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Altered somatostatin receptor 3 expression and functional dysregulation in tuberous sclerosis complex

Mirte Scheper [a,*,1], Alessandro Gaeta [b,1], Gabriele Ruffolo [b,c,**], Lilian J. Lissner [b], Marie Le Bihan [d], Jasper J. Anink [a], Floor E. Jansen [e], Wim van Hecke [f], Angelika Mühlebner [f], Dirk Schubert [d], James D. Mills [a,1], Eleonora Palma [b,1], Eleonora Aronica [a,1]

[a] Amsterdam UMC location University of Amsterdam, Department of (Neuro)Pathology, Amsterdam Neuroscience, Meibergdreef 9, Amsterdam, the Netherlands
[b] Department of Physiology and Pharmacology, laboratory affiliated to Pasteur Institute Italy, Cenci Bolognetti Foundation University of Rome Sapienza, Rome 00185, Italy
[c] IRCCS San Raffaele Cassino, Cassino 03043, Italy
[d] Department of Medical Neurosciences, Radboudumc, Donders Institute for Brain Cognition and Behaviour, Nijmegen 6525 HR, the Netherlands
[e] Department of Child Neurology, Brain Center University Medical Center, Member of ERN EpiCare, Utrecht 3584 BA, the Netherlands
[f] Department of Pathology, University Medical Center Utrecht, Utrecht, the Netherlands

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ABSTRACT

Somatostatin (SST), a neuropeptide primarily synthesized by GABAergic interneurons, modulates neuronal excitability and synaptic transmission through its interaction with somatostatin receptors (SSTRs). Dysregulation of SST signaling has been implicated in neurodevelopmental disorders, including tuberous sclerosis complex (TSC). However, its precise role in these pathologies remains incompletely understood. We investigated SST and SSTR expression across diverse brain cell types in control and TSC cortical samples using single-cell RNA sequencing (scRNA-seq). We conducted functional assessments of SST signaling using electrophysiological recordings in *Xenopus laevis* oocytes microtransplanted with human brain membranes. We pharmacologically modulated SST receptor activity to elucidate receptor-specific effects on GABAergic transmission. scRNA-seq analysis revealed that SST expression is predominantly confined to GABAergic interneurons, while SSTR1 and SSTR2 exhibit strong expression in both glutamatergic and GABAergic neuronal populations. In TSC samples, SSTR5 was upregulated in GABAergic neurons, SSTR2 in glutamatergic neurons, while SSTR3 was downregulated in both glutamatergic neurons and microglia. Functional experiments demonstrated that SST enhances GABAergic currents in control tissues through a receptor-mediated mechanism involving protein kinase C activation. In contrast, SST application in TSC samples resulted in a significant suppression of GABAergic currents. Pharmacological inhibition of SSTR3 further exacerbated this effect, suggesting a compensatory role for this receptor subtype. Our findings reveal a disruption of SST signaling in TSC, contributing to altered coordination of excitatory-inhibitory activity and epileptogenesis. Targeting SST signaling may represent a therapeutic strategy for restoring inhibitory network function in TSC and related disorders.

1. Introduction

Somatostatin (SST), a neuropeptide primarily known for its regulatory role in various physiological processes, exerts its influence on the brain through a complex interplay of receptors and signaling pathways (Robinson and Thiele, 2020). Initially identified for its inhibitory effects on growth hormone secretion, somatostatin's significance extends far beyond endocrine regulation, encompassing modulation of neurotransmission, neuronal excitability, and synaptic plasticity within the central nervous system (Song et al., 2021; Urban-Ciecko and Barth, 2016; Mòdol et al., 2024). These diverse functions are mediated through interaction with multiple somatostatin receptor (SSTR) subtypes, each exhibiting

* Corresponding author.
** Corresponding author at: Department of Physiology and Pharmacology, laboratory affiliated to Pasteur Institute Italy, Cenci Bolognetti Foundation University of Rome Sapienza, Rome, 00185, Italy.
E-mail addresses: m.scheper@amsterdamumc.nl (M. Scheper), gabriele.ruffolo@uniroma1.it (G. Ruffolo).
[1] Contributed equally

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distinct affinities and downstream signaling mechanisms (Olias et al., 2004; Kumar, 2005). All SSTRs are G-protein-coupled receptors (GPCRs) with seven transmembrane domains. Furthermore, its diverse functions are mediated through interaction with these specific receptors expressed on various brain cell types, including neurons and glial cells (Song et al., 2021; Breder et al., 1992). The intricate interplay between SST and its receptors regulates a multitude of physiological processes crucial for preserving brain homeostasis.

SST is synthesized primarily by a subset of GABAergic interneurons, known as SST-expressing interneurons (SST^+ INs), which are prominently located in the neocortex, hippocampus and amygdala. These interneurons integrate diverse inputs from glutamatergic neurons, other GABAergic interneurons, and modulatory systems involving neurotransmitters such as dopamine, serotonin, and acetylcholine (Urban-Ciecko and Barth, 2016; Tremblay et al., 2016; Cattaneo et al., 2019a). They project locally within the same region and, in some cases, send long-range projections to distal areas, thereby enabling the widespread coordination of neural activity. Within local circuits, SST^+ INs primarily innervate the dendrites of excitatory pyramidal neurons, exerting inhibitory control through the release of both γ-aminobutyric acid (GABA) and SST (Urban-Ciecko and Barth, 2016; Muñoz et al., 2017). Additionally, they form inhibitory loops by synapsing onto other interneurons, establishing feedback and feedforward connections that help maintain the coordination between excitation and inhibition across cortical and subcortical networks (Hostetler et al., 2023; Liguz-Lecznar et al., 2016).

The principal mode of inhibition exerted by SST^+ INs is via GABA release. GABA is the major inhibitory neurotransmitters in the mature brain and acts through ionotropic $GABA_A$ receptors and metabotropic $GABA_B$ receptors (Kanigowski et al., 2023). $GABA_A$ receptors are ligand-gated chloride channels that mediate rapid synaptic inhibition and are composed of multiple subunits that confer distinct function properties critical for synaptic specificity and network stability (Sallard et al., 2021). In contrast, $GABA_B$ receptors are GPCRs that mediate slower and more prolonged inhibition, often by modulating presynaptic neurotransmission release and postsynaptic membrane conductance (Chalifoux and Carter, 2011). Beyond GABA, SST^+ INs also release SST itself, which acts in a modulatory fashion by binding to SSTRs expressed on presynaptic terminals, postsynaptic neurons, and surrounding non-neuronal cells (Momiyama and Zaborszky, 2006). Activation of SSTRs can suppress neurotransmitter release, reduce neuronal excitability, and fine-tune local circuit dynamics. For example, SSTR activation on GABAergic terminals can inhibit GABA release through a negative feedback mechanism, while SSTRs on pyramidal neurons can modulate membrane potential and synaptic integration (Momiyama and Zaborszky, 2006). In this way, SST signaling complements the fast inhibition mediated by GABA, providing an additional layer of control over cortical excitability and temporal precision in network activity.

