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ORIGINAL RESEARCH

Tissue and Cerebral Biofluid Remodelling of tRNA-Derived Small RNA Signatures in Experimental and Human Epilepsy

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Abstract

Small noncoding RNAs are increasingly recognized as important regulators of gene expression in brain diseases including epilepsy. tRNA-derived small RNAs (tsRNAs) including tRNA fragments (tRFs) and tRNA halves (tiRNAs) are emerging as stable and regulated RNA species which respond to cellular stress and accumulate in disease states. Using experimental temporal lobe epilepsy (TLE) models and human TLE samples, we here determine whether tsRNAs are localized to specific tissue and biofluid compartments in the brain and how they are altered in epilepsy. We analysed small RNA sequencing datasets from hippocampal tissue, cerebrospinal fluid (CSF), and hippocampal interstitial fluid over time from rat TLE models, and human CSF from control subjects and TLE patients. Small RNA class composition, tsRNA subclass distributions, parent tRNA origins, and differential tsRNA expression profiles were systematically compared across intracellular and extracellular compartments, experimental epilepsy models, and human TLE samples. All differential expression analyses applied Benjamini-Hochberg correction. This revealed that small RNA composition varied strongly by compartment. Hippocampal tissue was dominated by microRNAs, while CSF and interstitial fluid contained significant proportions of tRNA-derived reads. In chronic epilepsy, hippocampal tissue showed a significant increase in overall tsRNA content and in the proportion of tRNA-derived reads, without a significant shift in tsRNA class composition. The CSF and interstitial fluid of epileptic animals showed fragment-level remodelling of tsRNA profiles, although compositional shifts did not reach significance after correction. The CSF from TLE patients also showed extracellular tsRNA populations with potential disease-associated shifts in small RNA composition. Fragments derived from parent tRNAs GlyGCC and GluCTC were recurrent contributors across compartments, though their prominence was compartment-dependent. Collectively, these findings suggest that tsRNAs represent a prominent, compartment-specific, and disease-associated layer of small RNA remodelling in experimental and human epilepsy. The findings may guide the choice of biofluid source and panel of RNAs for biomarker-based diagnostics or provide targets for new therapeutic approaches in drug-resistant epilepsies.

Keywords Circulating biomarker · Epileptogenesis · Experimental epilepsy · TRNA-derived small RNAs · Pilocarpine model · Perforant pathway stimulation · Extracellular RNA, Hippocampal microdialysis

Abbreviations

CSF	Cerebrospinal fluid
EEG	Electroencephalography
itRF	Internal tRNA-derived fragment
LiCl	Lithium chloride
miRNA	MicroRNA
ncRNAs	Non-coding RNAs
NGS	Next-generation sequencing
PPS	Perforant Pathway Stimulation
rRNA	Ribosomal RNA
SE	Status epilepticus
sncRNAs	Small non-coding RNAs
tiRNA	tRNA-derived stress-induced RNA (tRNA halves)
TLE	Temporal lobe epilepsy
tRF	tRNA-derived fragment

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tsRNA	tRNA-derived small RNA
RNASeq	RNA sequencing

Background

Epilepsy is a chronic neurological disorder characterized by recurrent seizures caused by hyperexcitable neuronal networks (Devinsky et al. 2018; Thijs et al. 2019). A significant proportion of patients fail to achieve adequate seizure control with currently available anti-seizure medicines and diagnosis relies heavily on clinical examination and history with high rates of mis-diagnosis which would benefit from biomarkers with mechanistic links to pathophysiology (Kearney et al. 2019; Henshall and Simonato 2024; Klein et al. 2024). Among the most common forms of focal epilepsy in adults is temporal lobe epilepsy (TLE), which is often associated with drug resistance. The administration of kainic acid, pilocarpine or perforant pathway stimulation in animals faithfully models aspects of the pathophysiology of human TLE and these models have been widely used to determine the cell and molecular processes underlying hippocampal pathology, epileptogenesis, and the establishment of chronic drug resistant epilepsy (Curia et al. 2008; Lévesque et al. 2016; Bumanglag and Sloviter 2018).

In the nervous system, small noncoding RNAs (sncRNAs) have emerged as important regulators of gene expression and protein translation. Most work in this domain has been focused on microRNAs (miRNAs), which regulate mRNA stability and translation. Alterations in levels of various miRNAs have been reported in experimental epilepsy models and in tissue, plasma, and cerebrospinal fluid (CSF) from people with epilepsy, supporting a role in disease-associated molecular changes that may be captured by small RNA profiles (Jimenez-Mateos and Henshall 2013; Henshall et al. 2016; Brennan and Henshall 2020; Wang and Zhao 2021). Functional studies in rodents confirm this, identifying miRNAs that serve to modulate seizure thresholds, seizure-induced injury, neuroinflammation, synaptic remodelling, as well as acting as biomarkers of disease (Brennan and Henshall 2020; Hansen et al. 2023; van Vliet et al. 2024). That said, small RNA sequencing datasets contain many classes of non-miRNA species, and their contribution to the biology of epilepsy remains less well understood (Li et al. 2018; Venø et al. 2020; Brennan et al. 2020; Wajahat et al. 2021; Vangoor et al. 2024; Winek and Soreq 2025).

Among emerging classes of sncRNAs, tRNA-derived small RNAs (tsRNAs) have been attracting increasing attention (Winek and Soreq 2025). Rather than representing random degradation products, tsRNAs are produced as discrete and reproducible molecules with defined structures and expression patterns (Shen et al. 2018; Xie et al. 2020; Kim et al. 2020; Weng et al. 2022). These include short fragments called tRNA-derived fragments (tRFs), internal tRFs (itRFs), and long fragments called tRNA halves or tiRNAs, which are generated by angiogenin-mediated cleavage in the anticodon loop (Shen et al. 2018; Su et al. 2019; Zong et al. 2021; Weng et al. 2022). This stress-associated cleavage of tRNAs has been linked to cellular stress responses, translation repression, and stress granule biology (Shen et al. 2018; Zong et al. 2021).

While still relatively understudied in epilepsy, several lines of evidence make tsRNAs biologically plausible candidates for further investigation (Magee et al. 2019; Zhang et al. 2023; Winek and Soreq 2025). Epileptic tissue is exposed to neuronal hyperexcitability, metabolic and oxidative stress, and inflammatory signalling, all of which could influence tsRNA generation (Shen et al. 2018; Liu et al. 2021; Weng et al. 2022; Winek and Soreq 2025). We previously reported that 5'tRFs change in plasma with respect to seizure state in human epilepsy and in a pilocarpine model of TLE, suggesting that specific tsRNAs can be detected in clinically accessible biofluids and may indicate disease states (Hogg et al. 2019; Soukupova et al. 2026).

tsRNAs have been shown to be released from neurons (Hogg et al. 2019, 2020; Mesquita-Ribeiro et al. 2021). However, how tsRNAs are distributed in vivo across brain tissue, interstitial/extracellular fluid, and CSF, if these distributions are altered in epilepsy, and whether specific parent tRNAs and fragment classes recur across different compartments, remain unclear.

In this work, we determined the tsRNA profiles in experimental models of TLE across hippocampal tissue, interstitial fluid and CSF, as well as in human CSF from TLE patients. The findings provide clarity on links between epileptogenic tissue and tsRNA formation and may be useful in the design of fluid-based biomarkers or therapeutic targeting of the system.

Methods

Data Overview and Study Design

In this study, we aimed to extensively characterize the small noncoding RNAs (ncRNAs) landscape, particularly focusing on tRNA-derived small RNAs (tsRNAs), across different biological compartments and experimental models of epilepsy. This study consists of three major analytical components. The first component examines sncRNA profiles in a pilocarpine model of epilepsy, focusing on rat hippocampal tissue and cerebrospinal fluid (CSF) to analyse both intracellular and extracellular alterations in chronic epilepsy conditions. The second component extends the analysis of

extracellular alteration to interstitial fluid utilizing microdialysis data from a perforant pathway stimulation (PPS) rat model. The third component analyses human CSF samples from TLE patients, which enables cross-species comparisons and translational interpretation.

