Transcriptomic analysis of dorsal and ventral subiculum after induction of acute seizures by electric stimulation of the perforant pathway in rats

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Abstract
Preconditioning is a mechanism in which injuries induced by non-lethal hypoxia or seizures trigger cellular resistance to subsequent events. Norwood et al., in a 2010 study, showed that an 8-h-long period of electrical stimulation of the perforant pathway in rats is required for the induction of hippocampal sclerosis. However, in order to avoid generalized seizures, status epilepticus (SE), and death, a state of resistance to seizures must be induced in the hippocampus by a preconditioning paradigm consisting of two daily 30-min stimulation periods. Due to the importance of the subiculum in the hippocampal formation, this study aims to investigate differential gene expression patterns in the dorsal and ventral subiculum using RNA-sequencing, after induction of a preconditioning protocol by electrical stimulation of the perforant pathway. The dorsal (dSub) and ventral (vSub) subiculum regions were collected by laser-microdissection 24 h after preconditioning protocol induction in rats. RNA sequencing was performed in a Hiseq 4000 platform, reads were aligned using the STAR and DESeq2 statistics package was used to estimate gene expression. We identified 1176 differentially expressed genes comparing control to preconditioned subiculum regions, 204 genes were differentially expressed in dSub and 972 in vSub. The gene ontology enrichment analysis showed that the most significant common enrichment pathway considering up-regulated genes in dSub and vSub was steroid metabolism. In contrast, the most significant enrichment pathway considering down-regulated genes in vSub was axon guidance. Our results indicate that preconditioning induces changes in the expression of genes related to synaptic reorganization, increased cholesterol metabolism, and astrogliosis in both dSub and vSub. Both regions also presented a decrease in the expression of genes related to glutamatergic transmission and an increase in expression of genes related to complement system activation and GABAergic transmission. The down-regulation of proapoptotic and axon guidance genes in the ventral subiculum suggests that preconditioning may induce a neuroprotective environment in this region.

KEYWORDS
hippocampus, preconditioning, subiculum
1 | INTRODUCTION

Preconditioning is a mechanism in which injuries induced by non-lethal hypoxia or seizures trigger cellular resistance to subsequent events (Amini et al., 2015; Kelly & McIntyre, 1994; Plamondon et al., 1999; Pohle & Raucu, 1994). This injury tolerance seems to be associated with increased expression of genes involved in anti-apoptotic functions, oxidative-stress, and heat shock functions (Kirino, 2002; Stenzel-Poore et al., 2003). Norwood et al., in a 2010 study (Norwood et al., 2010), showed that an 8-h-long period of electrical stimulation of the perforant pathway (PP) in rats is required for the induction of hippocampal sclerosis. However, in order to avoid generalized seizures, status epilepticus (SE), and death, a state of resistance to seizures must be induced in the hippocampus. The authors used a preconditioning paradigm, consisting of 2 daily 30-min stimulation periods, to successfully achieve this state of resistance. These authors suggested a decrease in glutamate release by CA3 and dentate gyrus (DG) neurons and an increased inhibition of these cells as possible mechanisms to explain how preconditioning by electrical stimulation of the PP induces resistance to seizures in the hippocampus (Norwood et al., 2010). Therefore, this would prevent electrical activity elicited by PP stimulation from spreading throughout the nervous system, triggering SE. However, the authors did not perform experiments investigating the molecular mechanisms and differentially expressed genes induced after the preconditioning in the hippocampus.

The perforant pathway consists of projections from the entorhinal cortex to the DG (Witter, 2007). Layer II neurons of the entorhinal cortex project to the molecular layer of the dentate gyrus that in turn project to the stratum lacunsum moleculare of CA3 and CA2. Layer III entorhinal cortex neurons project to CA1 and subiculum neurons (Witter & Moser, 2006). Since subicular pyramidal neurons are the main source of projections to deep layers of the entorhinal cortex (Chrobak et al., 2000), the subiculum has a great influence on the electrical output of the hippocampal formation.

The subiculum connects the hippocampus CA1 to the entorhinal cortex (Somjen, 1995; Stafstrom, 2005) and it has an important function in high amplification of neuronal response, short-term memory (Miashita, 2004), and spatial memory codification (Sharpe & Green, 1994a). Furthermore, the subiculum is anatomically divided into dorsal and ventral subiculum, which have specific characteristics associated with its morphology and functions. The dorsal subiculum processes information of space, movement, and declarative memory. Its neurons receive inputs from dorsal CA1 and the entorhinal cortex layer III neurons, projecting outputs to the mammillary nucleus and presubiculum neurons (O’Mara, 2005; Sharp & Green, 1994b). In comparison, the ventral subiculum is responsible for stress modulation through inhibitory projections to the hypothalamic system (Lowry, 2002; O’Mara, 2005; Sharp & Green, 1994b). Its neurons receive inputs from the ventral CA1 and the entorhinal cortex layer III neurons, projecting outputs to amygdaloid complexes and parasubiculum neurons (Lowry, 2002; Witter & Groenewegen, 1990).

Therefore, due to the importance of the subiculum in the hippocampal formation and its influence on the preconditioning mechanism, this study investigates differential gene expression patterns in the dorsal and ventral subiculum using RNA-sequencing, after induction of a preconditioning protocol by electrical stimulation of the perforant pathway.

2 | METHODOLOGY

2.1 | Animals

Three-month-old male Wistar rats were used for preconditioning protocol (n = 16), of which eight animals were electrically stimulated and eight animals received the placement of the electrodes without electrical stimulation, being characterized as a control group. Animals were housed in a 12-h light/dark cycle on a ventilated rack with free access to food and water throughout experimentation protocol. All the experiments were performed at the University of Campinas—UNICAMP and the experimental protocol was approved by UNICAMP’s research ethics committee (CEUA 3850-1 protocol) according to accepted ethical practices and legislation regarding animal research in Brazil (Brazilian federal law 11,794 from October 8th, 2008).