Previous studies have shown that neurons can release SST in a calcium-dependent manner (Tapia-Arancibia et al., 1989), even without external stimuli such as sensory input (Gamse et al., 1980) SST release can be triggered by membrane depolarization (Tapia-Arancibia and Astier, 1989), glutamate (Fontana et al., 1996; Rage et al., 1994), activate NMDA and AMPA receptors. Both *in vitro* experiments and *in vivo* studies, have identified factors that modulate SST release. Notably, striatal SST^+ INs co-release glutamate and GABA, generating excitatory-inhibitory sequences in postsynaptic neurons (Cattaneo et al., 2019b). This co-release is mediated by the activation of ionotropic AMPA/NMDA receptors expressed in axon terminals upon glutamate stimulation, primarily observed in the striatum. In contrast, cortical SST+ INs are known to co-release GABA and SST onto postsynaptic neurons upon activation (Liguz-Lecznar et al., 2016; Baraban and Tallent, 2004). Interestingly, GABA release from SST+ INs inhibits both GABA and SST spontaneous release (Gamse et al., 1980), regulated by $GABA_B$ receptors on the axon terminals of SST+ INs (Bonanno et al., 1999).

Understanding the significance of SST and receptor expression in different brain cell types is crucial, as it not only elucidates fundamental neurobiological mechanisms, but also has implications for neurological and neurodevelopmental disorders. Previous research has highlighted the involvement of SST^+ INs in neurodevelopmental disorders such as Tuberous Sclerosis Complex (TSC) and Focal Cortical Dysplasia (FCD). Several studies have emphasized the importance of SST interneurons in regulating cortical network activity and inhibitory control. For instance, studies using animal models have demonstrated alterations in SST^+ INs function associated with aberrant network synchronization and epileptogenesis (Malik et al., 2019), characteristic features of TSC and FCD. Furthermore, human post-mortem studies have revealed reductions in SST^+ INs density and alterations in SST receptor expression within epileptogenic cortical regions in patients with TSC and FCD (Scheper et al., 2024). Of relevance to our study, our prior research identified SST^+ INs as the most immature population within the pathological milieu of TSC, highlighting the vulnerability of SST^+ INs in disease pathology (Scheper et al., 2024). However, despite these advancements, significant gaps persist in our understanding of the broader implications of SST dysregulation in TSC pathology.

Hence, we aim to perform a comprehensive investigation into SST and its receptor expression across various brain cell types, in both control and TSC samples. By integrating differential expression analysis, functional experiments in oocyte models and pharmacological SSTR modulation, we seek to provide a thorough understanding of the role of SST in TSC, and this work may ultimately provide insights that contribute to the identification of novel therapeutic strategies.

2. Materials and methods

2.1. Study cohort

Surgical and postmortem brain tissues were selected from the archives of the Department of Neuropathology of the Amsterdam UMC (Amsterdam, The Netherlands) and the UMC Utrecht (Utrecht, The Netherlands). Cortical brain samples were obtained from patients undergoing surgery for intractable epilepsy and diagnosed with TSC cortical tubers (single-cell RNA sequencing: n = 5 patients, oocyte experiments: n = 6) or focal cortical dysplasia (single-cell RNA sequencing: n = 5). In addition, age- and tissue-matched autopsy control samples were collected (single-cell RNA sequencing: n = 3, oocyte experiments: n = 2) from individuals without a history of seizures or other neurological disease. Furthermore, in another set of experiments we used fetal brain samples (oocyte experiments: n = 3), which were obtained from spontaneous or medically induced abortions with appropriate maternal written consent for brain autopsy. Gestational ages were based on obstetric data, fetal and brain weights and standard fetal anthropometric measurements. All autopsies were performed within 9 h after death. Tissue was obtained and used in accordance with the Declaration of Helsinki and the Amsterdam UMC Research Code provided by the Medical Ethics Committee and according to the Amsterdam UMC and UMC Utrecht Biobank Regulations (W21–295; 21–174). Clinical information about the brain samples is summarized in supplementary table 1.

2.2. Single-cell RNA sequencing

2.2.1. Control and TSC single-cell RNA sequencing dataset

Single-cell RNA sequencing was performed at Single Cell Discoveries (https://www.scdiscoveries.com/) according to the 10x genomics Chromium Single Cell Gene Expression Flex protocol. Prior to loading the samples, the frontal cortex tissue was cut into slices, which were fixed, and cells were extracted. Cells were counted to ensure quality control. For each sample, 8000 cells were loaded, and the resulting sequencing libraries were prepared following a standard 10 × Genomics protocol.

2.2.2. Processing and analysis of scRNA-Seq data

All processing and analyses of the scRNA-Seq data have been previously described (Scheper et al., 2025). Sample reads were aligned to the human genome GRCh38 using Cell Ranger. Filtering of empty barcodes was done in Cell Ranger. The data from all samples were loaded in R (version 4.3.1) and processed using the Seurat package (version 5.0.1). More specifically, cells with at least 1000 UMIs per cell and less than 5 % mitochondrial gene content were retained for analysis. The data of all 10 × libraries was merged and processed together. The merged dataset was normalized for sequencing depth per cell and log transformed. After filtering, datasets were integrated using reciprocal principal component analysis (RPCA). The integrated data was subsequently scaled, and dimensionality reductions were performed. Clustering was computed using the FindNeighbors function (dims = 1:20), and FindClusters at a resolution of 0.5. To identify the cell types in separate clusters, marker genes for each cell type were used (Supplementary table 2). To perform differential expression analysis between control and TSC samples, we performed pseudo-bulk analysis. This approach involves aggregating cells within each biological sample to create 'pseudobulks'. Differential expression analysis was performed using the R package DESeq2 (Love et al., 2014). To control the false discovery rate, we applied the Benjamini-Hochberg correction, considering gene expression changes with an adjusted p-value < 0.05 as statistically significant.

2.2.3. Cortical development single-cell RNA sequencing dataset

We accessed the UCSC Cell Browser's Cortical Development Single-Cell data set to obtain single-cell RNA sequencing (scRNA-seq) data for our study (Speir et al., 2021; Velmeshev et al., 2023). The data set includes a comprehensive collection of gene expression profiles from various cell types present in the human cortex across different stages of development. The raw scRNA-seq data were downloaded from the UCSC Cell Browser. Preprocessing steps included quality control to remove low-quality cells and potential doublets. Cells with high mitochondrial gene content or low unique molecular identifier (UMI) counts were excluded. The data were then normalized and log-transformed to ensure comparability across cells. To accurately identify and annotate cell types, we employed marker-based classification. Known marker genes for distinct cortical cell types, such as neurons, glial cells, and progenitor cells, were used to assign cell identities. The annotation was further validated by comparing our results with the reference annotations provided by the UCSC Cell Browser. We performed differential expression analysis to compare gene expression profiles between control and TSC samples similar to our previous description. Additionally, we performed age-expression correlation analyses.