A consistent bioinformatics approach was adopted to analyse all datasets to enable comparable interpretation across species, models, and compartments.

Datasets and Sample Description

In this study, four small RNA Sequencing datasets were included, spanning in-house generated and publicly available data. All datasets from animals correspond to chronic stages of epilepsy, consistent with the original study designs.

Rat Hippocampal Tissue – Chronic Pilocarpine Epilepsy

Hippocampal tissue from adult rats subjected to pilocarpine-induced status epilepticus (SE) and corresponding controls were obtained at the chronic phase (approximately 3 months post SE). The dataset included control ($n=10$) and pilocarpine-exposed ($n=10$) animals. A lithium-pilocarpine protocol was used to induce status epilepticus as previously described (Baloun et al. 2020). In short, 24 h prior to administering pilocarpine, animals received lithium chloride (LiCl; 127 mg/kg, intraperitoneal). A single intraperitoneal injection of pilocarpine (45 mg/kg in adult rats) was used to induce status epilepticus, while control animals were treated with saline. Seizure activity was monitored behaviourally. Status epilepticus was defined by the onset of sustained motor seizures characterized by forelimb clonus. Approximately 2 h after onset, seizures were terminated, to reduce mortality. Animals that exhibited sustained convulsive seizures were included. Subsequently, animals were monitored for spontaneous seizure activity. Hippocampal tissue was collected at the chronic stage of epilepsy which was approximately 3 months post status epilepticus. The sequencing data for this dataset can be found in the GEO database (accession number GSE124332). This dataset was part of a previously published investigation, and for more details, see the original study (Baloun et al. 2020).

Rat Cerebrospinal Fluid – Early Chronic Pilocarpine Epilepsy

CSF samples were obtained from rats ($n=3$) at baseline (naïve/pre-SE) and again 14 days after pilocarpine-induced SE (as above, see 2.1; $n=3$). To evaluate changes in tsRNAs, 100 µL of CSF were collected as previously described (Soukupová et al. 2023). Small RNA sequencing was subsequently performed on 20–50 µL of the matched samples from each individual animal.

Rat Hippocampal Interstitial Fluid – Chronic PPS Model

Samples from extracellular interstitial fluid (IF) were obtained via microdialysis from rat hippocampus in a rat model of chronic mesial temporal lobe epilepsy with hippocampal sclerosis induced by perforant pathway stimulation (PPS). At ~45 days post-stimulation, samples were collected. The dataset included ($n=6$) sham controls and PPS-exposed animals ($n=7$). Perforant pathway stimulation and microdialysis were performed as described previously (Siebenbrodt et al. 2022). Animals were surgically implanted with stimulation electrodes targeting perforant pathway and recording electrodes in the hippocampus. The delivery of stimulation was in accordance with established paradigms leading to the development of epileptiform activity and spontaneous seizures. An additional guide cannula for microdialysis probes was implanted above the right hippocampus to perform repetitive intrahippocampal microdialysis to collect interstitial cellular fluid at defined timepoints. Intracranial electrodes and a subcutaneous EEG transmitter allowed continuous EEG-monitoring. Animals were maintained until the chronic phase (~45 days post-stimulation, 30 days after the first spontaneous seizure), at which point samples were collected.

Human Cerebrospinal Fluid – Temporal Lobe Epilepsy

CSF samples were obtained from two sites, including University Hospital Magdeburg and the University Hospital Erlangen (Table 1). The control group ($n=10$; mean age 55.2 ± 13.5 years; 5 M/5F) comprised individuals without a confirmed diagnosis of epilepsy or primary neurological disease. Control donors had a range of non-epileptic conditions, including sensory symptoms ($n=5$), systemic or oncological comorbidities ($n=2$), and neurological or neurovascular comorbidities ($n=3$). This heterogeneity should be considered when interpreting group comparisons. The TLE group ($n=10$; mean age 50.0 ± 15.5 years; 5 M/5F) consisted of patients with a confirmed diagnosis of TLE, including cryptogenic TLE ($n=3$), focal TLE of other or undefined aetiology ($n=4$), hippocampal sclerosis ($n=1$), tumour-associated TLE ($n=1$; DNET/ganglioglioma differential diagnosis), and one case with undefined aetiology. Lateralisation was documented in four TLE patients (three right, one left). Patients were diagnosed with TLE by the referring clinical teams at each site according to standard clinical criteria, including seizure semiology, EEG, and neuroimaging findings, as described in (Raoof et al. 2017).

Table 1 Clinical characteristics of the human CSF cohort

Characteristic	Control CSF donors ($n=10$)	TLE patients ($n=10$)
Age, years (mean ± SD)	55.2 ± 13.5	50.0 ± 15.5
Sex (M/F)	5/5	5/5
Sample origin (Magdeburg/Erlangen)	5/5	5/5
Clinical diagnosis		
Paraesthesia/sensory symptoms	5	–
Systemic/oncological comorbidity	2	–
Neurological/neurovascular comorbidity	3	–
Cryptogenic TLE	–	3
Focal TLE (other/undefined aetiology)	–	4
Hippocampal sclerosis	–	1
Tumour-associated TLE (DNET/ganglioglioma DD)	–	1
Unknown/not specified	–	1
Lateralisation (TLE patients)		
Right	–	3
Left	–	1
Not specified	–	6

Age SD calculated from individual values

Controls were selected based on no confirmed diagnosis of epilepsy or primary neurological disease; comorbidities are summarized by category

Lateralization was recorded only where explicitly documented in the source data

Small RNA Sequencing

Rat Hippocampal Tissue (Pilocarpine model): The original study (Baloun et al. 2020) describes the protocols for tissue collection, extraction of RNA, library preparation, and sequencing in detail. In brief, according to manufacturer's protocol, TRI Reagent® was used to isolate total RNA from frozen hippocampal tissue. Quantity and quality of RNA were assessed using NanoDrop and Qubit fluorometric assays. Small RNA libraries were prepared using the NEXTflex Small RNASeq Kit (Bio Scientific) as per manufacturer's instructions. Libraries were then size-selected, pooled, and sequenced on the Illumina NextSeq 500 platform. Rat CSF (Pilocarpine model): Total RNA was isolated from 25 to 50 µL CSF samples using miRNeasy Serum/Plasma Advanced Kit (Qiagen), with miND spike-in controls (TAmiRNA) during extraction. To maximize concentration, RNA elution volume was 12 µL. Small RNA libraries were prepared using QIAseq miRNA Library Kit (Qiagen) using bead-based library size selection that allows inclusion of tiRNAs and finally sequenced on a Novaseq X plus Illumina sequencer. All animals in this dataset were male and were randomly assigned to the control or pilocarpine groups. Rat Hippocampal Interstitial Fluid (PPS model): Microdialysis samples were analysed using next generation sequencing (NGS). NGS was performed by TAmiRNA (Vienna, Austria). RNA isolation was performed from 50 µL of microdialysate using the miRNeasy Mini Kit with glycogen and enhanced washing. RNA-Spike-in controls were added at equimolar amounts to all samples prior to the extraction. 8.5 µL total RNA and 1 µL NGS spike-ins were used for library preparation using the RealSeq Biofluids Kit. Sequencing was performed on Illumina NextSeq 550 in high output mode. All animals in this dataset were male. Human CSF (TLE Cohort): Total RNA was extracted from 350 µL CSF samples of TLE patients using miRNeasy Serum/Plasma Advanced Kit (Qiagen) using an elution volume of 12 µL. Library preparation was performed using QIAseq miRNA Library Kit (Qiagen) using bead-based library size selection that allows inclusion of tiRNAs and subsequently sequenced on a Novaseq X plus Illumina sequencer.