2.2 | Surgery and electric stimulation of the perforant pathway

For electrodes implantation, rats were anesthetized using a gas mixture of isoflurane/oxygen (2%/98% respectively) at 2 L/min using an acrylic induction box. The anesthesia was maintained during the entire surgical procedure using a mask adapted to an Angle Two (Leica Microsystems) stereotaxic apparatus. Subsequently, rats were positioned into the stereotaxic apparatus and the skull was exposed by an incision on the scalp where the Bregma was localized and perforations on bone were performed. Next, two bipolar stainless steel, polyamide covered, 0.125 mm stimulation electrodes (P1tec, Roanoke) were placed into the perforant pathway with the following coordinates: ±4.5 mm lateral, +7.6 mm posterior, and −3 mm ventral.

Electrical activity was recorded by stainless steel, polyamide covered, 0.25 mm monopolar electrodes (P1tec, Roanoke) placed into the dentate gyrus with the following coordinates: ±2 mm lateral, −3 mm posterior, −3.5 mm ventral, and −2 mm lateral. In addition, a reference electrode was positioned above the dura mater in the coordinates: −1 mm lateral, +3 mm posterior. The final position of recording and stimulation electrodes was based on the occurrence of population spike evoked by granular neurons of DG (Norwood et al., 2010). Furthermore, two perforations in the spaces between reference, stimulation, and recording electrodes were performed to fix stainless steel screws to hold and stabilize all elements into a dental acrylic cement.

Seven days after the electrodes’ placement surgery, freely moving awake rats were electrically stimulated in the perforant pathway as previously described (Norwood et al., 2010) for 30-min in two consecutive days. The electric stimulation was performed using a Grass Astro-Med S88 stimulus generator (Grass® Technologies), using,
paired pulses, 0.1-ms pulse duration with interpulse interval of 40 ms, and pulse amplitude of 20 V, and the recordings were performed using a miniature preamplifier and digitizer with 32 channels (Intan Technologies) and the signal was digitized at 10 kHz.

One day after the last preconditioning stimulation session, rats were deeply anesthetized with an isoflurane/oxygen mixture (2%/98%). Subsequently, they were quickly decapitated, and their brains were immediately removed and froze at −60°C using n-hexane and dry ice.

2.3 | Laser microdissection

Frozen brains were processed in a cryostat (Leica Biosystems) to obtain 60 μm serial sections throughout the entire hippocampus using PEN membrane-covered slides (Life Technologies®, Thermo Fisher Scientific). Subsequently, slides were stained with Cresyl Violet, dehydrated with an ethanol series, and stored at −80°C. A Palm (Zeiss®) system was used to delimitate the dorsal subiculum (dSub) and ventral subiculum (vSub), and the tissue was mechanically collected in separate tubes using a surgical microscope (Zeiss®) and ophthalmic forceps. The scheme of the rat brain showing the dorsal and ventral hippocampus is shown in Figure 1a, the dorsal and ventral subiculum regions localization in Figure 1b, the subiculum circuit network in Figure 1c, and microdissected dorsal and ventral subiculum regions are shown in Figure 1d, and Figure 1e.

2.4 | RNA extraction, cDNA library preparation, and RNA-sequencing

The RNA was isolated from microdissected samples using Trizol’s manufacturer protocol for RNA isolation (Thermo Fisher Scientific). RNA samples were submitted to a Bioanalyzer run prior to cDNA library construction and an average RIN (RNA Integrity Number) of 7 was obtained. Subsequently, the cDNA libraries were produced using the TruSeq Stranded mRNA LT library preparation kit according to manufacturer instructions (Illumina). Sequencing was performed in an Hiseq 4000 platform (Illumina) available from Macrogen Inc, producing an average of 17.8 million reads per sample, 95% of bases over Q30.

Reads were aligned to the Rattus norvegicus Ensembl Rnor 5.0 assembly genome using the STAR aligner tool (https://github.com/alexdobin/STAR). The average sequencing alignment rate was 83%. The DESeq2 statistics package (http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html) and the limma package (https://bioconductor.org/packages/release/bioc/html/limma.html) were used to estimate gene expression and for statistical analysis purposes. A list of differentially expressed genes with a statistical significance of $p < .05$ (after correction for multiple tests) was generated. The list of differentially expressed genes was submitted to enrichment analysis using clusterProfiler package (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html, RRID:SCR_016884) (Yu et al., 2012) and the functional profiles were classified into KEGG Pathways. ClusterProfiler uses a web api to access the latest version of the KEGG database from the KEGG website (https://www.genome.jp/kegg/). Therefore, for the present manuscript, the analysis performed in February 2022 was based on the KEGG database that reports its last update in 2021-12-23. For all enrichment analyses, pathways were considered significantly different when $p < .05$ (after adjustment for multiple comparisons).

2.5 | Immunolabeling

For immunolabeling, three control ($N = 3$) and three preconditioning ($N = 3$) rats were transcardially perfused with 4% formaldehyde 48 h after the last stimulation. Brains were processed for histology and immunolabeling for GFAP using fluorescent secondary antibodies. Images were acquired in an epifluorescence microscope (Leica®). A total of three tissue sections (40um) were used per condition.

Tissue sections were incubated free-floating in blocking solution (PBS containing 0.05% sodium azide, Triton X 100 0.2% and albumin 2%) for 1 h. Subsequently, sections were incubated in blocking solution containing primary antibody (1:500 mouse anti-GFAP monoclonal antibody Abcam, catalog number #AB53554) for 24 h. Then, samples were washed three times with PBS and incubated for 24 h in blocking solution containing secondary antibody (1:1000 donkey anti-goat IgG antibody, ThermoFisher, catalog number #A11058). Samples were then washed three times with PBS and mounted with Vectashield in standard glass slides. Measurement of GFAP-labeled area in sections containing dSub and vSub was performed using ImageJ (version 1.53), integrated pixel density for labeled area was measured in a 1 × 1 mm square positioned over the area in each of the three sections containing the subiculum for each animal. Integrated pixel densities for each section for the same biological replicate were combined.

3 | RESULTS

In the present study, we performed a precondition paradigm for inducing acute seizures by electrical stimulation of the perforant pathway (PP) in rats. Samples were submitted to laser microdissection and the dorsal and ventral subiculum were separated and removed. RNA was isolated and sequenced followed by statistical and in silico analysis by the DESEQ2 package comparing the control and preconditioning groups.

