, including potassium channels, were positively correlated with PGES duration (4 voltage-gated, 2 inward rectifiers, 1 two 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 7 potassium channels in hippocampus by RNAseq.[29] Our SUDEP proteomics studies[24,28] did not detect the same potassium channels in the current study. Five different potassium channels were detected but were similar in frontal cortex and hippocampus,[28] and three potassium channels in brainstem nuclei were detected and not altered between SUDEP and non-SUDEP epilepsy.[24] Follow up studies should investigate whether the altered potassium channel RNA corresponds to functional changes, 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 to clinical history. PGES duration also 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 extra-oral 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. Further, 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 non-sensory 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.[1,5,49] 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).[50] RNAseq did not correlate *HTR1A* or *HTR2A* (5HT1A or 5HT2A) to PGES. Protein changes may not correspond to transcript changes,[30] although overall pathway analysis may be more comparable. Hippocampal sclerosis was present in most cases for protein analyses (n=13/16, predominantly ILAE type 1), however neuronal loss was not associated with decreased serotonin receptors. Patients were not evaluated for pathogenic gene variants.

In summary, increased 5HT2A protein and potassium channel signaling transcripts in TLE patients were associated with prolonged PGES. Future studies should investigate serotonin receptor protein expression (5HT1A, 5HT2A, 5HT3B, 5HT4) and potassium channels in patients with PGES and in SUDEP cases, particularly in hippocampal subregions and the brainstem.

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Disclosure of Conflicts of Interest

The authors declare no conflicts of interest.

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Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Data Availability

The data that support the findings of this study are available in the public repositories listed in the methods, supplemental files, and from the authors upon reasonable request.

Author Contributions:

Conception and design of study: OD

Acquisition and analysis of data: DL, SD, JL, DF, JM, YL, MJ, JA, JB, SI, EV, BD, CS, RT, MN, TW, MT, EA, MB

Drafting significant portion of manuscript or figures: DL, OD

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Figure Legends

Figure 1. 5HT1A and 5HT2A protein expression in whole homogenate from the hippocampus and temporal cortex by 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 to PGES duration. **D)** Quantification of 5HT1A relative to actin in temporal cortex (n = 9) indicates no correlation to PGES duration. **E)** Quantification of 5HT2A relative to actin in hippocampus (n = 16) indicates a positive correlation to PGES duration. **F)** Quantification of 5HT2A relative to actin in temporal cortex (n = 9) indicates no correlation to PGES duration.

Figure 2. 5HT1A protein expression in subregions of the hippocampus and temporal cortex by 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 seconds and **D-F)** PGES ≥ 50 seconds. **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.

Figure 3. 5HT2A protein expression in subregions of the hippocampus and temporal cortex by 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 seconds and **D-F)** PGES ≥ 50 seconds. **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.

Figure 4. Serotonin receptor transcript expression from RNAseq in the hippocampus by PGES duration. A) Expression of each of the serotonin receptors detected in the hippocampus (n = 16) and 2 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, 2 correlated to PGES duration. *HTR3B* had a negative correlation

with PGES duration ($p = 0.043$, $R^2 = 0.26$). **C)** *HTR4* had a positive correlation with PGES duration ($p = 0.049$, $R^2 = 0.25$).

Supplemental Data

Supplemental Figure 1. WGCNA of RNAseq in the hippocampus. A) Clustering of TLE patients is indicated from RNAseq expression data in the hippocampus (n = 16) with the corresponding clinical history. PGES, epilepsy onset, epilepsy duration, and age are indicated from low (white) to high (red) values. Sex is indicated for females (purple) and males (green). **B)** After WGCNA, the top two transcripts that correlated with PGES duration were **B)** *PPP1R17* ($p < 0.0001$, $R^2 = 0.76$) and **C)** *DHRS7* ($p < 0.0001$, $R^2 = 0.75$) with negative correlations. **D)** WGCNA resulted in identification of 27 modules (M-color), with clustering indicated on the left by eigenprotein adjacency. Module trait analysis indicated 4 significant modules (p value is indicated) correlated with PGES duration, 3 modules with epilepsy onset, 1 module with epilepsy duration, and 1 module with age at surgery. Heatmap indicates correlation values, blue is a negative correlation and red is a positive correlation. Top GO annotations below 5% FDR are noted for the significant modules on the right.