Small RNA Sequencing Analysis

Generation of Count Matrices

tsRNAsearch, a publicly available and validated Nextflow-based pipeline developed by our team, was used to process raw small RNA sequencing data from all datasets to identify and quantify tRNA-derived small RNAs (Donovan et al. 2021). In brief, standard trimming procedures, embedded in the pipeline, were used to remove sequencing adapters. Reads alignment to a customized noncoding RNA (ncRNA) reference, optimized for accurate mapping of tRNA-derived fragments, was performed, enabling discrimination between different tsRNA classes such as 5' tRNA halves (5' tiRNAs), 5' tRFs, 3' tRNA halves (3' tiRNAs), 3' tRFs, and internal fragments (itRFs), while minimizing false-positives. Subsequently, reads were collapsed to generate summaries at count-level for each tsRNA species across samples. Additionally, nucleotide-level coverage profiles were generated using integrated depth calculation methods, allowing for a detailed assessment of read distribution along parent tRNA sequences. While built-in statistical testing functionality is provided in tsRNAsearch pipeline, we limited its use to preprocessing and quantification steps, exporting count matrices for downstream statistical analysis.

Differential Expression Analysis

Differential expression analysis of tsRNAs was performed using DESeq2 version 1.48.1 (Love et al. 2014) in R version 4.5.1. Count data generated from tsRNAsearch were used as input. The median-of-ratios method implemented in DESeq2 was used for normalization to account for library size differences and compositional biases. Prior to testing, low-count fragments were removed using a single harmonised filter applied identically to all datasets, retaining

fragments with at least 10 counts in at least half the samples of the smaller group. For the rat CSF dataset, in which the same three animals were sampled before and after status epilepticus, a paired design was used with animal included as a blocking factor (~animal + condition) so that each animal served as its own control. Because DESeq2 models counts with a negative-binomial distribution, it does not assume normality of the underlying data. Multiple-testing correction was applied throughout using the Benjamini-Hochberg procedure, and statistical significance was defined as a Benjamini-Hochberg-adjusted p-value < 0.05 together with an absolute log2 fold-change cutoff of $|\log2FC| \geq 0.5$, applied identically across datasets. For the human cohort, a sensitivity analysis was additionally performed in which sex was included as a covariate (~sex + condition) and the results were compared with the primary model. All differential expression results are reported in supplementary information (Supplementary Tables S1-S4).

Data Visualization and Statistical Analysis

All downstream analyses and visualizations were performed in R, primarily using the tidyverse version 2.0.0 and ggplot2 version 4.0.2 packages. Small RNA class composition across conditions was visualized using pie charts, illustrating relative contributions of major sncRNA classes to the total read pool. Group differences in small-RNA class, parent-tRNA and biotype composition were tested using non-parametric tests, including the Mann-Whiteny U test for independent groups (tissue, interstitial fluid and human CSF) and the paired Wilcoxon signed-rank test for the paired rat CSF dataset, with Benjamini-Hochberg correction across the categories compared within each comparison. Individual per-sample composition values were retained and are displayed as data points in the supplementary composition figures (Supplementary Figures S1-S3) and tabulated in Supplementary Table S5. As a secondary computational reanalysis of existing datasets, group assignments followed the source studies; for the rat CSF dataset, library preparation was carried out without knowledge of group assignment (blind to condition). All datasets were processed through the single tsRNAsearch pipeline with identical parameters to minimise processing bias.

tsRNA subclass distributions were plotted using stacked bar plots, where each bar represents an individual sample, and coloured segments indicate the fractional contribution of different tsRNA classes (e.g., 5' tiRNA, 5' tRFs, itRFs, 3' tRFs, 3' tiRNA). Absolute tsRNA read counts were overlaid as line plots on a secondary y-axis. Parent tRNA species contributions were visualized using stacked bar plots showing fractional contributions of reads derived from individual tRNA isoacceptors. Finally, results from differential expression analysis were visualized using volcano plots, displaying log2 fold-change versus statistical significance for individual tsRNA fragments.

Results

Small Non-coding RNA Landscapes in Pilocarpine Model of Chronic Epilepsy

Rat Hippocampal Tissue in Chronic Pilocarpine Epilepsy

We first explored the global landscape of small noncoding RNAs (sncRNAs) in hippocampal tissue from rats that underwent pilocarpine-induced epilepsy. In this model, rats undergo an initial status epilepticus, and spontaneous recurrent seizures emerge over the subsequent weeks. Across experimental groups, miRNA dominated the sncRNA pool, consistent with the well-established abundance of miRNAs in the brain. Specifically, miRNA reads represented the majority of mapped small RNAs in controls (86.8 ± 6.8%), with tRNA-derived reads representing a minority (10.3 ± 6.2%). A notable shift in the relative composition was observed in samples from rats with pilocarpine-induced epilepsy. Although miRNAs remained the dominant sncRNA species, the proportion of tRNA-derived reads was significantly increased (to 16.4 ± 6.6%), with a corresponding decrease in miRNA (to 79.9 ± 6.9%; both Mann-Whitney with Benjamini-Hochberg correction, BH-adjusted $p = 0.028$). Ribosomal RNA did not change significantly, whereas the residual "other" ncRNA fraction showed a small but significant increase (1.9 ± 0.5% to 2.4 ± 0.4%, BH-adjusted $p = 0.028$) (Fig. 1A; Supplementary Figure S3). These findings indicate that chronic epilepsy is associated with an altered relative proportion of specific small RNAs in the hippocampus, with a particular relative augmentation of tRNA-derived species.

We next examined the profile of tRNA-derived small RNAs (tsRNAs) by subclass. 5' tRNA halves (5' tiRNAs) were the most prevalent class in both control and pilocarpine-treated animals (64.1 ± 12.2% and 71.5 ± 2.7%), with internal tRNA fragments (itRFs) the next most abundant (20.6 ± 5.1% and 23.5 ± 3.9%). The total tsRNA content (as reflected by the absolute tRNA read counts) was significantly higher in samples from pilocarpine-treated animals compared to controls, increasing 2.7-fold (50,049 to 136,934 mean reads; Mann-Whitney $p = 0.031$) (Fig. 1B). The 5' tiRNA fraction was nominally higher in epileptic rats than controls, and the 3' tRF fraction nominally lower (14.9% to 4.8%), but neither difference reached significance after correction ($p = 0.141$ and $p = 0.089$, respectively; both BH-adjusted $p = 0.187$). No tsRNA class showed a