2.3. Xenopus laevis' oocytes and voltage clamp recordings

Xenopus laevis oocytes' injection was executed as previously described (Eusebi et al., 2009). Briefly, cell membranes were isolated from brain tissue and injected into the oocytes to directly transplant the native receptors, embedded in their own membranes, with their original glycosylation pattern and auxiliary proteins (Eusebi et al., 2009). Human brain tissues collected were snap-frozen in liquid nitrogen without delay, then immediately processed when received or stored at −80°C for further use. To obtain neuronal membranes, we used samples weighing 10–30 mg. Tissues were homogenized in a membrane buffer solution (200 mM glycine, 150 mM NaCl, 50 mM EGTA, 50 mM EDTA, and 300 mM sucrose; plus 20 μL of protease inhibitors "P2714; Sigma"; pH 9, adjusted with NaOH). Afterwards, the material was centrifuged for 15 min at 9500 x g. Then, the supernatant was centrifuged for 2 h at 100.000 x g with an ultra-centrifuge (Beckman-Coulter). Finally, the pellet was washed with sterile water, re-suspended in assay buffer (glycine 5 mM) and immediately used or stored at −80°C until use. The *Xenopus* oocytes cytoplasmatic injection of membranes was performed as previously described (Eusebi et al., 2009). Oocytes were injected with membrane samples (≃ 50–100 nL each oocyte; 0.2–10 mg protein/mL) and stored at 16°C in a saline solution (Barth's solution) enriched with antibiotics until the electrophysiological recordings were performed.

The use of *Xenopus laevis* frogs, the surgical procedures for oocytes extraction and use conformed to the Italian Ministry of Health guidelines (authorization no 427/2020-PR). All the animal procedures followed the recommendations of the ARRIVE guidelines and the methods are reported accordingly.

The "two-electrodes voltage-clamp'' electrophysiological technique was performed 24–48 h after oocytes' cytoplasmic injection and 48–72 h from the intranuclear one, at room temperature. The microtransplanted oocytes were clamped with two microelectrodes filled with 3 M KCl while placed in a recording chamber (0.1 mL volume), constantly perfused with oocyte Ringer solution (OR: NaCl 82.5 mM; KCl 2.5 mM; CaCl2 2.5 mM; MgCl2 1 mM; Hepes 5 mM, adjusted to pH 7.4 with NaOH). Neurotransmitters application was controlled by computer (Biologique RSC-200; Claix, France) using a gravity driven multi-valve perfusion system (9–10 mL/min) to regulate the precise duration of each application. GABA (50–250 μM) was applied for 5 s to oocytes to elicit inward GABA-evoked currents (I_{GABA}).

The stability of I_{GABA} was assessed by two consecutive applications of neurotransmitter, separated by a 4 min washout, and only the cells with a < 5 % variation in the current amplitude were considered in the statistical analysis.

SST 1 μM was either co-applicated with GABA or incubated for 2 h, as specified for each experiment. Inhibitors and modulators (Cyclosomatostatin, c-SST; GF 109203X; MK-4256) were pre-incubated for 30 min before being co-incubated with SST for 2 h. In a specific set of experiments, we performed intranuclear injection in oocytes of human complementary DNA (cDNAs) encoding for α1, β2 and γ2 $GABA_A$ subunits (pcDNA3 vector) (Ruffolo et al., 2022). Human cDNA encoding for $GABA_A$ was a gift by Dr. Keith Wafford.

3. Results

3.1. Expression levels of SST and its receptors in the frontal cortex

Understanding the role of SST within the frontal cortex of young individuals is essential as this brain region underlies higher-order cognitive functions such as decision-making, attention, and social behavior. Dysfunctions in SST signaling may contribute to cognitive deficits observed in neurodevelopmental disorders, making this investigation particularly relevant for understanding and potentially addressing these conditions. To do this, we determined the expression level of SST and its receptors (*SSTR1–5*) using a single cell sequencing cohort that consisted of 3 control and 5 TSC samples of prefrontal cortical tissue. This dataset has been described in detail previously (Scheper et al., 2025). For this analysis, we investigated these expression levels in seven different cell types including: GABAergic neurons, glutamatergic neurons, astrocytes, microglia, oligodendrocytes, oligodendrocyte precursor cells (OPCs), and endothelial cells.

In line with our expectations, the gene encoding for the somatostatin neuropeptide (*SST*) was expressed mostly in GABAergic neurons and showed a higher expression than its receptors (Fig. 1) across both control and TSC samples. Next, we looked at the expression of different SST receptors in the frontal cortex. *SSTR1* and *SSTR2* were both mostly expressed in GABAergic and glutamatergic neurons, with *SSTR1* also showing expression in OPCs. Expression of *SSTR3–5* was significantly lower that the expression of *SSTR1* and *SSTR2*. However, *SSTR3* did show a relatively increased expression in glutamatergic neurons. These results are all in line with the idea that SST functions mostly in a network between GABAergic and glutamatergic neurons.

3.2. Expression of SST receptors in TSC and FCD

Next, we aimed to investigate the variations in SST receptor

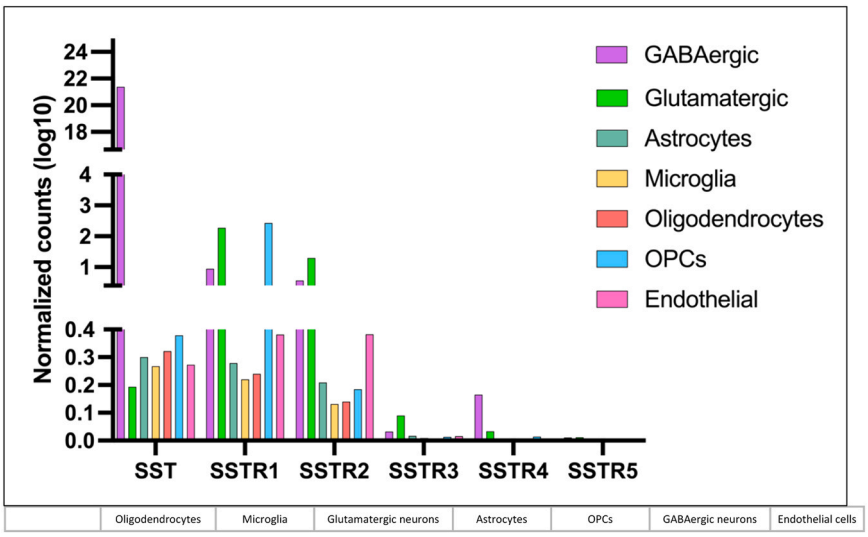

Fig. 1. Expression levels of somatostatin (*SST*) and somatostatin receptor subtypes 1–5 (*SSTR1–5)* across all cell types identified in single-cell RNA sequencing. The x-axis represents the different genes (*SST, SSTR1, SSTR2, SSTR3, SSTR4*, and *SSTR5*), while the y-axis indicates the average normalized counts across both TSC and control samples. Each color on the graph corresponds to a different cell type, providing insights into the expression patterns of *SST* and its receptor subtypes across diverse cell populations. $n = 8$. The table underneath shows exact values shown in bar plot.

expression between control and TSC samples, as well as control and FCD samples, to discern specific alterations in the SST expression and signaling that may contribute to disease onset and progression. Specifically, we used differential expression analysis to determine these differences in the five cell types that show the most expression of these receptors and therefore included: GABAergic neurons, glutamatergic neurons, astrocytes, microglia, and oligodendrocytes.

This analysis revealed distinctive pattern of upregulation and downregulation in different cell types across the frontal cortex. Specifically, *SSTR5* is upregulated in TSC within GABAergic neurons (Log2F = 1.691, padj = 0.0415) (Fig. 2A), suggesting a potential role in the inhibitory of SST neurons themselves or other GABAergic populations. Conversely, *SSTR2* displayed upregulated expression in glutamatergic neurons (Log2F = 1.264, padj = 7.41E-08) (Fig. 2B), possibly indicating it involvement in the regulation of excitatory signaling pathways. Intriguingly, *SSTR3* showed downregulation in glutamatergic neurons (Log2F = −0.855, padj = 0.0017), and microglia (Log2F = −2.511, padj = 0.0267) (Fig. 2B and C), suggesting a complex role in these cell types, possibly linked to altered sensitivity to somatostatin signaling. Notably, no significant differences in SST receptors were observed in astrocytes and oligodendrocytes.