We found 1176 differentially expressed genes comparing control to preconditioned subiculum regions, in which 204 genes were differentially expressed in dSub and 972 in vSub. Considering the vSub differentially expressed genes in the preconditioned group, 399 genes were down-regulated, and 573 genes were up-regulated (Figure 2a), while in dSub, 71 genes were down-regulated, and 132 genes were up-regulated (Figure 2d). We also performed the statistical analysis using limma-trend software. The results demonstrated 792 differentially expressed genes comparing the control group to the preconditioning group, in which 62 genes were differentially expressed in dSub and 729 genes were differentially expressed in
Considering dSub differentially expressed genes in the preconditioned group, 18 genes were up-regulated and 44 were down-regulated, while in vSub, 277 genes were up-regulated and 452 were down-regulated. The analysis of enrichment pathways considering DEGs list generated by limma is presented in the Appendix S1 item 10.1. The complete list of differentially expressed genes is presented in the Appendix S2. Gene count tables and raw files (fastq) for all samples are available at GEO (GSE178409).

Furthermore, the principal component analysis demonstrated clustering of samples according to the condition, control and preconditioning, in dSub and vSub (Figure 1g).

The most significant common enriched pathways considering dSub up-regulated genes in the preconditioning group were butanoate metabolism, fatty acid metabolism, steroid biosynthesis, carbon metabolism, pyruvate metabolism, and glycolysis/glucogenesis (Figure 2b), while in vSub the enriched pathways considering up-regulated genes
in the preconditioning group were terpenoid backbone biosynthesis, gap junction, and aminoacyl-tRNA biosynthesis (Figure 2c).

The most significant enriched pathway considering dSub down-regulated genes in the preconditioning group were arrhythmogenic right ventricular cardiomyopathy, muscle contraction, and hypertrophic cardiomyopathy (Figure 2e). In contrast, in vSub the most significantly enriched pathway considering down-regulated genes in the preconditioning group was axon guidance (Figure 2f). The differentially expressed genes associated with each enriched pathway are listed in Table 1. The complete list of enriched pathways is presented in Table S1.

Moreover, we found expression of cell population markers Gfap (Glial fibrillary acidic protein), C3 (Complement C3), and Gad2 (Glutamate Decarboxylase 2) genes up-regulated in dSub and vSub (Figure 4a–c), the Glis2 (Glutaminase 2) gene down-regulated in dSub and vSub (Figure 4d), and the Gt1-1 (Solute Carrier Family 6 Member 1) gene down-regulated in vSub (Figure 4e).

Considering the up-regulation of Gfap (Figure 4a), we performed immunolabeling for this gene in coronal tissue sections containing dSub and vSub 48 h after preconditioning (Figure 3). We quantified the GFAP-labeled area in tissue sections containing dSub or vSub. For control dSub, we observed an average integrated pixel density of 255.94 ± 106.9 and for stimulated dSub 234.75 ± 76.4 (average ± standard error). For control vSub we observed an average integrated pixel density of 81.42 ± 20.8 and for stimulated vSub
When comparing control and preconditioned vSub samples we observed a statistically significant increase in the average GFAP-labeled area (Student’s t test, p < .05).

| TABLE 1 | Differentially expressed genes in dSub and vSub according to the enrichment pathways after induction of preconditioning |
|----------|---------------------------------------------------------------------------------------------------------------|
| Butanoate metabolism | Up-regulated exclusively in vSub |
|                     | Bdh1 |
| Common up-regulated genes in dSub and vSub | Aacs; Gad2; Hmgcs1; Acat2I1; Acat2 |
| Fatty acid metabolism | Up-regulated exclusively in dSub |
|                     | Elovl5 |
| Up-regulated exclusively in vSub | Acsl3; Scd2; Fads1; Acaca; Aasn; Acat2 |
| Common up-regulated genes in dSub and vSub | Fads2; Acat2I1; Scd |
| Steroid biosynthesis | Up-regulated exclusively in vSub |
|                     | Sc5d; Dhcr24; Lss; Sdqle; Nsdh1; Hsd17b7; Ebp; Tm7sf2; |
| Common up-regulated genes in dSub and vSub | Msmo1; Dhcr7; Cyp51 |
| Carbon metabolism | Up-regulated exclusively in vSub |
|                     | Tkt; Pgam1; Pdhb; Taldo1; Me1; Pfk; Pgls; Psph; Acat2 |
| Common up-regulated genes in dSub and vSub | Acss2; Eno1; Acat2I1; Pfkp; Me3 |
| Pyruvate metabolism | Up-regulated exclusively in vSub |
|                     | Pdhb; Me1; Acaca; Acat2 |
| Common up-regulated genes in dSub and vSub | Acss2; Idha; Acat2I1; Me3 |
| Glycolysis/Gluconeogenesis | Up-regulated exclusively in vSub |
|                     | Pgam1; Pdhb; Galm; Pfk1; Loc688778; Aldh3b1 |
| Common up-regulated genes in dSub and vSub | Acss2; Idha; Eno1; Pfkp |
| Biosynthesis of unsaturated fatty acids | Up-regulated exclusively in dSub |
|                     | Fads2; Elovl5; Scd |
| Terpenoid backbone biosynthesis | Up-regulated exclusively in vSub |
|                     | Pmvk; Mvk; Hmgcr; Fdps; Idl1; Hmgcs1; Acat2I1; Acat2; Mvd |
| Gap junction | Up-regulated exclusively in vSub |
|                     | Map2k1; Tuba4a; Tubb5; Map2k2; Tuba1b; Tubb3; Tuba8; Tubb4b; Tubb2a; Tuba1a; Itpr3; Tubb2b |
| Aminoacyl-tRNA biosynthesis | Up-regulated exclusively in vSub |
|                     | Eprs; Nar1; Yars1; Sars1; Mars1; Aars1; Gars1; Hars1; Wars1; Cars |
| Axon guidance | Down-regulated exclusively in vSub |
|                     | Ilk; Rock2; Efnb3; Ephra4; Pard3; Ptch1; Mapk3; Camk2g; Sema4c; Slat2; Rnd1; Pkncc1; Sema3f |
| Arrhythmogenic right ventricular cardiomyopathy | Down-regulated exclusively in dSub |
|                     | Cacng7; Cacng3; Atp2a3; Itga8 |
| Muscle contraction | Down-regulated exclusively in dSub |
|                     | Cacng7; Atp2a3; Atp1a1; Cacng3 |
| Hypertrophic cardiomyopathy | Down-regulated exclusively in dSub |
|                     | Cacng7; Cacng3; Atp2a3; Itga8 |