Supplemental Figure 2. Potassium channel transcript expression from RNAseq in the hippocampus by PGES duration. A) Expression of potassium channel transcripts in the hippocampus (n = 16) that were identified by WGCNA in the M-skyblue3 module, with a positive correlation to PGES duration ($p = 3.98 \times 10^{-2}$, corr. = 0.52). Z-score is indicated on the heatmap, with higher values represented in red and lower values in blue.

Supplemental Table 1. Detailed Case History

Supplemental Table 2. WGCNA RNAseq Gene and Module Correlations in Hippocampus

Supplemental Table 3. WGCNA RNAseq GOanRichment Analysis in Hippocampus

Table 1. Case History Summary

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

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. n = number of cases; M = male; F = female; mean ± standard deviation is indicated

Figure 1.

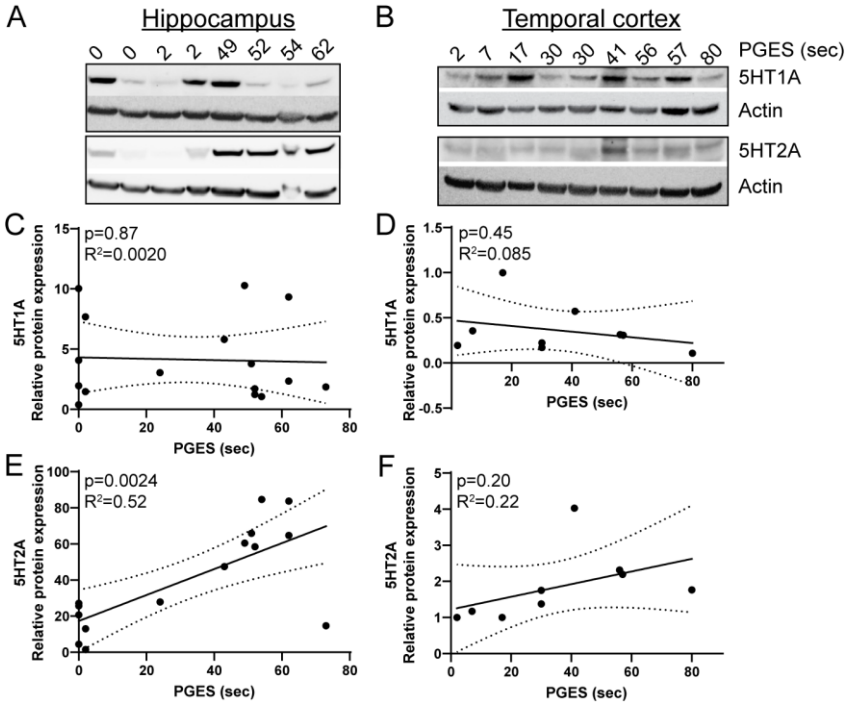

A
Hippocampus
0 0 2 2 49 52 54 62
B
Temporal cortex
2 7 17 30 30 41 56 57 80
PGES (sec)
5HT1A
Actin
5HT2A
Actin
C
p=0.87
R²=0.0020
5HT1A Relative protein expression
PGES (sec)
D
p=0.45
R²=0.085
5HT1A Relative protein expression
PGES (sec)
E
p=0.0024
R²=0.52
5HT2A Relative protein expression
PGES (sec)
F
p=0.20
R²=0.22
5HT2A Relative protein expression
PGES (sec)

Figure 2.