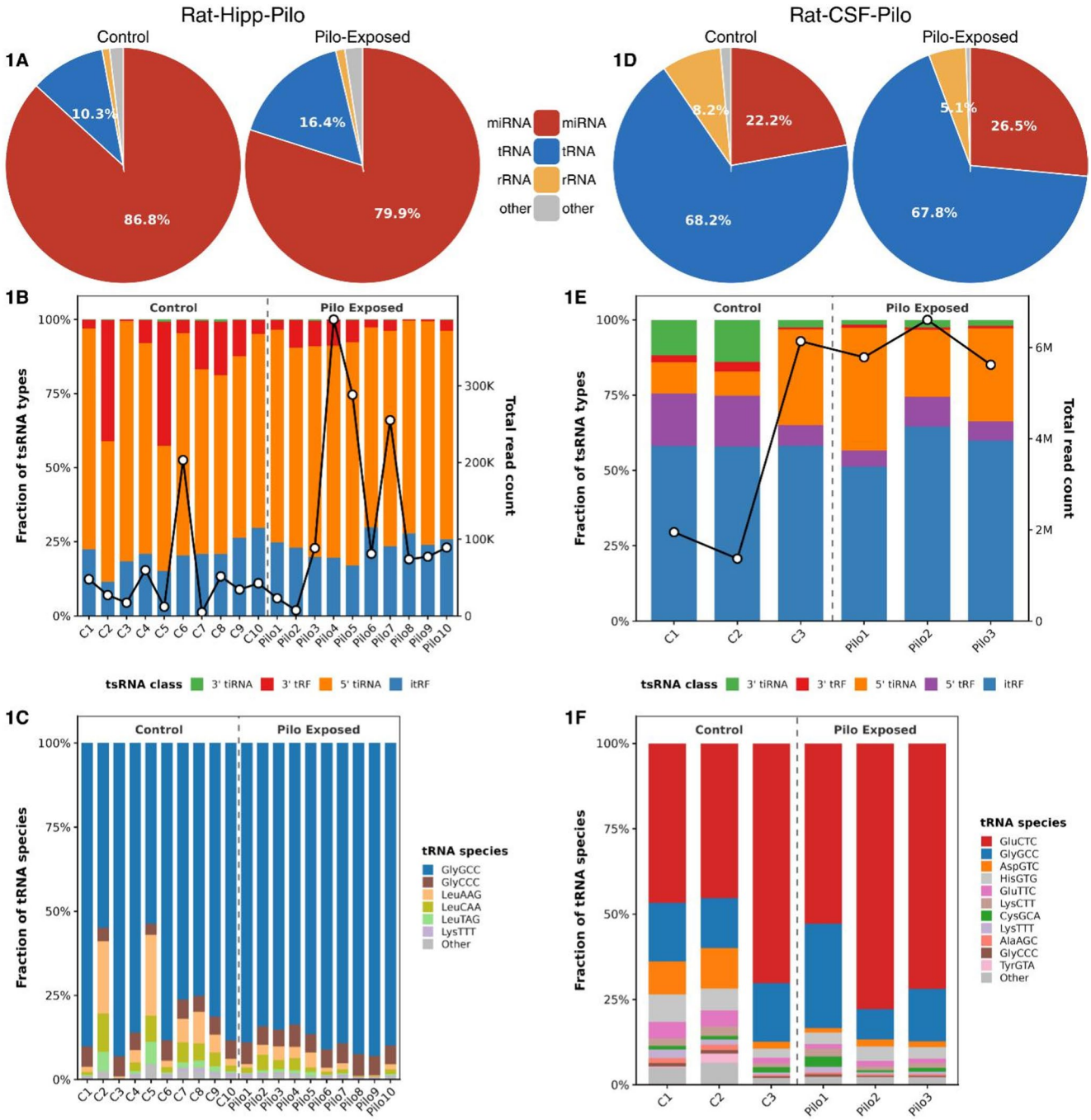


Fig. 1 Small RNA Composition and tsRNA Profiling in Rat Pilocarpine Models (Tissue and CSF). **A** Pie charts of overall small RNA class composition in rat hippocampal tissue (Rat-Hipp-Pilo) for control (n = 10) and pilocarpine-treated animals (n = 10). The proportions of major sncRNA classes, including miRNAs, tRNA-derived reads, and other small RNA categories, are shown. **B** Stacked bar plots of tsRNA subclass composition in Rat-Hipp-Pilo samples, with colours indicating tsRNA types (5' tiRNAs, 5' tRFs, 3' tRFs, internal tRFs, 3' tiRNAs). The overlaid line indicates total absolute tRNA reads per sample (right y-axis). **C** Parent tRNA species contributions to tsRNA reads in Rat-Hipp-Pilo. Bars show fractional contributions of specific tRNA isoacceptors to total tsRNA content per sample. **D** Pie charts of sncRNA classes in rat CSF (Rat-CSF-Pilo) at baseline (naïve state; *n* = 3) and 14 days post-pilocarpine-induced SE (*n* = 3), showing predominance of tRNA fragments. **E** tsRNA subclass composition and total tRNA reads in Rat-CSF-Pilo. **F** Parent tRNA species contributions in Rat-CSF-Pilo tsRNAs

significant compositional shift between groups (all BH-adjusted $p \geq 0.187$) (Supplementary Figure S1).

Reads derived from GlyGCC were most abundant overall following the analysis of tsRNA parent tRNA species. Several leucine isoacceptors and GlyCCC, with lower contributions from other species such as TyrGTA and ThrAGT followed in abundance. GlyGCC-derived tsRNAs were nominally higher in samples from pilocarpine-treated animals than controls, whereas several leucine-derived tsRNAs (e.g., LeuAAG, LeuCAA, LeuTAG) were nominally more prominent in controls (Fig. 1C); however, no parent-tRNA proportion differed significantly between groups after correction (all BH-adjusted $p \geq 0.218$) (Supplementary Figure S2). GlyCCC-derived reads were similar across conditions. The prominence of GlyGCC tsRNAs is in line with previous observations that certain Gly-derived tsRNAs robustly accumulate in stress and disease contexts and may take part in modulating translation or stress responses (Deng et al. 2022; Jirström et al. 2025).

Differential expression analysis (DESeq2 with Benjamini-Hochberg correction; 1,777 fragments tested) identified eight fragments as significantly altered in epileptic tissue (BH-adjusted $p < 0.05$ and $|\log2 \text{ fold change}| \geq 0.5$). All eight were downregulated relative to controls, and all were 3' tRFs, predominantly derived from LeuAAG; no fragment was significantly upregulated (Fig. 2A). This loss of specific 3' tRFs is directionally consistent with the nominal reduction in the 3' tRF fraction noted above, although the class-level shift itself did not reach significance.

Rat Cerebrospinal Fluid in Early Chronic Pilocarpine Epilepsy

To explore the small RNA landscape in the extracellular environment, we profiled small RNAs in CSF samples collected from the same three animals at baseline and during the early phase of epilepsy (14 days post-SE); given the small, paired sample ($n = 3$), this dataset was analysed with a paired design (see Methods) and is treated as exploratory throughout. This time point corresponds to around the time that spontaneous seizures first emerge in the model. Strikingly, the sncRNA composition in CSF was dominated by tRNA-derived sequences, making up 68.2±2.5% of reads at baseline and 67.8±4.4% following pilocarpine-induced SE (Fig. 1D). In contrast, miRNAs made up 22.2% of reads at baseline and 26.5% in the post-pilocarpine early samples; none of these compositional differences was significant (paired Wilcoxon with BH correction, all $p \geq 0.66$). Ribosomal RNAs (rRNA) fragments represented the main additional component and comprised approximately 5–10% of reads in samples. These descriptive patterns suggest a subtle, however coordinated shift in the small RNA composition in the extracellular space following the epileptogenic insult, which was explored further at the tsRNA fragment level below.

We next explored in detail the tRNA species content of rat CSF. Total tRNA read content in CSF samples was markedly higher than observed in tissue, with individual samples reaching as high as ~6 million reads (Fig. 1E).

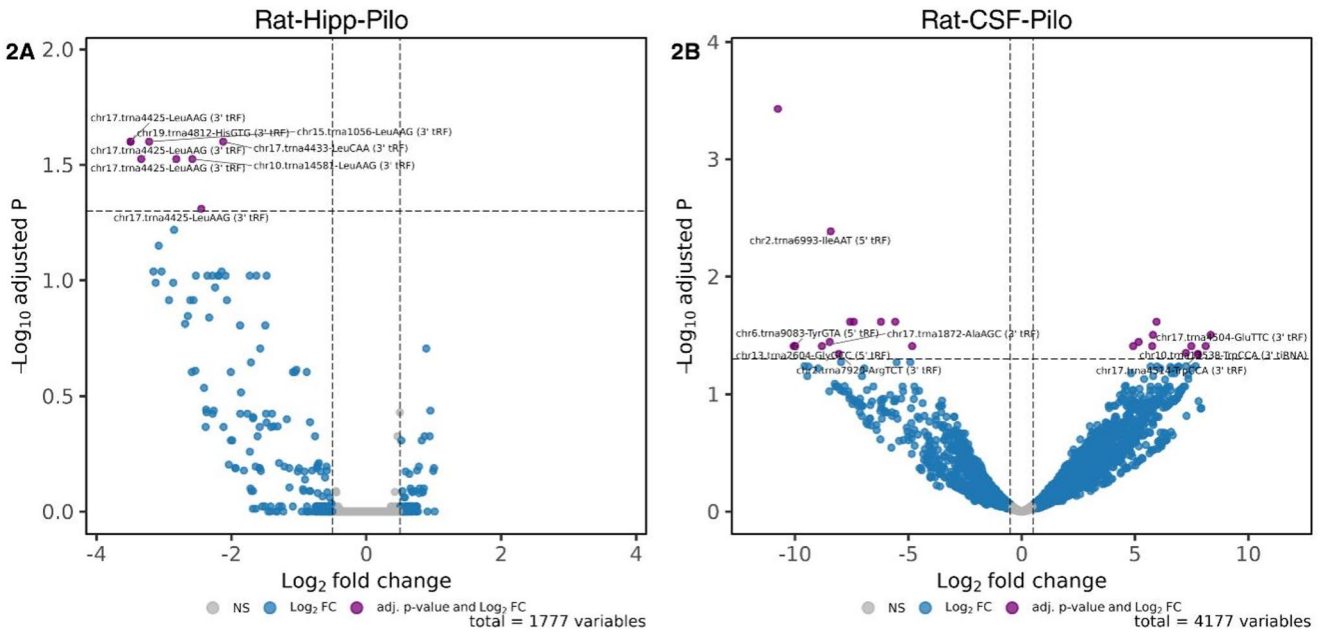


Fig. 2 Differential Expression Analysis of tsRNAs in Rat Pilocarpine Models (Tissue and CSF). **A** Volcano plot of differential tsRNA expression in Rat-Hipp-Pilo (pilocarpine-treated animals, n = 10 vs control, n = 10), highlighting the eight significantly downregulated 3' tRFs (no fragment was significantly upregulated after correction). **B** Volcano plot of differential tsRNA expression in Rat-CSF-Pilo, indicating major shifts in tsRNA profiles between the baseline ($n = 3$) and early chronic phases ($n = 3$)