Increased cholesterol metabolism, and astrogliosis in both the dorsal and ventral subiculum. Both regions also presented changes in gene expression associated with glutamate transmission, complement system, and GABAergic transmission. In addition, alteration in the expression of proapoptotic and axon guidance-related genes in the ventral subiculum suggests that preconditioning may induce a neuroprotective environment in this region. These altered mechanisms may explain the lack of neuronal loss in the subiculum observed in many MTLE experimental models (Knopp et al., 2005; Stafstrom, 2005).

We observed the up-regulation of Gad2 gene (Figure 4a) and down-regulation of Gat-1 gene (Figure 4b), suggesting an increase in GABAergic transmission and the down-regulation of Grip2 and Gls2

169.77 ± 30.19 (average ± standard error). When comparing control and preconditioned vSub samples we observed a statistically significant increase in the average GFAP-labeled area (Student’s t test, p < .05).

4 | DISCUSSION

The present study explores, for the first time, the transcriptome of laser microdissected dorsal and ventral subiculum after a preconditioning paradigm through the induction of acute seizures by electrical stimulation of the perforant pathway (PP) in rats. Our results indicate that preconditioning induces synaptic reorganization, increased cholesterol metabolism, and astrogliosis in both the dorsal and ventral subiculum. Both regions also presented changes in gene expression associated with glutamate transmission, complement system, and GABAergic transmission. In addition, alteration in the expression of proapoptotic and axon guidance-related genes in the ventral subiculum suggests that preconditioning may induce a neuroprotective environment in this region. These altered mechanisms may explain the lack of neuronal loss in the subiculum observed in many MTLE experimental models (Knopp et al., 2005; Stafstrom, 2005).
gene, suggesting a possible decrease in glutamatergic transmission in both subiculum areas. These findings are in agreement with the hypothesis presented by Norwood et al. (2010) that preconditioning by PP electrical stimulation could result in an increase in inhibition and/or reduction of excitation in the hippocampus. The glutamic acid decarboxylase, GAD2, is present in presynaptic terminals and participates in GABA synthesis. Given its function, the GAD2 plays an important role in central GABA synapses, regulating GABA production (Pan, 2012; Zhao & Gammie, 2014). Thus, the up-regulation of Gad2 gene expression might increase GABA production in presynaptic neurons.

GAT-1 is the most expressed membrane surface transporter of the mammalian brain (Bowery, 2007; Conti et al., 2004). The Gat-1 expression is necessary for excitability regulation, synaptic process (Minelli et al., 1995), and reuptake of GABA from the synaptic cleft. The down-regulation of the gat-1 gene reduces GABA’s reuptake, resulting in enhancement of GABA transmission (Kang et al., 2001). Our data suggest that preconditioning induces increased GABA production by up-regulation of the Gad2 gene, resulting in increased GABAergic transmission in both subiculum areas. However, in vSub, the GABAergic transmission would be further intensified owing to down-regulation of the Gat-1 gene, resulting in reduced GABA reuptake.

**FIGURE 3** Immunolabeling of the dorsal and ventral subiculum region 48 h after preconditioning, using N = 3 animals per condition and n = 3 sections per condition. Scale bar (480 μm) at the bottom right of each figure. In red astrocytes labeled using GFAP. (a) Control dorsal subiculum. (b) Preconditioning dorsal subiculum. (c) Control ventral subiculum. (d) Preconditioning ventral subiculum. (e) Mean integrated pixel density for each biological replicate (N = 3). Labeled area was measured in a 1 × 1 mm square positioned over the area in each of the three sections containing the subiculum for each animal. Integrated pixel densities for each section for the same biological replicate were combined. Values for each biological replicate are plotted in the graph as open diamonds. Star marks statistically significant difference when comparing SV to PV (Student’s t test, p < .05).
The present results also revealed up-regulation of the C3 gene in both subiculum areas after preconditioning (Figure 4c). The C3 is one of the most abundant molecules of the complement system (Stephan et al., 2012). In the CSN, the complement system participates in synaptic pruning (Hua & Smith, 2004; Janeway Jr et al., 2001; Stephan et al., 2012), and in the hippocampus, C3 might be associated with the pruning of glutamatergic synapses (Perez-Alcazar et al., 2014; Salter et al., 2020).

The Gls2 gene, the glutaminase gene encoding the liver-type isoforms, encodes the GLS2 enzyme, a glutaminase (GA). GA enzymes are considered the main producer of presynaptic glutamate in the brain (Kvamme, 2018; Márquez et al., 2017), participating in glutamate biosynthesis in mitochondria (Suzuki et al., 2010), neurotransmission, and metabolism (Mattson, 2008). The down-regulation of the Gls2 gene suggests reduced glutamate production in dorsal and ventral subiculum neurons. Our data also show Grip2 gene down-regulated.
The most enriched pathways in both subiculum areas were associated with increased cholesterol biosynthesis (Figure 2b,c). Cholesterol has an important role in the central nervous system (CNS) representing 20%–30% of all lipids of the brain (Pfrieger, 2003) and it is locally synthesized by oligodendrocytes, astrocytes, and neurons. In neurons, cholesterol also participates in the production of myelin (Russell et al., 2009), dendritic formation (Fester et al., 2009; Russell et al., 2009); synaptic connections, and axon guidance (de Chaves et al., 1997; Goritz et al., 2005). However, cholesterol production in neurons is inefficient compared to the production in astrocytes (Dietschy, 2009; Nieweg et al., 2009).