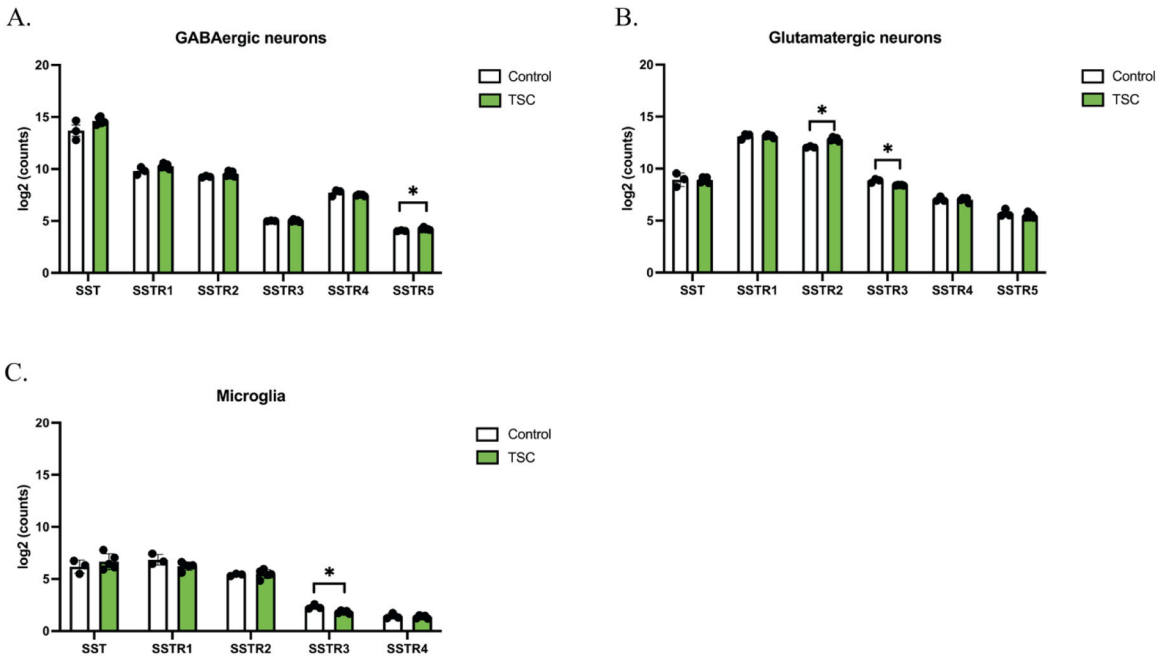

Fig. 2. Differential expression analysis between control and tuberous sclerosis complex (TSC) samples across various cell types. (A) Expression levels of somatostatin (SST) and somatostatin receptor subtypes 1–5 (*SSTR1–5*) in GABAergic neurons. Upregulation of *SSTR5* is observed. (B) Expression levels of *SST* and *SSTR1–5* in glutamatergic neurons. (C) Expression levels of *SST* and *SSTR1–5* in microglia. Other cell types were not included in the analysis due to their low overall expression levels of the genes of interest. Data are presented as mean ± SEM. Statistical analysis was performed using DESeq2 with Benjamini-Hochberg correction for multiple testing. Control: $n = 3$, TSC: $n = 5$.

3.3. Expression of SST and receptors in cortical development

To investigate whether the changes in receptor expression observed in TSC tissue were due to developmental immaturity, we examined the expression patterns of SST receptors across cortical development. Our initial analysis focused on comparing samples from the 3rd trimester to those from the first year postnatal period, using differential expression analysis to identify genes exhibiting altered expression across these developmental stages. Among SST receptors, *SSTR3* showed significant differential expression between the two groups, displaying unique dynamics compared to other receptors, which maintained stable expression levels through these stages. The differential expression of *SSTR3* was evident in a pseudobulk analysis across all cell types and was particularly pronounced in glutamatergic neurons. In contrast, other cell types did not exhibit this expression change, suggesting a cell-type-specific pattern.

Next, we analyzed the developmental trajectory of *SSTR3* expression by correlating its expression levels with age across all time points, from the 2nd trimester through the 3rd trimester and into the first decade postnatal. This analysis revealed a robust positive correlation between *SSTR3* expression and age ($R = 0.73$, $p = 1.2e\text{-}10$) (Fig. 3). In comparison, similar analyses of *SSTR1*, *SSTR2*, *SSTR4*, and *SSTR5* showed no or weaker correlations with age. These results highlight a progressive increase in *SSTR3* expression during cortical development, distinguishing it from other SST receptors.

3.4. Somatostatin effect on GABA-evoked currents

To investigate the effects of SST on GABA-evoked currents (I_{GABA}) in healthy controls and TSC patients, we analyzed tissues at different developmental stages by microtransplanting cellular membranes from brain tissues into *Xenopus* oocytes. Specifically, we compared prenatal controls, postnatal controls, and samples from TSC patients to determine how SST modulates I_{GABA} under normal and pathological conditions. In prenatal controls (Fig. 4A), the application of SST (1 μM, 2 h) significantly increased the GABA-evoked current amplitude, compared to baseline (24.9 ± 8.5 % of current potentiation after the incubation; $n = 26$; $p < 0.05$, paired *t*-test). Similarly, in postnatal controls (Fig. 4B), SST led to a significant enhancement of the GABA-evoked current, indicating a modulatory effect of SST in healthy control samples across developmental stages (23.8 ± 3.4 %, after incubation; $n = 15$; $p < 0.001$, paired *t*-test). However, in tissue from TSC patients (Fig. 4C), SST resulted in a significant decrease in the GABA-evoked current amplitude compared to baseline (-22.52 ± 2.1 % after incubation; $n = 34$; $p < 0.001$, paired *t*-test). These findings suggest a differential modulation by SST between controls and TSC samples.

Next, to determine whether the observed changes in GABA current were mediated through SST receptor activity, we first tested the direct effect of SST on GABA-evoked currents in oocytes expressing recombinant human α1β2γ2 GABA-A receptors. Acute co-application of SST (1 μM) and GABA (50 μM) did not alter the amplitude of GABA-evoked currents (+2.8 ± 1.7 % 1 μM SST; $p > 0.05$; paired *t*-test; $n = 14$), as shown by representative current traces and bar graph quantification ($p > 0.05$; Fig. 4D). Similarly, pre-incubation with SST (1 μM, 2 h) did not result in any significant change in GABA-evoked currents compared to controls (3.7 ± 2.1 %; $p > 0.05$, paired *t*-test; $n = 10$; Fig. 4E-F). These findings indicate that SST does not directly modulate $GABA_A$ receptor function suggesting that the changes in GABA currents observed in control tissues are likely not mediated by a direct interaction between SST and $GABA_A$ receptors. Instead, these results point to the involvement of SST receptor-mediated signaling pathways in the regulation of GABAergic activity within a more complex, native cellular environment.