While the absolute tRNA read content among baseline samples showed some variability, the overall magnitude of the signal remained notably greater than that observed in the hippocampal tissue dataset. This elevation relative to tissue may reflect the high stability and extracellular persistence of tRNA fragments in biofluids (Costa et al. 2023). Across both conditions, internal tRFs (itRFs) represented the most abundant tsRNA class in the matched control and early epilepsy CSF samples. The second most abundant class was 5' tiRNAs, showing a tendency to be higher in the early chronic post-SE state compared to baseline. 5' tRFs and 3' tiRNA, in contrast, were generally more abundant in baseline samples, whereas 3' tRFs comprised only a minor fraction in all samples, regardless of the disease stage.

GluCTC-derived fragments, followed by GlyGCC, with additional contributions from species such as AspGTC, HisGTG, and LysCTT accounted for the largest fractions of CSF tRNA reads, as shown by the parent tRNA species analysis (Fig. 1F). The prominence of GluCTC tsRNAs contrasts with the patterns seen in the hippocampus and indicates extracellular selectivity or differential release mechanisms for specific parent tRNAs.

In the paired analysis, 24 fragments were significantly differentially expressed in the CSF in the early phase of epilepsy relative to baseline (Fig. 2B); 13 were downregulated and 11 upregulated. Downregulated fragments were most often derived from TyrGTA and AspGTC, whereas upregulated fragments included those from HisGTG, GluTTC and TrpCCA, and both sets spanned internal, 5' and 3' classes rather than a single fragment type. In particular, while the most abundant parent tRNA overall was GluCTC, relatively few GluCTC-derived fragments were significantly altered, indicating that abundance and differential regulation are not necessarily coupled. Together, these findings suggest that the transition into chronic epilepsy in the pilocarpine model is characterised by a selective reshaping of extracellular tsRNA profiles, characterised by selective, fragment-level remodelling; given the paired sample of three animals, these fragment-level results are exploratory.

Collectively, our analysis of the sncRNA landscape of the rat brain reveals substantial differences in miRNA and tRNA between the hippocampus and CSF and compartment-specific changes in the pilocarpine model of TLE; in the hippocampus this is a significant increase in overall tsRNA abundance rather than a significant shift in class proportions. In the CSF, we observed a high proportion of tRNA-derived reads dominated by specific parent tRNA (e.g., GluCTC, GlyGCC). Thus, extracellular biofluids inherently carry distinct sncRNA profiles and pilocarpine accentuates these differences, especially by altering tsRNA subclass distributions and selectively modulating specific tsRNA fragments rather than consistently increasing all tRNA-derived species.

Extracellular Small RNA Dynamics in Chronic Epilepsy Models and Human Temporal Lobe Epilepsy

Rat Hippocampal Interstitial Fluid in Chronic PPS Epilepsy

To study extracellular small RNAs in a different biofluid compartment, we analysed hippocampal interstitial fluid obtained via microdialysis. Samples were obtained from sham controls and perforant pathway stimulation-exposed (PPS-exposed) rats at ~45 days post-stimulation, a toxin-free model of chronic TLE (Venø et al. 2020). Consistent with CSF findings, tRNA-derived sequences constituted the largest component of interstitial fluid from control rats (51.3 ± 18.1%; Fig. 3A). Interstitial fluid contained lower amounts of rRNA and miRNAs, with other sncRNAs contributing smaller proportions. In PPS-exposed animals the tRNA-derived fraction was nominally lower (46.8%), with nominal increases in other classes, but no compositional change was significant after correction (all BH-adjusted $p \geq 0.55$).

Within the subset of tsRNA, absolute tRNA fragment counts varied across samples, with a minority of control samples exhibiting particularly high counts. While such variation may partly reflect biological heterogeneity or sampling differences, the overall tsRNA subclass distribution patterns were similar between conditions (Fig. 3B). itRFs and 5' tiRNAs were the most abundant in interstitial fluid, with 5' tRFs and 3' tRFs present at moderate levels. 3' tiRNAs were the least abundant subclass. In general, control samples showed somewhat higher absolute tsRNA counts than PPS-exposed samples. However, the broad subclass landscape in interstitial fluid was not altered.

Multiple isoacceptors including CysGCA, AlaTGC, LysCTT, and GluCTC contributed substantially to the interstitial fluid pool as indicated by the analysis of parent tRNA species (Fig. 3C). Certain species (e.g., CysGCA and AlaTGC) occupied larger fractions overall, followed by LysCTT and GluCTC, showing consistent patterns across conditions, and GlyGCC appeared relatively more prominent in PPS-exposed samples. This landscape suggests both shared and condition-associated features in the sources of interstitial fluid tsRNAs.

Differential expression analysis of PPS-exposed interstitial fluid identified 23 significantly altered fragments, all of which were downregulated; no fragment was significantly upregulated (Fig. 4A). The downregulated set was dominated by 3' tRFs and also spanned internal, 5' tRF and 3' tiRNA classes, most often derived from ArgCCT, TyrGTA