Neurons and astrocytes differ in the cholesterol synthesis pathways, neurons mainly use the Kandutsch–Russell pathway, while astrocytes use the block pathway (Petrov et al., 2016). Our data show up-regulation of genes participating in cholesterol biosynthesis in neurons and astrocytes. For instance, the gene Nsdhl participates in the Kandutsch–Russell pathway in neurons and the gene Iss participates in the block pathway in astrocytes. However, most of the differentially expressed genes participate in both pathways, suggesting that increased cholesterol biosynthesis may occur in neurons and astrocytes in both subiculum areas.

Furthermore, RNAseq data showed up-regulation of the Gfap gene (Figure 4f), and we also found an increase in its immunolabeling in dSub and vSub after preconditioning (Figure 3). The quantification of GFAP-labeled area indicates an increase of GFAP expression in preconditioning compared to control in dSub and vSub. Reactive astrogliosis is characterized by hypertrophy and increase of astrocytes proliferation in injured regions, resulting in protection from new insults (Stevens, 2008), increased neuronal survival (Barreto et al., 2011), and synaptic reorganization (Barker & Ullian, 2010). Therefore, Gfap gene up-regulation in dSub and vSub highlights that preconditioning induces astrogliosis in both regions, but mainly in vSub as shown by immunolabeling (Figure 3c,d) and Gfap expression in RNAseq data (Figure 4a). The increase in the expression of genes related to cholesterol biosynthesis might indicate an increased membrane production by reactive astrocytes, a possibility also supported by the observation of increased GFAP labeling in the subiculum of preconditioned rats. However, RNAseq data alone are not sufficient to completely support a network reorganization hypothesis, and more experiments should be done to evaluate additional parameters such as altered microglial activity or interneuronal function.

The axon guidance gene ontology includes many genes that are essential for the correct function of synapses, influencing positively or negatively the development and remodeling of these neuronal structures (Stoeckli, 2018). Semaphorins, netrins, slits, repulsive guidance molecules, and ephrins are proteins expressed throughout axons (Kolodkin & Tessier-Lavigne, 2011; Pasterkamp & Kolodkin, 2013). Our data show the Sema3f, Pknc1, and Slt2 genes down-regulated (Table 1), and up-regulation of Sema5a (Table S1) in vSub after preconditioning. The Sema3f gene codes semaphorin 3F and 5A respectively.

The axon guidance process is necessary to establish functional neuronal connections during brain development, however, axon guidance molecules also expressed in the adult brain contribute to functional and structural synaptic plasticity (Glasgow et al., 2021). During the postnatal development, molecules involved in axon guidance that are expressed in excitatory and inhibitory synapses play a role in refining neuronal circuits, establishing an excitatory-inhibitory balance. For example, semaphorins 3F and 5A act as chemorepulsive signals by binding to the plexin–neuropilin receptor complex, activating signaling cascades depending on focal adhesion kinase (de Castro et al., 1999; Giger et al., 2000; Huber et al., 2005; Kolodkin et al., 1993; Ng et al., 2013; Walz et al., 2007). Semaphorin 3F has been shown to inhibit neurite outgrowth and collapse several populations of hippocampal axons (de Castro et al., 1999; Chédotal et al., 1998). Semaphorin 3F mRNA is abundantly expressed in adult granule cells of the dentate gyrus and pyramidal neurons of CA1 and CA3 regions (Bagri et al., 2003), modulating hippocampal basal synaptic transmission (Carulli et al., 2021). Semaphorin 5A also plays an inhibitory role on neurite outgrown (Oster et al., 2003), and in excitatory synapses on both developmentally born and adult-born granule cells of the dentate gyrus (Duan et al., 2014).

Ephrins are surface ligands that bind to Eph receptors, resulting in intracellular signaling promoting growth cone collapse and axon repulsion (Oster et al., 2003; Wegmeyer et al., 2007). The B-subfamily ephrins signaling mechanism involves activation of the kinase domain and phosphorylation of the cytoplasmic tail of ephrin-Bs (Hruska & Dalva, 2012). Studies have demonstrated the regulation of AMPA receptors at synapses in ephrinB3 expressing hippocampal neurons (Antion et al., 2010; Aoto et al., 2007; McClelland et al., 2010). The study performed by Aoto et al. (2007) showed that postsynaptic ephrinB3 expression promoted the formation of glutamatergic synapses on the shafts, and ephrinB3 knockout mouse resulted in reduced glutamatergic synapses abundance in the CA1 region. Although our data did not find differentially expressed AMPA receptors genes, the down-regulation genes associated with synapse dynamics and up-regulation of semaphorin 5A suggest that signaling for glutamatergic synapses pruning in the ventral subiculum might be taking place after preconditioning.

We also found an increase in glycolysis metabolism in the dSub and vSub. Glucose is the primary energy source for the nervous system (Jha & Morrison, 2018) being extremely important in the neurotransmission processes. The increased supply of glucose is used for...
excitatory and inhibitory neurons. Astrocytes take up glucose from the blood capillaries by glucose transporters (GLUTs), and the glucose is stored as glycogen or metabolized into pyruvate in the glycolysis process. The pyruvate is converted to lactate and it is transported to neurons and converted into pyruvate in mitochondria for aerobic energy (Jha & Morrison, 2018). Our results indicate up-regulation of Pfk1, Pgaml1, Eno1, and Ldha genes (Table 1). These participate in both astrocytes and neuron glycolysis pathways (Yellen, 2018). Thus, suggesting increased energy production might occur in both cells as a possible consequence of the preconditioning stimulation in the dorsal and ventral subiculum.

Moreover, we observed a down-regulation of Tp53bp2 gene (Figure 4g) in the ventral subiculum. The gene Tp53bp2 encodes the apoptosis stimulating protein of p53–2 (ASPP2) (Iwabuchi et al., 1994; Samuels-Lev et al., 2001; Takahashi et al., 2004) enhancing damage-induced apoptosis (Lopez et al., 2000; Yang et al., 1999) through the stimulation of p53 transactivation of proapoptotic target genes (Chen et al., 2005). Once the p53 protein is activated, it may induce hypoxic–ischemic and excitotoxic neuronal death (Crumrine et al., 1994). Thus, this gene was associated with proapoptotic functions in neurons, suggesting that its down-regulation might induce a neuroprotective environment in the ventral subiculum, which corroborates with the hypothesis of increased GABAergic transmission in this region. The CA3 transcriptome profile of preconditioning animals treated with systemic kainic acid presented down-regulation of genes associated with calcium signaling, ion channels, excitatory neurotransmitters receptors (Jimenez-Mateos et al., 2008), ubiquitous metabolism, apoptosis, and post-translational modifications (Borges et al., 2007). These results suggest the preconditioning neuroprotective role.