To further investigate whether the SST-mediated GABA-evoked current increase was due to specific intracellular signaling pathways, we co-applied a protein kinase C (PKC) inhibitor (GF 1 μM) with SST (1 μM, 2 h) in oocytes transplanted with prenatal tissues. In the presence of the PKC inhibitor, the potentiation effect of SST was abolished, resulting in a nonsignificant current change of 4.4 ± 4.1 % ($n = 14$, $p > 0.05$) (Fig. 5A). These findings indicate that SST enhances GABA signaling in control tissue through a receptor-mediated mechanism that requires PKC activation. Having established that SST-mediated potentiation of GABA-evoked currents requires PKC activation, we sought to confirm whether this effect was specifically dependent on SSTR activation. To address this, we applied the non-selective SSTR inhibitor, c-SST (10 μM),

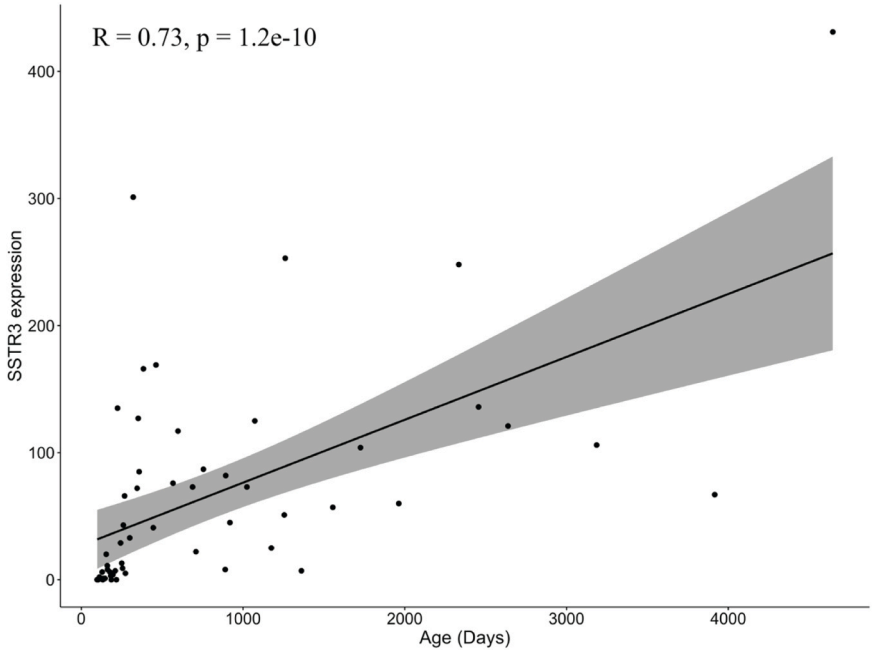

Fig. 3. Age-expression correlation of *SSTR3*. Correlation analysis depicting the relationship between age and expression levels of *SSTR3* during cortical development. The y-axis represents the expression of *SSTR3* in counts, while the x-axis illustrates age in days, starting from the day of conception. Spearman correlation was performed to assess the association. $n = 55$ patients.