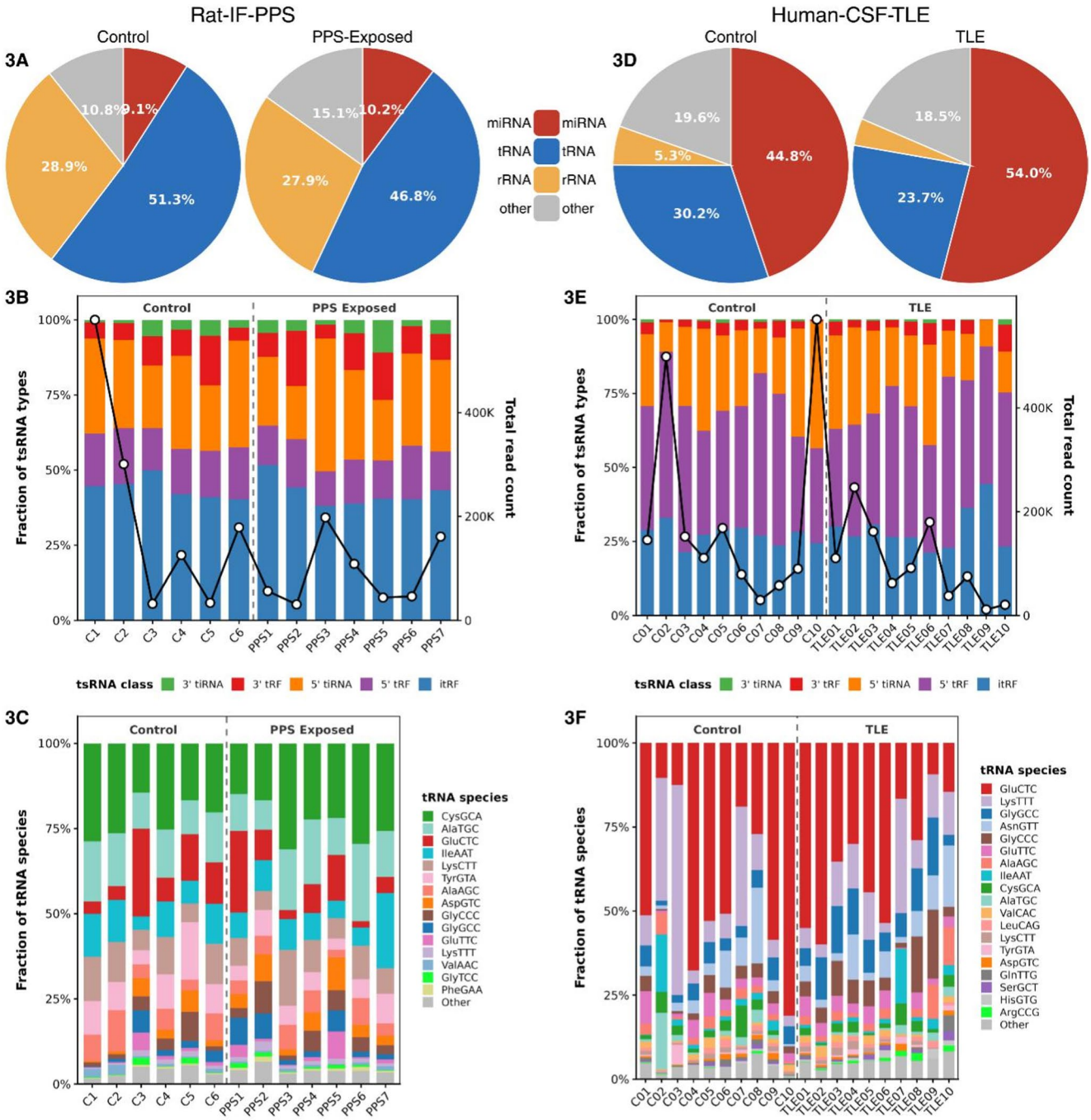


Fig. 3 Extracellular tsRNA Profiles in PPS Rat IF and Human CSF in TLE. **A** Pie charts of small RNA classes in rat hippocampal interstitial fluid (Rat-IF-PPS) for control ($n=6$) and PPS-exposed ($n=7$), showing changes in tRNA, miRNA, and other sncRNAs. **B** tsRNA subclass composition and total tRNA reads per sample in Rat-IF-PPS, highlighting relative distributions of itRFs, tRFs, and tiRNAs. **C** Parent tRNA species contributions to the tsRNA pool in Rat-IF-PPS. **D** Pie charts of small RNA class composition in human CSF for healthy controls ($n=10$) and TLE patients ($n=10$), with miRNA and tRNA proportions indicated. **E** tsRNA class composition and total tRNA reads in human CSF samples. **F** Parent tRNA species distribution in human CSF tsRNAs. For the human cohort (panels D-F), each group, neurologically healthy controls and patients with TLE, comprised five male and five female participants. Sex was not used to stratify the composition comparisons shown here (see Discussion, limitations)

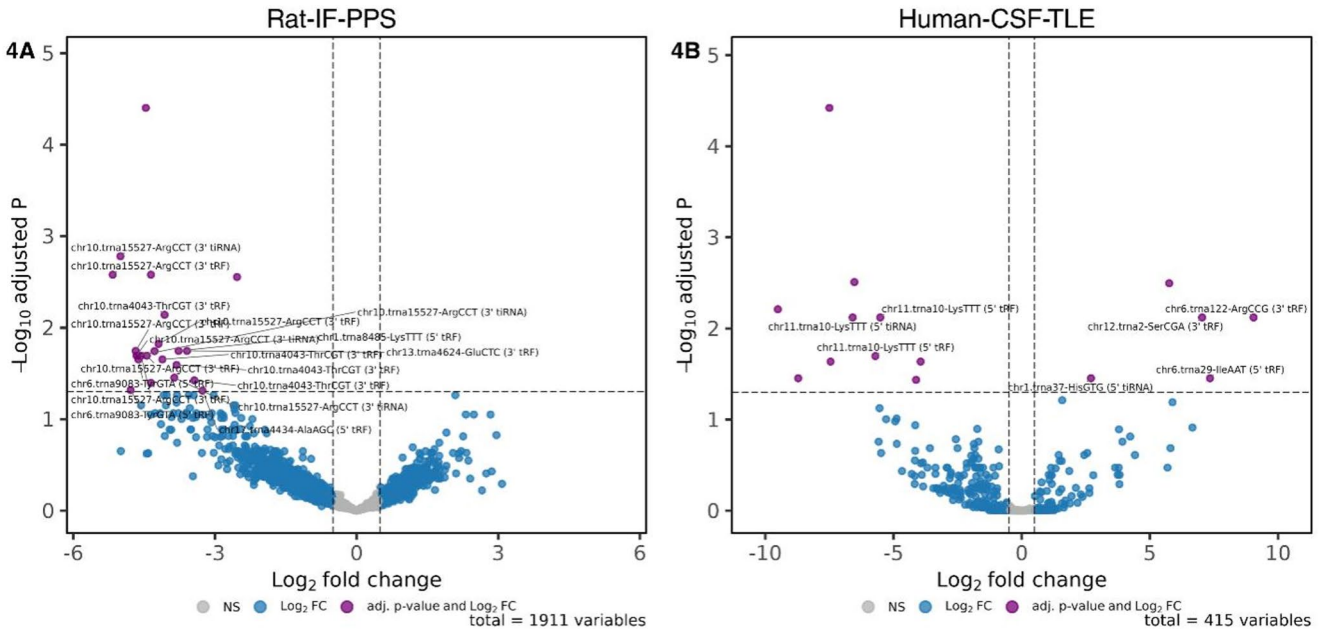


Fig. 4 Differential Expression Analysis of Extracellular tsRNAs in PPS Rat IF and Human CSF in TLE. **A** Volcano plot of differential tsRNA expression in Rat-IF-PPS (PPS-exposed, $n=7$ vs. control, $n=6$), illustrating the predominance of downregulated fragments. **B** Differential tsRNA expression in human CSF (TLE, $n=10$ vs. healthy, $n=10$), showing distinct patterns of up- and downregulated fragments

and ThrCGT. These shifts suggest that chronic epilepsy modulates tsRNA profiles in interstitial fluid, with potential implications for local communication within the hippocampal microenvironment and exchange with other fluid compartments (e.g., CSF).

sncRNA Landscape in Human CSF from TLE Patients

To explore the translational relevance of the rat model data, we characterized small RNAs in CSF from neurologically healthy controls ($n=10$; five male, five female) and patients with TLE ($n=10$; five male, five female). In contrast to the rat findings, miRNAs comprised the largest sncRNA fraction in CSF from control individuals (44.8%; Fig. 3D). This was followed by tRNA-derived reads, and snRNAs, with other sncRNAs making up the remainder of the sncRNA pool. The proportion of miRNA reads was nominally higher in CSF samples from TLE patients (54.0%) and the relative abundance of tRNA-derived reads nominally lower (30.2% to 23.7%), but this compositional shift was not significant after correction.

In the tsRNA pool, absolute read content was generally higher in controls than in TLE patients, with notable variability across individuals (Fig. 3E). The most abundant subclasses in control CSF included 5' tRFs, 5' tiRNAs, and itRFs, whereas TLE patients exhibited relatively elevated representation of 3' tRFs. 3' tiRNAs remained scarce in both groups.

Parent tRNA species composition in human CSF showed that GluCTC-derived fragments were the most abundant overall, followed by LysTTT and GlyGCC (Fig. 3F). Compared to controls, TLE patient CSF displayed a nominally higher fraction of GlyGCC-derived fragments (4.6% to 10.1%, $p=0.009$), but this did not survive correction (BH-adjusted $p=0.073$) and no parent-tRNA shift was significant.