Analyzing the genes associated with cardiac and muscle contraction in dSub after the preconditioning, we observed down-regulated genes of arrhythmogenic right ventricular cardiomyopathy, muscle contraction, and hypertrophic cardiomyopathy pathways (Figure 2e). The gene Cacng3, a calcium voltage-dependent channel subunit 3, was present in these pathways. The calcium voltage-gated channels are important to modulate the entry of Ca2+ in excitable cells by G-protein and second messenger regulation (Dolphin, 2018). According to UNIPROT, the biological processes of the Cacng3 gene (voltage-dependent calcium channel gamma-3 subunit) are associated with the regulation of AMPA glutamate receptors. The studies performed by (Dolphin, 2018; Everett et al., 2007) demonstrated that expression of the Cacng3 gene on the brain was associated with susceptibility of patients to develop childhood absence epilepsy (CAE). Therefore, we suggest that the Cacng3 channel might participate in AMPA glutamate receptors function modulation by reducing the influx of calcium in cells in response to glutamate. The down-regulation of Cacng3 gene associated with the down-regulation of Glu2, might result in reduced calcium influx in neurons from the dorsal subiculum region.

RNA-Seq from the present study also provided data concerning molecular mechanisms specific to each subiculum subregion after preconditioning stimulation. Overall, gene expression changes were more prominent in the ventral subiculum and, as expected, unique alterations in gene ontology pathways were more frequent in this subregion. For instance, gene expression data in the ventral subiculum show increased expression of genes associated with the glycolysis metabolism pathway (Figure 2c), down-regulation of genes associated with axon guidance (Figure 2f), and Tp53bp2 proapoptotic gene down-regulation (Figure 4g).

In conclusion, these findings suggest preconditioning by PP electrical stimulations might induce synaptic reorganization, complement system activation, possibly decreased glutamatergic transmission, and possibly increased GABA transmission in subiculum areas. In the ventral subiculum, the down-regulation of proapoptotic genes and axon guidance indicates that preconditioning induces a neuroprotective environment in this region. Although preconditioning induced similar altered mechanisms in both subiculum areas, changes were more noticeable in the ventral subiculum and further studies would be necessary to further explore such region-specific effects. Moreover, the results indicate that changes in the gene expression levels in the subiculum induced by preconditioning by PP electrical stimulation may correlate to plastic changes that may play a role in regulating hippocampal electrical output during seizures. However, RNAseq data alone are not sufficient to completely support a network reorganization hypothesis, and more experiments should be done to evaluate additional parameters. Nevertheless, the data generated by this study point possible directions for further explorations.

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CONFLICT OF INTEREST
None of the authors has any conflict of interest.

AUTHORS’ CONTRIBUTIONS
Beatriz B. Aoyama contributed to conceptualization, methodology, investigation, validation, visualization, formal analysis, writing—original draft of the manuscript. Gabriel G. Zanetti carried out the investigation. Elayne V. Dias contributed to conceptualization, methodology, formal analysis, writing—original draft, and supervision. Maria C. P. Athié contributed to conceptualization, formal analysis, writing—original draft of the manuscript. Iscia Lopes-Cendes carried out conceptualization, formal analysis, and writing—original Draft. André S. Vieira carried out conceptualization, methodology, resources, project administration, funding acquisition, supervision, and writing—original draft of the manuscript.

DATA AVAILABILITY STATEMENT
The raw data, transcriptome data, and its analyzes are available upon request to the corresponding author. Gene count tables and raw files (fastq) for all samples are available at GEO (GSE178409).
Dolphins, A. C. (2018). Voltage-gated calcium channels: Their discovery, function and importance as drug targets. *Brain and Neuroscience Advances* 2(October). https://doi.org/10.1177/2398212818794805.

Duan, Y., Wang, S. H., Song, J., Mironova, Y., Ming, G. L., Kolodkin, A. L., & Giger, R. J. (2014). Semaphorin 5A inhibits synapticogenesis in early postnatal- and adult-born hippocampal dentate granule cells. *eLife*, 3(October). https://doi.org/10.7554/eLife.04390.

Everett, K. V., Chioza, B., Aicardi, J., Aschauer, H., Brouwer, O., Callenbach, P., Covais, A., Dulac, O., Eeg-Olofsson, O., Feucht, M., & Friis, M. (2007). Linkage and association analysis of CACNG3 in childhood absence epilepsy. *European Journal of Human Genetics: EHJG*, 15(4), 463–472.

Fuster, L., Zhou, L., Bülow, A., Huber, C., Von Lossow, R., Prange-Kiel, J., Jarry, H., & Rune, G. M. (2009). Cholesterol-promoted synapticogenesis requires the conversion of cholesterol to estradiol in the hippocampus. *Hippocampus*, 19(8), 692–705.

Giger, R. J., Cloutier, J. F., Sahay, A., Prinjha, R. K., Levengood, D. V., Moore, S. E., Pickering, S., Simmons, D., Rastan, S., Walsh, F. S., & Kolodkin, A. L. (2000). Neurexin-2 is required in vivo for selective axon guidance responses to secreted Semaphorins. *Neuron*, 25(1), 29–41.

Glasgow, S. D., Ruthazer, E. S., & Kennedy, T. E. (2021). Guiding synaptic plasticity: Novel roles for Netrin-1 in synaptic plasticity and memory formation in the adult brain. *The Journal of Physiology*, 599(2), 493–505.

Goritz, C., Mauch, D. H., & Pfrieger, F. W. (2005). Multiple mechanisms mediate cholesterol-induced synapticogenesis in a CNS neuron. *Molecular and Cellular Neuroscience*. https://doi.org/10.1016/j.mcn.2005.02.006.