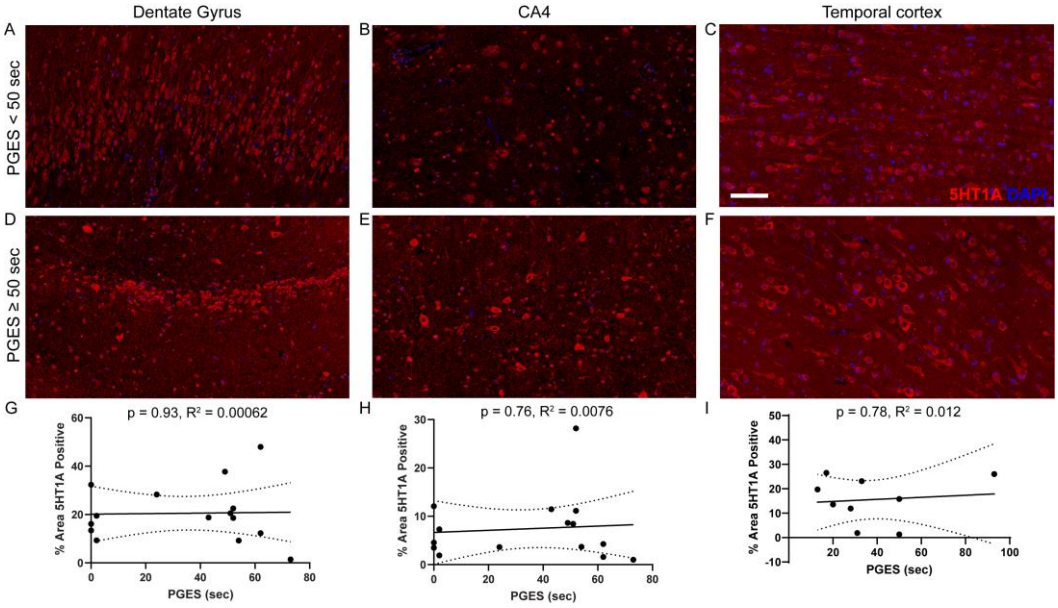

Dentate Gyrus
CA4
Temporal cortex
A
B
C
PGES < 50 sec
5HT1A DAPI
D
E
F
PGES ≥ 50 sec
G
H
I
p = 0.93, R² = 0.00062
p = 0.76, R² = 0.0076
p = 0.78, R² = 0.012
% Area 5HT1A Positive
PGES (sec)

Figure 3.