Differential expression analysis identified multiple tsRNAs that were altered in CSF from TLE patients (Fig. 4B). Fifteen fragments reached significance, including five upregulated and ten downregulated in patients. Downregulated fragments were predominantly internal tRFs, including the most strongly altered fragment overall, an itRF derived from GluTTC (log2 fold change = −7.5, BH-adjusted $p=3.8\times10^{-5}$), together with fragments from LysTTT and AlaTGC; upregulated fragments derived from ArgCCG, SerCGA and HisGTG among others. This leading itRF-GluTTC signal remained significant when the model was adjusted for sex (Supplementary Table S7).

Both rat interstitial fluid in the PPS model and human CSF exhibited robust extracellular tsRNA populations, but with distinct distribution patterns. In the rat interstitial fluid, tRNA-derived sequences dominated and decreased in relative proportion with epileptogenic stimulation, whereas in human CSF, the relative proportion of tRNA fragments was lower overall and reduced further in TLE compared to healthy controls. In both systems, internal fragments and 5' subclasses were prominent, although their relative ratios differed.

Comparing landscapes across species and compartments, several consistent themes emerge. First, small-RNA composition is strongly compartment-specific: hippocampal tissue is miRNA-dominated (86.8% miRNA, 10.3% tRNA-derived in controls), whereas every biofluid is relatively tRNA-enriched by comparison (rat CSF 68.2%, rat IF 51.3%, human CSF 30.2% tRNA-derived), with tissue differing significantly from each biofluid ($p \leq 0.01$). The dominant tsRNA class also differs by compartment (i.e., 5' tiRNAs in tissue, internal tRFs in rat CSF and interstitial fluid, and 5' tRFs in human CSF), with no 5' tRF fragments detected in tissue after filtering. GluCTC and GlyGCC parent tRNAs repeatedly contribute high fractions of extracellular tsRNAs, though their prominence is compartment-dependent (GlyGCC dominates tissue but is a minor component of interstitial fluid; GluCTC dominates CSF but is essentially absent from tissue), and condition-associated expression shifts often involve these species. Human TLE CSF and rat PPS interstitial fluid share relative decreases in absolute or proportional tRNA fragment content in disease, paralleling the increase in miRNA representation observed in human CSF. While interspecies differences and compartmental contexts caution against overinterpretation, the convergence on specific tsRNA patterns suggests that select tRNA fragments may be responsive to epileptic pathology across systems.

Discussion

This study presents a multi-compartment and cross-species analysis of small noncoding RNA landscapes in experimental and human epilepsy, with a special focus on tRNA-derived small RNAs (tsRNAs). We identified pronounced compartment-specific differences in small RNA composition, with tsRNAs representing a dynamically regulated component of the small RNA repertoire, across rat hippocampal tissue, rat hippocampal interstitial fluid, rat CSF, and human CSF. While intracellular tissue profiles were dominated by miRNAs, high abundance of tRNA-derived sequences was present in extracellular compartments, especially in rat IF and CSF. Specific tsRNA subclasses and parent tRNA species, including GlyGCC and GluCTC, emerged as recurrent features across datasets, with condition-specific changes suggesting selective remodelling of tsRNA landscapes in epilepsy. Taken together, these findings support the view that tsRNAs represent a regulated and compartment-dependent component of RNA biology in the epileptic brain.

It is increasingly evident that TLE is associated with major shifts in brain and biofluid levels of various small noncoding RNA species (Simonato et al. 2021; Henshall and Simonato 2024; Winek and Soreq 2025). Determining the composition and changes in sncRNA content in experimental and human epilepsy is important to improve our understanding of the formation, function and movement of such species in epilepsy and thus improve our mechanistic understanding of their value as biomarkers and potential therapeutic targets. Identifying species-specific differences is of further importance to ensure commonly used models reflect human-relevant molecular findings. This research also addresses the broader issue of whether and how the integrity and function of fluid barriers and material exchange changes in TLE, including roles for systems such as glymphatics (Abbott et al. 2018).

The relative increase and redistribution of tRNA-derived fragments in epileptic conditions, especially in the hippocampal tissue following pilocarpine exposure, is one of the central observations of this study. Neuronal hyperexcitability, metabolic stress, and inflammation are well-established features in the context of epilepsy (Vezzani et al. 2011; Pitkänen and Engel 2014; Sanz and Garcia-Gimeno 2020; Mukhtar 2020). The increase in overall tsRNA abundance in hippocampal tissue from pilocarpine-exposed animals, accompanied by a non-significant trend toward higher 5' tiRNA representation, is consistent with a stress-responding cleavage program that may reflect altered translational control or cellular adaptation to chronic epileptic activity. Notably, the coinciding reduction of specific 3' tRFs suggests that tsRNA remodelling is not uniform and instead involves selective shifts in cleavage patterns or fragment stability. Similar selective processing has been reported in other systems, where distinct classes of tsRNAs are generated depending on cleavage position, ribonuclease activity, parent tRNA identity, and tRNA modification state (Fu et al. 2009; Yamasaki et al. 2009; Shen et al. 2018; Oberbauer and Schaefer 2018; Wen et al. 2021).

A notable feature from our results is the clear divergence between intracellular and extracellular landscapes of small RNA, with a high proportion of tRNA-derived fragments, often exceeding miRNA abundance in rat IF and CSF. Human CSF showed a more mixed profile where miRNAs were the largest fraction. However, tsRNAs still constitute a substantial component. These observations are consistent with other studies (Dhahbi et al. 2013; Tosar and Cayota 2020), which have reported the enrichment of tRNA-derived fragments and other non-miRNA species in biofluids such as serum, plasma, and other extracellular compartments. Several mechanisms may explain this enrichment in tsRNAs in extracellular fluids. First, tsRNAs may be more chemically stable than other RNA species, partly due to post-transcriptional modifications inherited from their parent tRNAs (Motorin and Helm 2011). Second, they may be packaged more efficiently into extracellular vesicles than

other RNAs, which could be facilitated by selective packaging and release mechanisms or by protein binding such as Argonaute proteins (Valadi et al. 2007; Arroyo et al. 2011). In the CSF dataset we analysed, both the high absolute read counts and dominance of itRFs suggest that tsRNA profiles in the extracellular space are indeed shaped by both production and stability. The relative decrease of tRNA-derived reads and increase in miRNAs in the CSF samples from pilocarpine-treated rats further indicate that epilepsy-related changes extend beyond intracellular compartments to affect extracellular RNA composition. Notably, the analysis from the matched CSF samples collected from the same animals at two different timepoints reinforces this interpretation by showing that such changes occur within the same animals over time, reducing the likelihood that they reflect inter-individual variability.

In multiple datasets, certain parent tRNA species, most notably GlyGCC and GluCTC, comprise a large fraction of tsRNA reads in several datasets. This cross-compartment and cross-species recurrence suggests that tsRNA generation is not a random process and instead reflects selective processing, stabilization, or detectability of specific tRNAs due to modifications. Previous studies have similarly demonstrated that tsRNA populations are abundantly composed of fragments derived from a limited subset of tRNAs, including glycine and glutamic acid isoacceptors (Lee et al. 2009; Kumar et al. 2016; Tosar et al. 2018; Drino et al. 2020). While we do not completely understand the reasons for this selectivity, it may relate to the differences in tRNA abundance, modification patterns, or susceptibility to cleavage by specific ribonucleases. For example, under stress conditions, certain tRNAs are more prone to angiogenin-mediated cleavage, which leads to preferential generation of specific tiRNAs (Yamasaki et al. 2009).

With regard to the parent tRNA of origin, we use this term to denote the parental tRNA isodecoder from which a fragment derives (for example, tRNA-Gly-GCC or tRNA-Glu-CTC), rather than any genomic parent-of-origin or imprinting effect. The recurrence of fragments from a small number of parent tRNAs is more parsimoniously explained by the biology of tRNA fragmentation than by imprinting, i.e., the abundance of the parent tRNA, sequence- and structure-specific cleavage by stress-activated ribonuclease such as angiogenin, and tRNA modifications that either protect from or expose cleavage sites. Consistent with a compartment-selective rather than a uniform programme, the prominence of these parents was compartment-dependent in our data, i.e., GlyGCC-derived fragments dominated hippocampal tissue but were a minor component of interstitial fluid, whereas GluCTC-derived fragments dominated CSF but were essentially absent from tissue.