Hruska, M., & Dalva, M. B. (2012). Ephrin regulation of synapse formation, function and plasticity. *Molecular and Cellular Neurosciences*, 50(1), 35–44.

Hu, J. Y., & Smith, S. J. (2004). Neural activity and the dynamics of central nervous system development. *Nature Neuroscience*. https://doi.org/10.1038/nn1218.

Huber, A. B., Kania, A., Tran, T. S., Gu, C., Garcia, N. D., Lieberam, I., Johnson, D., Jessell, T. M., Ginty, D. D., & Kolodkin, A. L. (2005). Distinct roles for secreted Semaphorin signaling in spinal motor axon guidance. *Neuron*, 48(6), 949–964.

Iwabuchi, K., Bartel, P. L., Li, B., Marraccino, R., & Fields, S. (1994). Two cellular proteins that bind to wild-type but not mutant p53. *Proceedings of the National Academy of Sciences of the United States of America*, 91(13), 6098–6102.

Janeway, C. A., Jr., Travers, P., Walport, M., & Shlomchik, M. J. (2001). The complement system and innate immunity. *Garland Science*.

Jha, M. K., & Morrison, B. M. (2018). Glia-neuron energy metabolism in neuronal crosstalk: Implications for neuroprotection from brain injury. *Frontiers in Synaptic Neuroscience*.

Kang, T. C., Kim, H. S., Seo, M. O., Park, S. K., Kwon, H. Y., Kang, J. H., & Won, M. H. (2001). The changes in the expressions of γ-aminobutyric acid transporters in the gerbil hippocampal complex following spontaneous seizures. *Neuroscience Letters*. https://doi.org/10.1016/s0304-3908(01)02088-2.

Kelly, M. E., & DC, M. I. (1994). Hippocampal kindling protects several structures from the neuronal damage resulting from Kainic acid-
induced status epilepticus. Brain Research. https://doi.org/10.1016/0006-8993(94)91927-5

Kirino, T. (2002). Ischemic tolerance. Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism, 22(11), 1283–1296.

Knopp, A., Kivi, A., Wozny, C., Heinemann, U., & Behr, J. (2005). Cellular and network properties of the subiculum in the pilocarpine model of temporal lobe epilepsy. The Journal of Comparative Neurology, 483(4), 476–488.

Kolodkin, A. L., Matthes, D. J., & Goodman, C. S. (1993). The Semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. Cell, 75(7), 1389–1399.

Kolodkin, A. L., & Tessier-Lavigne, M. (2011). Mechanisms and molecules of neuronal wiring: A primer. Cold Spring Harbor Perspectives in Biology. https://doi.org/10.1101/cshperspect.a001727

Kvamme, E. (2016). Glutamate and glutamate receptors: Volume I. CRC Press.

Lopez, C. D., Ao, Y., Rohde, L. H., Perez, T. D., O’Connor, D. J., Lu, X., Ford, J. M., & Naumovski, L. (2000). Proapoptotic p53-interacting protein 33Bp2 is induced by UV irradiation but suppressed by p53. Molecular and Cellular Biology, 20(21), 8018–8025.

Lowry, C. A. (2002). Functional subsets of serotonergic Neurones: Implications for control of the hypothalamic-parietal-adelenal Axis. Journal of Neuroendocrinology, 14(11), 911–923.

Mao, L., Takamiya, K., Thomas, G., Lin, D. T., & Huganir, R. L. (2010). GRIP1 and 2 regulate activity-dependent AMPA receptor recycling via exocyst complex interactions. Proceedings of the National Academy of Sciences of the United States of America, 107(44), 19038–19043.

Márquez, J., Campos-Sandoval, J. A., Peñalver, A., Matés, J. M., Segura, J. A., Blanco, E., Alonso, F. J., Rodríguez, F., & de Fonseca, F. R. (2017). Glutamate and brain Glutaminases in drug addiction. Neurochemical Research, 42(3), 846–857.

Mattson, M. P. (2008). Glutamate and neurotrophic factors in neuronal plasticity and disease. Annals of the New York Academy of Sciences, 1144(11), November, 97–112.

McClelland, A. C., Hruska, M., Coenen, A. J., Henkemeyer, M., & Márquez, J., Campos-Sandoval, J. A., Peñalver, A., Matés, J. M., O’Mara, S. (2005). The subiculum: What it does, what it might do, and what neuroanatomy has yet to tell us. Journal of Anatomy. https://doi.org/10.1111/j.1469-7580.2005.00446.x

Ng, T., Ryu, J. R., Sohn, J. H., Tan, T., Song, H., Ming, G.-L., & Goh, E. L. K. (2012). Transcriptional control of Gad2. Transcription, 3(2), 68–72.

Pan, Z. Z. (2012). Transcriptional control of Gad2. Transcription, 3(2), 68–72.

Pasterkamp, R., & Kolodkin, A. L. (2013). SnapShot: Axon guidance. Cell. https://doi.org/10.1016/j.cell.2013.03.031

Stenzel-Poore, M. P., Stevens, S. L., Xiong, Z., Lessov, N. S., Harrington, C. A., Mori, M., Mellor, R., Rosenzweig, H. L., Tobar, E., Shaw, T. E., & Chu, X. (2014). Altered cognitive performance and synaptic function in the hippocampus of mice lacking C3. Experimental Neurology, 253(March), 154–164.

Petrov, A. M., Kasimov, M. R., & Zefirov, A. L. (2016). Brain cholesterol metabolism and its defects: Linkage to neurodegenerative diseases and synaptic dysfunction. Acta Neuroae, 8(1), 58–73.

Pfrieger, F. W. (2003). Cholesterol homeostasis and function in neurons of the central nervous system. Cellular and Molecular Life Sciences. https://doi.org/10.1007/s00018-003-3018-7

Plamondon, H., Blondeau, N., Heurteaux, C., & Lazdunski, M. (1999). Mutually protective actions of Kainic acid epileptic preconditioning and sublethal global ischemia on hippocampal neuronal death: Influence of adenosine A1 receptors and KATP channels. Journal of Cerebral Blood Flow & Metabolism. https://doi.org/10.1097/00004679-199912000-00002

Pohle, W., & Rauca, C. (1994). Hypoxia protects against the neurotoxicity of Kainic acid. Brain Research. https://doi.org/10.1016/0006-8993(94)91693-4

Russell, D. W., Halford, R. W., Ramirez, D. M. O., Shah, R., & Kotti, T. (2009). Cholesterol 24-hydroxylase: An enzyme of cholesterol turnover in the brain. Annual Review of Biochemistry, 78, 1017–1040.