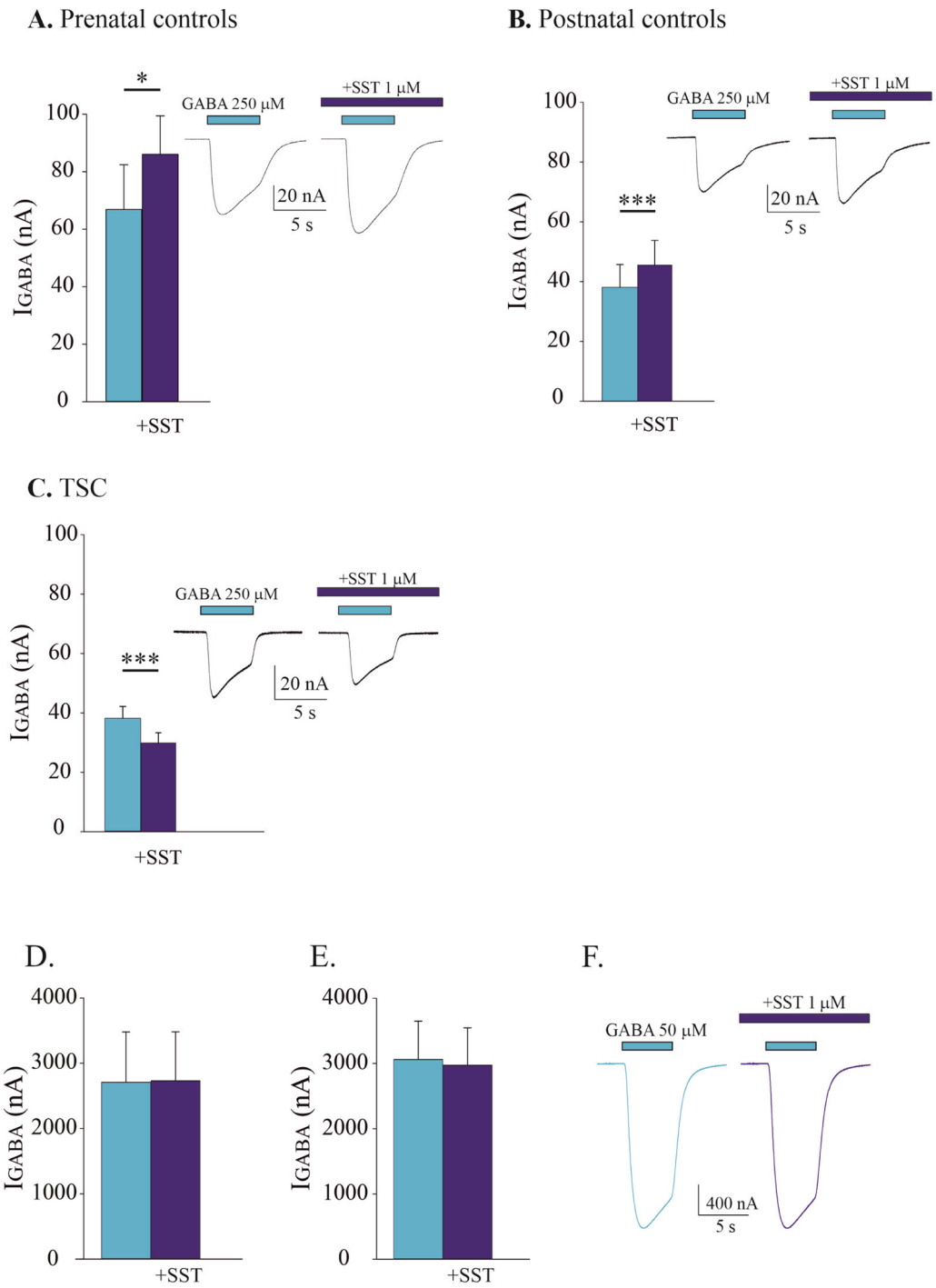

Fig. 4. Differential modulation of $GABA_AR$ mediated by somatostatin (SST). The graph shows the amplitudes (as mean ± s.e.m) of GABA-evoked currents (250 µM) before and after incubation with 1 µM Somatostatin for 2 h. (A) The bar graph shows the increase of GABA current amplitude in prenatal control tissues (from 66.8 ± 3.5 nA, before to 86.7 ± 4.2 nA, after the incubation; n = 26; $p < 0.05$, *paired t-test*). (B) The bar graph shows the increase of GABA current amplitude in postnatal control tissues (from 38.0 ± 7.6 nA, before to 45.5 ± 3.3 nA, after incubation; n = 15; $p < 0.001$, paired *t*-test). (C) The bar graph shows the decrease of GABA current amplitude in TSC tissues (from 38.0 ± 3.9 nA, before vs to 30.0 ± 3.3 nA, after incubation; n = 34; $p < 0.001$, *paired t-test*). Inset on the right, for each panel, representative GABA current traces before and after 1 µM SST for 2 h of incubation for each tissue. **(D)** The bar graph represents the mean ± s.e.m. of the I_{GABA} amplitudes evoked from oocytes intranuclearly injected with α1β2γ2 cDNAs before (blue) and after (purple) the acute application of 1 µM SST (from 2709.4 ± 771.0 nA, before, to 2730.2 ± 751.7 nA, after n = 14; $p > 0.05$, *paired t-test*). **(E)** The bar graph represents the mean ± s.e.m. of the I_{GABA} amplitudes evoked from oocytes before (blue) and after (purple) the incubation with 1 µM SST (n = 10; 3061.7 ± 586.8 nA, before and 2972.2 ± 573.7 nA, after the incubation; $p > 0.05$, *paired t-test*). **(F)** Representative GABA current traces (50 µM) before (blue trace) and after the incubation (purple trace).

in oocytes injected with human postnatal tissue. We found that, the application of c-SST blocked this effect (−9.2 ± 4.3 %, n = 16, $p > 0.05$) (Fig. 5B).

Taken together, these results confirm that SST-mediated modulation of GABA currents is receptor-dependent and relies on the activation of SSTRs. Together with the observed PKC dependence, these findings highlight a specific receptor-mediated signaling pathway through which SST regulates GABAergic activity in control tissue.

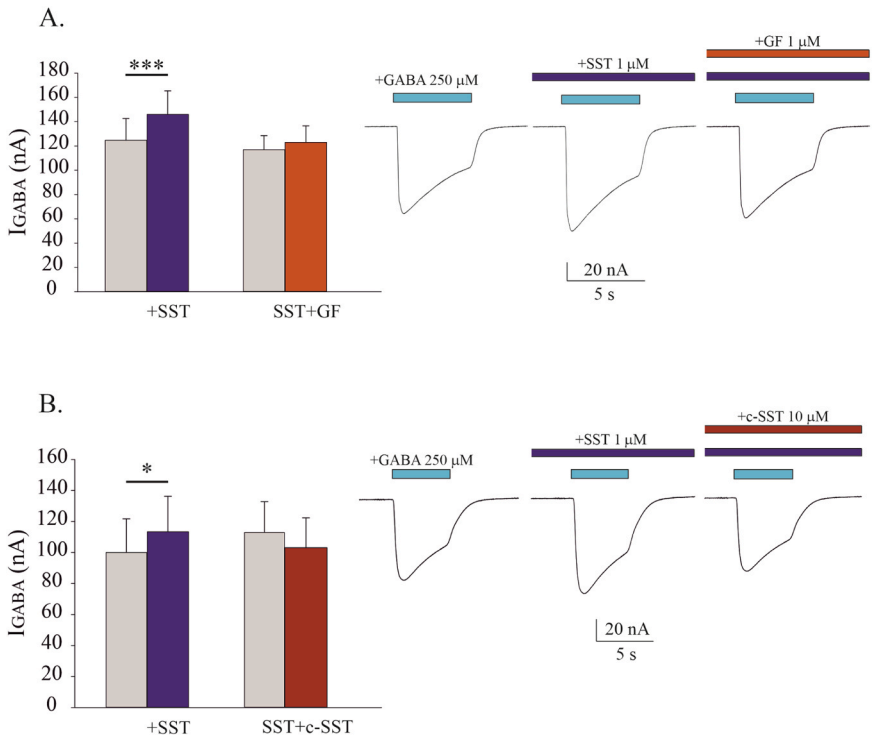

Fig. 5. SST's effect is counteracted by kinase inhibitor GF and non-selective SSTR inhibitor c-SST. (A) Left. The graph shows the variation of GABA-evoked current (as % ± s.e.m) in oocytes injected with human prenatal tissues after the incubation with 1 µM SST alone (purple bar), or in combination with 1 µM GF (orange bar) (I_{GABA} 124.6 ± 17.9 nA, before and 146 ± 19.4 nA, after the incubation with 1 µM SST with an increase of 30.7 ± 13.3; n = 11; $p < 0.001$, *paired t-test vs* 116.9 ± 11.6 nA, before and 123 ± 13.5 nA, after the incubation with 1 µM GF with a % increase of 4.4 ± 4.1; n = 14; $p > 0.05$, *paired t-test*). Right. Representative GABA current traces (250 µM) before and after the incubation with SST alone or in combination with GF. (B) Left. The graph shows the variation of GABA-evoked current (as % ± s.e.m) in oocytes injected with human prenatal tissues after the incubation with 1 µM SST alone (purple bar), or in combination with 10 mM c-SST (red bar) (I_{GABA} 100 ± 21.7 nA, before and 113.4 ± 22.8 nA, after the incubation with 1 µM SST with an increase of 21.9 ± 10.5; n = 14; $p < 0.05$, *paired t-test vs* 112.9 ± 19.9 nA, before and 103.1 ± 19.3 nA, after the incubation with 10 µM c-SST with a % decrease of 9.2 ± 4.3; n = 16; $p > 0.05$, *paired t-test*). Right. Representative GABA current traces (250 µM) before and after the incubation with SST alone or in combination with c-SST.

3.5. Somatostatin effect is modulated by the specific block of SSTR3

To investigate the effect of somatostatin (SST) and the selective SST receptor type 3 (SSTR3) antagonist MK-4256 on GABAergic currents, we performed voltage-clamp recordings on oocytes microtransplanted with membranes from control and TSC tissues.

In control samples (Fig. 6a), the application of SST significantly enhanced GABAergic currents by approximately 20 % compared to baseline GABA application ($p < 0.05$). In contrast, the co-incubation with MK-4256 (1 µM) blocks the effect on GABAergic currents, reducing the amplitude slightly, though this effect did not reach statistical significance.

Furthermore, in TSC (Fig. 6b), the effect of SST leads to a significant reduction in GABAergic current by approximately 20 % ($p < 0.01$), indicating a loss of the facilitatory effect observed in control samples.

Notably, MK-4256 produced an even stronger suppression of GABAergic currents in TSC, with a reduction of nearly 30 % ($p < 0.001$), suggesting that, in TSC, when SSTR3 is completely and specifically blocked, it is possible that the activation of other SST receptors is responsible of further decrease of GABA-evoked currents. These findings suggest that SSTR3 may activate a protective signaling pathway.

4. Discussion

Our study provides novel insights into the potential role of somatostatin (SST) and its receptors in the frontal cortex, implicating their importance in both normal cortical development and the pathophysiology of tuberous sclerosis complex (TSC). Through single-cell sequencing and electrophysiological approaches, we dissected the cell-type–specific expression patterns of SST and its receptors, as well as their functional impact on postsynaptic GABAergic signaling across control and TSC samples.