It remains to be established how such fragments might act on downstream targets. By analogy with characterised tsRNA functions, candidate mechanisms include loading into Argonaute proteins and miRNA-like, seed-based silencing of messenger RNAs; displacement of RNA-binding proteins from their target transcripts; and interference with translation through competition with the ribosome or translation-initiation machinery. We attempted to define candidate targets computationally (Supplementary Table S8), but sequence-based target prediction did not yield robust, interpretable pathway enrichment once the well-documented bias toward transcripts with long, GC-rich 3'UTRs was controlled for. We therefore refrain from drawing target-level conclusions and note that establishing the direct targets and mechanisms of the disease-associated fragments identified here will require dedicated functional studies.

A distinction between abundance and differential regulation is also highlighted by our results. While the overall CSF is dominated by GluCTC-derived fragments, few of these fragments were significantly altered between conditions. Other tRNAs such as AspGTC and GluTTC contributed disproportionately to the differentially expressed fragments. This suggests that tsRNAs that are highly abundant are not necessarily the ones that are most dynamically regulated, and that disease-associated changes may preferentially influence a subset of tsRNAs with distinct biological roles, stability, or release dynamics.

We extended our analysis to rat interstitial fluid and human CSF to assess whether extracellular tsRNA patterns observed in the pilocarpine model generalize across models and species. Both compartments showed robust tsRNA populations, with itRFs and 5'-derived fragments representing major classes. Across both compartments, epilepsy-related conditions were associated with a relative reduction in tRNA-derived reads and an increase in miRNA representation, although these compositional shifts did not reach significance after correction. The patterns nonetheless suggest that extracellular tsRNA remodelling may represent a recurrent, if subtle, feature of epilepsy-associated extracellular RNA changes.

This work has numerous strengths, including the integration of multiple datasets covering intracellular and extracellular compartments, the use of a consistent analytical approach, and the inclusion of both experimental models and human samples. The use of baseline and early chronic CSF samples collected from the same rats further strengthens the interpretation of extracellular changes. Still, limitations must be acknowledged. The datasets were generated for different purposes and were generated independently, which introduces potential variability in the handling of samples, protocols for sequencing, and quality of data. In some datasets, especially the rat CSF, sample sizes were

relatively small. Differential expression analyses apply Benjamini-Hochberg correction throughout, and composition changes that do not survive correction are reported as non-significant trends rather than as findings. Additionally, the present sequencing analysis was not itself accompanied by orthogonal validation and relies on sequencing-based inference. The cross-dataset comparison, due to these limitations, should be considered hypothesis-generating. We note, however, that the two parent tRNAs that recur most prominently here, GluCTC and GlyGCC, have been independently confirmed by quantitative PCR in a separate cohort of the same pilocarpine model (Soukupova et al. 2026), concordant with the exploratory CSF findings reported here. Two different rat models were used, including pilocarpine-induced status epilepticus and perforant pathway stimulation, reflecting the absence of a single ideal model of temporal lobe epilepsy and the availability of matched biofluid data. Because the two differ in their mode of epileptogenesis (chemoconvulsant versus electrical) (Curia et al. 2008; Sloviter and Bumanglag 2013), molecular features that recur across both are less likely to reflect the aetiology of any one model, although we did not perform direct quantitative comparisons of effect sizes between them. Likewise, human TLE represents a heterogeneous clinical condition with varying aetiologies and histories of treatment (Pitkänen et al. 2015). In particular, detailed antiseizure-medication histories were not available and could not be disentangled from disease state, so medication effects on the molecular signatures cannot be excluded. All rodent datasets used male animals only, so sex-specific differences in tsRNA responses could not be assessed and remain to be examined. In the human cohort, sex was included as a covariate for the disease-associated fragments (Supplementary Table S7) but the CSF composition comparisons (Fig. 3D-F) were not stratified or formally tested by sex. With five male and five female participants per group, the cohort is balanced but not powered for a sex-stratified composition analysis. Therefore, we cannot exclude sex as a factor contributing to these patterns, which remains to be examined in larger, sex-balanced cohort. Additionally, sampling differences (e.g., microdialysis versus lumbar CSF), species-specific RNA biology, and pre-analytical sample handling may influence observed profiles. Studies in extracellular RNA have shown that RNA profiles can vary significantly between biofluids and are affected by isolation and profiling methodology (Tosar et al. 2021). Even with these limitations, the recurrent identification of GlyGCC- and GluCTC-derived sequences across datasets supports the notion that certain tsRNAs are consistently represented in extracellular compartments. The strongest conclusions of this work relate to compartment-specific differences and within-dataset disease-associated changes. Longitudinal and paired tissue-CSF studies would be valuable in linking RNA dynamics in the intracellular and extracellular spaces. Lastly, larger human cohorts and the integration of supplementary biofluids such as plasma will be required in the future for assessing the potential of tsRNAs as epilepsy biomarkers.

Conclusions

Although the work is of a primarily descriptive nature, potential biological implications can be considered. First, the enrichment of tsRNAs in the extracellular compartments raises the possibility that these molecules, in the brain, may participate in intercellular communication. Extracellular RNAs such as miRNAs and tRFs have been proposed to act as signalling molecules that can influence recipient cells (Valadi et al. 2007; Mittelbrunn and Sánchez-Madrid 2012).

Second, differential regulation of certain tsRNA subclasses suggests that distinct cleavage pathways may be engaged in epileptic conditions. For instance, the increased overall abundance of tsRNAs in tissue may reflect stress-induced repression of translation, while the reduction of specific 3' tRFs may reflect altered degradation, stability, or export pathways.

Finally, the accumulation of disease-associated tsRNA signatures in human CSF raises the possibility that tsRNAs could potentially serve as candidate extracellular RNA biomarkers for epilepsy diagnosis. That said, given the variability observed across samples and the modest effect sizes for many fragments, extensive validation in larger and more controlled cohorts would be required for such applications.

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Author Contributions S.Z. performed formal analysis, software development, and visualisation, curated the data, contributed to the investigation and methodology, and wrote the original manuscript. K.S. contributed to data curation, investigation, and methodology. M.Sk. contributed to the investigation and methodology. M.T.V. contributed to the investigation, data curation, software development, and provision of resources. H.H. and P.K. contributed to the conceptualisation of the study, methodology, and provision of resources. M.Si. and F.R. contributed to the conceptualisation of the study, funding acquisition, and provision of resources. D.H. contributed to the conceptualisation of the study and funding acquisition. J.K. contributed to funding acquisition. J.H.M.P. conceptualised and designed the study, supervised the project, administered the project, acquired funding, and contributed to writing and revision of the manuscript. All authors contributed to writing - review and editing of the manuscript and read and approved the final version.

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Data Availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

Ethical Approval All animal experiments were conducted in accordance with national and international guidelines for the care and use of laboratory animals. Rat Hippocampal Tissue (Pilocarpine model): The authors of the original study obtained ethical approval as described in the original study (Baloun et al. 2020). Rat CSF (Pilocarpine model): All rat procedures were performed in accordance with the EU Directive 2010/63/EU, the ARRIVE guidelines, and the NC3Rs (National Centre for the Replacement, Refinement and Reduction of Animals in Research) principles for the ethical use and care of animals. Ethical approval for all procedures was obtained from the University of Ferrara Institutional Animal Care and Use Committee and the Italian Ministry of Health (Authorization: D.M. 603/2022-PR) prior to the commencement of the study. Rat Hippocampal Interstitial Fluid (PPS model): All procedures on animals were performed in accordance with the ARRIVE guidelines and with the guidelines of the European Community (Directive 2010/63/EU). The animal study protocol was approved by the local regulatory authority (Regierungspräsidium Gießen, protocol code 75-2012, approved 30 August 2012). Human CSF (TLE Cohort): Human CSF samples were collected and used in accordance with the Declaration of Helsinki. Ethical approval was obtained from the local medical ethics committees at the University of Magdeburg (approval no. 07/17) and the Friedrich-Alexander-University Erlanger-Nürnberg (approval no. 128/14Bc). Written informed consent was provided by all participants prior to sample collection.

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