Salter, E. W., Lei, G., Choi, S. L., Ralph, L. T., Zhang, L., Jin, F., Kadia, A., Wang, J., Georgiou, J., & Collingridge, G. L. (2020). Collingridge. Complement C3-dependent glutamatergic synapse elimination in the developing hippocampus is region- and synapse-specific. bioRxiv, 2020.05.20.106930. https://doi.org/10.1101/2020.05.20.106930

Samuels-Lev, Y., O’Connor, D. J., Bergamaschi, D., Trigiante, G., Hsieh, J. K., Zhong, S., Campagne, I., Naumovski, L., Crook, T., & Lu, X. (2001). ASPP proteins specifically stimulate the apoptotic function of p53. Molecular Cellular, 8(4), 781–794.

Sharp, P. E., & Green, C. (1994a). Spatial correlates of firing patterns of single cells in the subiculum of the freely moving rat. The Journal of Neuroscience. https://doi.org/10.1523/jneurosci.14-04-02339.1994

Sharp, P. E., & Green, C. (1994b). Spatial correlates of firing patterns of single cells in the subiculum of the freely moving rat. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 14(4), 2339–2356.

Somjen, G. G. (1995). Synaptic plasticity. Molecular, cellular and functional aspects. Electroencephalography and Clinical Neurophysiology. https://doi.org/10.1016/1052-8040(95)77349-9

Stafstrom, C. E. (2005). The role of the subiculum in epilepsy and Epileptogenesis. Epilepsy Currrents. https://doi.org/10.1535/jnepsci.11.535-7511.2005.00049.x

Wegmeyer, H., Egea, J., Rabe, N., Gezelius, H., Filosso, A., Enjin, A., Varroneaux, F., Deininger, K., Schnüttgen, F., Brose, N., & Klein, R. (2003). Effect of Ischaemic preconditioning on genomic response to cerebral Ischaemia: Similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. The Lancet, 362(9389), 1028–1037.

Stephan, A. H., Barres, B. A., & Stevens, B. (2012). The complement system: An unexpected role in synaptic pruning during development and disease. Annual Review of Neuroscience, 35, 369–389.

Stevens, B. (2008). Neuron-astrocyte signaling in the development and plasticity of neural circuits. Neuro-Signals, 16(4), 278–288.

Stoeckli, E. T. (2018). Understanding axon guidance: Are we nearly there yet? Development, 145(10), https://doi.org/10.1242/dev.151415

Suzuki, S., Tanaka, T., Poyurovsky, M. V., Nagano, H., Mayama, T., Ohkubo, S., Lokshin, M., Hosokawa, H., Nakayama, T., Suzuki, Y., & Sugano, S. (2010). Phosphate-activated Glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen...
species. *Proceedings of the National Academy of Sciences of the United States of America*, 107(16), 7461–7466.

Takahashi, N., Shinya Kobayashi, X., Jiang, K. K., Imai, K., Hibi, Y., & Okamoto, T. (2004). Expression of 53BP2 and ASPP2 proteins from TP53BP2 gene by alternative splicing. *Biochemical and Biophysical Research Communications*, 315(2), 434–438.

Walz, A., Feinstein, P., Khan, M., & Mombaerts, P. (2007). Axonal wiring of guanylate cyclase-D-expressing olfactory neurons is dependent on Neuropilin 2 and Semaphorin 3F. *Development*, 134(22), 4063–4072.

Wegmeyer, H., Egea, J., Rabe, N., Gezelius, H., Filosa, A., Enjin, A., Varoqueaux, F., Deininger, K., Schnütgen, F., Brose, N., & Klein, R. (2007). EphA4-dependent axon guidance is mediated by the RacGAP α2-Chimaerin. *Neuron*. [https://doi.org/10.1016/j.neuron.2007.07.038](https://doi.org/10.1016/j.neuron.2007.07.038)

Witter, M. P., & Groenewegen, H. J. (1990). The subiculum: Cytoarchitectonically a simple structure, but Hodologically complex. *Progress in Brain Research*, 83, 47–58.

Witter, M. P. (2007). The perforant path: projections from the entorhinal cortex to the dentate gyrus. *Progress in Brain Research*, 163, 43–61. [https://doi.org/10.1016/S0079-6123(07)63003-9](https://doi.org/10.1016/S0079-6123(07)63003-9).

Witter, M. P., & Moser, E. I. (2006). Spatial representation and the architecture of the entorhinal cortex. *Trends in Neurosciences*, 29(12), 671–678.

Wyszynski, M., Valtschanoff, J. G., Naisbitt, S., Dunah, A. W., Kim, E., Standaert, D. G., Weinberg, R., & Sheng, M. (1999). Association of AMPA receptors with a subset of glutamate receptor-interacting protein in vivo. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 19(15), 6528–6537.

Yang, J. P., Hori, M., Takahashi, N., Kawabe, T., Kato, H., & Okamoto, T. (1999). NF-kappaB subunit p65 binds to 53BP2 and inhibits cell death induced by 53BP2. *Oncogene*, 18(37), 5177–5186.

Yellen, G. (2018). Fueling thought: Management of glycolysis and oxidative phosphorylation in neuronal metabolism. *The Journal of Cell Biology*, 217(7), 2235–2246.

Yu, G., Wang, L., Han, Y., & He, Q. (2012). clusterProfiler: An R package for comparing biological themes among gene clusters. *OMICS: A Journal of Integrative Biology*, 16(5), 284–287.

Zhao, C., & Gammie, S. C. (2014). Glutamate, GABA, and glutamine are synchronously upregulated in the mouse lateral septum during the postpartum period. *Brain Research*, 1591(December), 53–62.

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