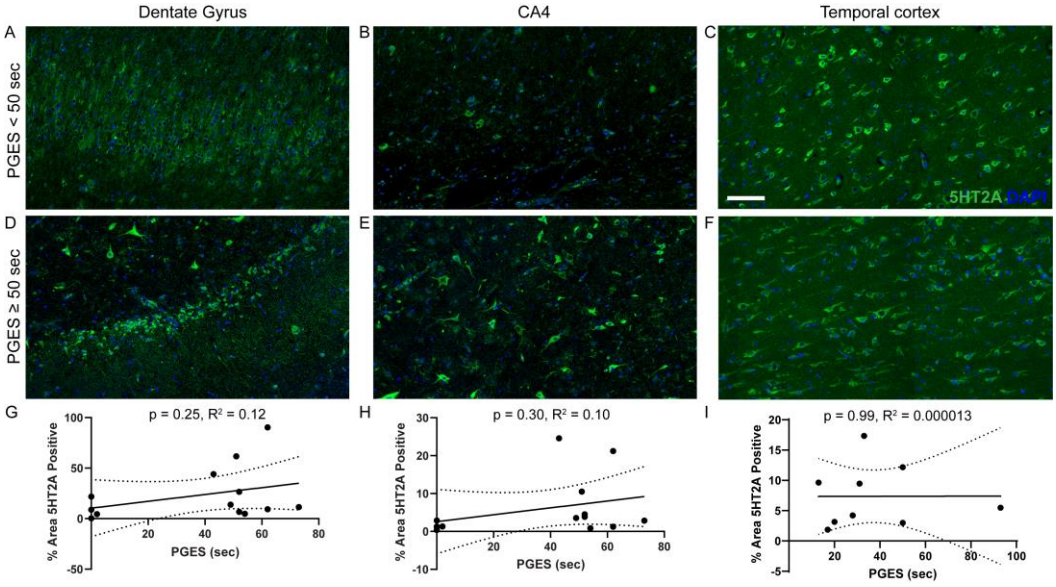
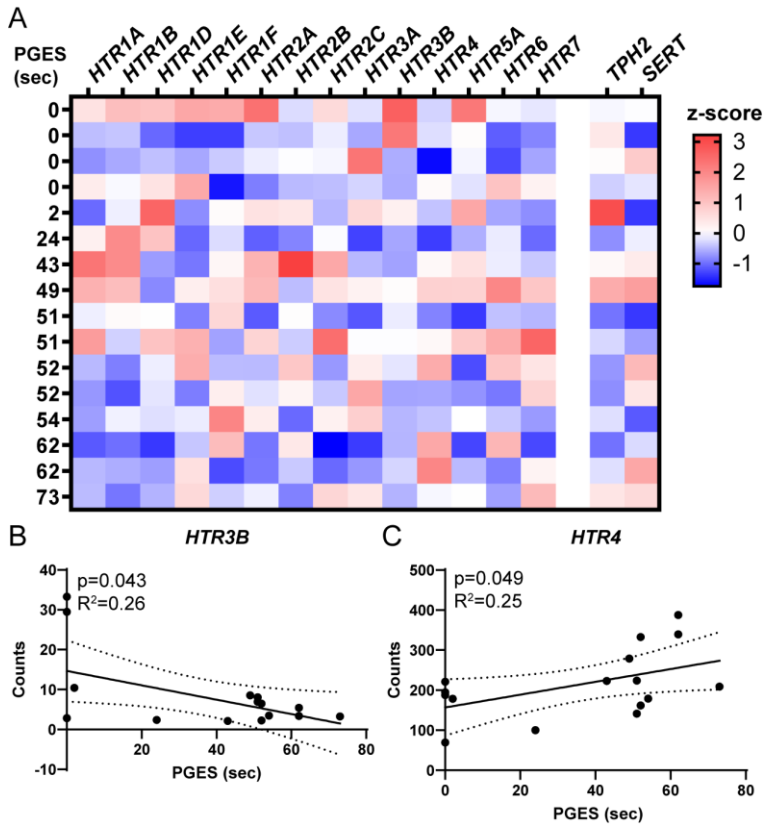
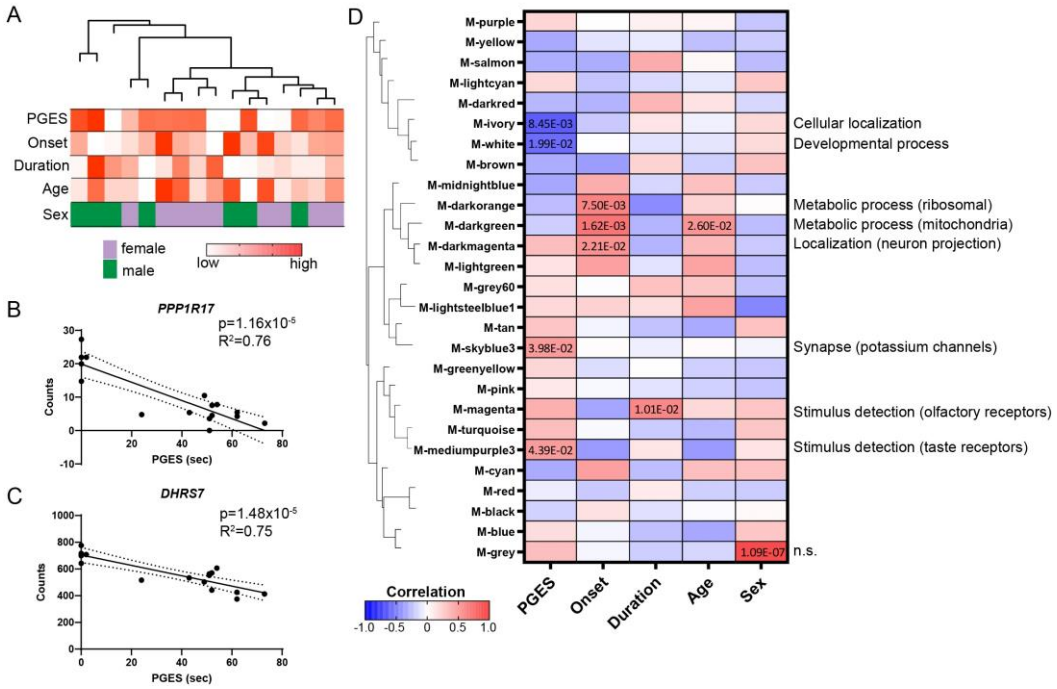

Dentate Gyrus
CA4
Temporal cortex
A
B
C
PGES < 50 sec
5HT2A DAPI
D
E
F
PGES ≥ 50 sec
G
p = 0.25, R² = 0.12
% Area 5HT2A Positive
PGES (sec)
H
p = 0.30, R² = 0.10
% Area 5HT2A Positive
PGES (sec)
I
p = 0.99, R² = 0.000013
% Area 5HT2A Positive
PGES (sec)

Figure 4.


A
PGES (sec)
HTR1A
HTR1B
HTR1D
HTR1E
HTR1F
HTR2A
HTR2B
HTR2C
HTR3A
HTR3B
HTR4
HTR5A
HTR6
HTR7
TPH2
SERT
0
0
0
0
2
24
43
49
51
51
52
52
54
62
62
73
z-score
3
2
1
0
-1
B
HTR3B
p=0.043
R²=0.26
Counts
PGES (sec)
C
HTR4
p=0.049
R²=0.25
Counts
PGES (sec)

Supplemental Figure 1.



Supplemental Figure 2.