In line with previous studies, our data confirm that SST is predominantly expressed in GABAergic neurons, while its receptors, particularly SSTR1 and SSTR2, show robust expression in both GABAergic and glutamatergic neurons. This distribution supports the notion that SST acts as a neuromodulator in maintaining the balanced coordination of inhibitory and excitatory signaling (Song et al., 2021). Notably, our differential expression analysis revealed significant alterations in SST receptor profiles in TSC tissues. The upregulation of *SSTR5* in GABAergic neurons and *SSTR2* in glutamatergic neurons, and *SSTR3* is downregulated in both glutamatergic neurons and microglia. Given the overall low expression of SSTR5 across cortical cell types, the magnitude of this change was modest and should be interpreted with caution, as its functional contribution may be limited compared to the more abundantly expressed SSTR2 and SSTR3. However, even relatively modest transcript-level changes in G-protein–coupled receptors such as SSTRs can have disproportionate functional effects, since small shifts in receptor availability or signaling efficacy may substantially alter downstream cascades and network excitability. Moreover, these modest changes at the RNA level may reflect more pronounced effects at the protein or signaling level, particularly in the context of mTOR-driven dysregulation of translation. Nevertheless, even subtle shifts in low-abundance receptors could reflect compensatory or cell-type–specific adaptations within the SST signaling network. Taken together, these findings suggest a reorganization of SST signaling in TSC, which may contribute to the disrupted coordination of excitatory/inhibitory signaling, a hallmark of TSC-related neurodevelopmental disorders and

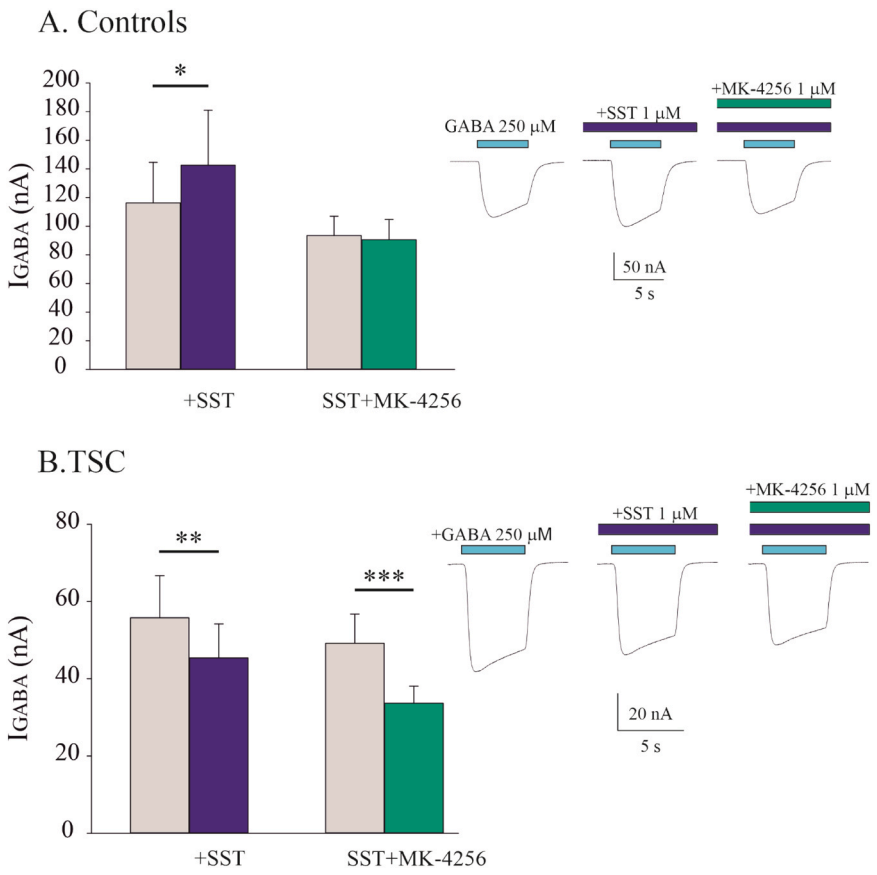

Fig. 6. Effect of SSTR3-selective antagonist MK-4256 on GABA currents. (A) Control. Left. The graph shows the variation of GABA-evoked current (as % ± s.e.m) in oocytes injected with human prenatal tissues after the incubation with 1 µM SST alone (blue bar) or in combination with 1 µM MK-4256 (green bar) (I_{GABA} 116.2 ± 28.3 nA, before and 142.6 ± 38.4 nA, after the incubation with 1 µM SST with a % increase of 19.3 ± 8.6; n = 10; $p < 0.05$, *paired t-test*; 93.52 ± 13.4 nA, before and 90.6 ± 14.1 nA, after the incubation with 1 µM MK-4256 with a % decrease of 4.4 ± 6.3; n = 11; $p > 0.05$, *paired t-test*). Right. Representative GABA current traces (250 µM) before and after the incubation with SST alone or in combination with MK-4256. (B) TSC. Left. The graph shows the variation of GABA-evoked current (as % ± s.e.m) in oocytes injected with human TSC tissues after the incubation with 1 µM SST alone (blue bar) or in combination with 1 µM MK-4256 (green bar) (I_{GABA} 52.5 ± 12.2 nA, before and 44.4 ± 9.8 nA, after the incubation with 1 µM SST with a % decrease of 15.6 ± 5.3; n = 14; $p = 0.007$, *paired t-test*; 49.2 ± 7.6 nA, before and 33.7 ± 4.4 nA, after the incubation with 1 µM MK-4256 with a % decrease of 24.5 ± 4.2; n = 20; $p < 0.001$, *paired t-test*). Right. Representative GABA current traces (250 µM) before and after the incubation with SST alone or in combination with MK-4256.

epileptogenesis.

Functionally, our electrophysiological data on transplanted human brain cellular membranes further reinforce and provide additional support for these molecular findings. In control cellular membranes, SST application enhances GABAergic currents, an effect that is reversed in TSC samples. This differential effect is striking, as it suggests a shift in receptor function or altered downstream signaling cascades in TSC. In control cellular membranes, the potentiation of GABA currents by SST was shown to be receptor-mediated and dependent on PKC activation. Indeed, previous studies report that SSTRs signaling can activate PKC downstream (Pittaluga et al., 2021) and $GABA_A$Rs function is frequently modulated by PKC-mediated phosphorylation (Palma et al., 2005; Brandon et al., 2000; Roseti et al., 2015). When a PKC inhibitor was co-applied, the SST-induced enhancement was abolished, confirming that intracellular signaling through PKC is essential for the SST effect. In contrast, the lack of any direct modulation by SST in a heterologous expression system (oocytes expressing human α1β2γ2 $GABA_A$ receptors) indicates that the action of SST in native tissue relies on a complex cellular context, likely involving native SSTR expression and associated intracellular signaling pathways. Moreover, experiments using a non-selective SSTR inhibitor (c-SST) in human prenatal tissue further validated the receptor dependency of the SST effect. SST application initially increased GABA currents significantly, but subsequent addition of c-SST blocks this effect. Together, these results emphasize that SST modulates GABAergic currents through a finely tuned balance of receptor-mediated processes, regulated by intracellular pathways, particularly involving PKC. The use of a selective SSTR3 antagonist, MK-4256, provided additional insight into the network-specific effects of SST signaling. In control samples, SST application enhanced GABAergic currents by approximately 20 % relative to baseline. However, when MK-4256 was co-applied, the enhanced current returned to baseline levels. This finding indicates that under normal conditions, the SST-mediated potentiation of GABA-evoked currents is largely dependent on SSTR3.

Among the SSTRs, SSTR3 is uniquely enriched in neurons and is the only subtype that localizes specifically to the primary cilia of both excitatory and inhibitory neurons, implicating it in ciliary signaling and neuronal homeostasis (Händel et al., 1999; Schulz et al., 2000). Activation of SSTR3 is linked to the inhibition of adenylyl cyclase through Gi/o proteins, leading to decreased intracellular cAMP levels and modulation of ion channel activity and gene expression (Patel, 1999). During brain development, SSTR3 expression shows dynamic changes and is particularly enriched in cortical and hippocampal regions, where it contributes to neuronal differentiation, migration, and synaptic plasticity (Guadiana et al., 2016).

Blocking this receptor is sufficient to reverse the effect of SST, highlighting SSTR3 may play an important role in mediating SST's effect on GABAergic transmission. Notably, in TSC, SST has the opposite effect,

decreasing the GABAergic currents by about 20 %. When MK-4256 is incubated with SST in these TSC, the suppression of GABA currents becomes even more pronounced, reaching nearly a 30 % decrease. While these observations could suggest a complex interplay among different SST receptor subtypes, the precise mechanisms remain unclear. It is possible that SSTR3 plays a modulatory role, but without direct evidence, any compensatory effects or interactions with other SST receptors and downstream signaling pathways remain speculative. Further studies are required to elucidate the receptor-specific mechanisms underlying these differential responses.

Recent preclinical studies have provided further mechanistic insight into how TSC-related mTORC1 hyperactivation alters GABAergic signaling at the receptor level. In *Tsc1*-deficient mouse models, alterations in $GABA_A$ receptor subunit composition and synaptic localization have been observed (Zhao and Yoshii, 2019). This study reported reduced surface expression of $GABA_A$ receptor α1 subunit in layer 2/3 pyramidal neurons of *Tsc1*-deficient mice, resulting in diminished miniature inhibitory postsynaptic currents (mIPSCs) and increased network excitability (Zhao and Yoshii, 2019). Similarly, a study demonstrated a reduction in postsynaptic $GABA_B$ receptor–mediated inhibition in the medial prefrontal cortex of $Tsc2^{+/-}$ mice, implicating disrupted inhibitory signaling as a key contributor to cortical dysrhythmia (Bassetti et al., 2020). Pharmacological enhancement of GABAergic tone through vigabatrin treatment has been shown to mitigate seizure activity in $Tsc1^{GFAPCKO}$ mice, further supporting the functional relevance of inhibitory pathway deficits (Zhang et al., 2013). Beyond receptor expression, *Tsc1* loss in interneurons influences their developmental trajectory and intrinsic electrophysiological properties, *Tsc1* deletion in the somatostatin lineage reduces fast-spiking properties and represses parvalbumin expression, revealing subtype-specific vulnerabilities within inhibitory circuits (Malik et al., 2019). Collectively, these findings indicate that TSC-related mTORC1 hyperactivation perturbs inhibitory synaptic function through multiple mechanisms, including altered receptor trafficking and subunit composition, interneuron developmental impairments, and changes in postsynaptic responsiveness. These results provide a mechanistic framework that complements our human data and underscore the broader impact of GABAergic and SST-related signaling perturbations in TSC pathophysiology.

Beyond TSC, alterations in SST signaling have been implicated in a variety of neurodevelopmental disorders. Reduced SST expression and dysfunctional SST interneurons have been observed in autism spectrum disorder (ASD) and schizophrenia which, like TSC, involve excitatory/inhibitory imbalances, cognitive impairments, and seizures. SST deficits in these disorders are linked to abnormal connectivity and network imbalance. Our results support the idea that disrupted SST signaling represents a shared mechanism across neurodevelopmental disorders. However, the receptor-specific alterations identified in TSC suggest a distinct pathophysiological signature that could offer opportunities for targeted therapeutic modulation. In TSC, the reorganization of SST receptor expression may not only contribute to the characteristic epileptogenic network activity but also to the broader spectrum of neurodevelopmental abnormalities seen in these patients.

The developmental trajectory of our findings adds another layer of complexity. The gradual increase in *SSTR3* expression during cortical maturation, observed from the prenatal to postnatal stages, marks a critical window for SST-driven inhibitory circuit formation. In TSC, the aberrant regulation of SST receptors suggests that developmental immaturity or a disrupted maturation process of these receptors could contribute to the onset of neurological deficits. Since TSC is a neurodevelopmental disorder characterized by seizures and cognitive impairments, altered SST receptor expression and function may play a pivotal role in the disease's pathogenesis.

Interestingly, the lack of significant differences in SST receptor expression in FCD samples compared to controls suggests that the alterations in SST signaling may be specific to the molecular pathology of TSC. One potential explanation is that in TSC, germline mutations in *TSC1* or *TSC2* result in constitutive mTORC1 hyperactivation across all affected cells, causing widespread developmental abnormalities that secondarily impact SST receptor expression. By contrast, MTOR-mutant FCD arises from somatic mosaic mutations, which are restricted to subsets of cortical cells and may therefore not drive broad transcriptional changes in SST signaling. Furthermore, while receptor transcript levels were unchanged in FCD, functional alterations may still occur at post-transcriptional or post-translational levels, such as through receptor trafficking, localization, or downstream signaling efficacy. These differences suggest that although both disorders converge on mTOR dysregulation, the consequences for SST signaling are shaped by the timing, distribution, and nature of the underlying genetic insult. This specificity not only advances our understanding of the disease mechanisms underlying TSC but also paves the way for targeted therapeutic interventions. Modulating SST receptors activity and thereby restoring the balance between excitatory and inhibitory signaling, could represent a promising strategy for ameliorating the neurodevelopmental and epileptogenic outcomes associated with TSC.

Despite these significant findings, our study has several limitations. First, the sample sizes, especially for the TSC and FCD tissues, were relatively small, which may limit the translatability of our findings. However, it should be considered that TSC is a rare disease, and the availability of high-quality resected human tissue is inherently limited, making large-scale studies in this population challenging. Moreover, while single-cell sequencing provides valuable insights into gene expression at the cellular level, it does not capture dynamic changes in protein levels or receptor function over time. The electrophysiological assessments, although informative, were performed in ex vivo cellular membranes and heterologous expression systems that may not fully recapitulate the in vivo complexity of cortical networks. However, it has been clearly showed that the oocytes microtransplantation preserves the native receptor properties as previously published (Palma et al., 2003). Furthermore, our study primarily focuses on the frontal cortex, and it remains unclear whether similar alterations in SST signaling occur in other brain regions affected by tuberal tissues.

Future studies should aim to address these limitations by expanding the sample sizes and incorporating longitudinal analyses to capture developmental dynamics more comprehensively. In vivo models, such as conditional knockout or transgenic animals, could be employed to more directly investigate the causal roles of SST and its receptors in TSC pathogenesis. Furthermore, exploring the intracellular signaling cascades downstream of SST receptor activation in both neurons and microglia will be critical for understanding the mechanistic underpinnings of the observed effects. Finally, given the parallels between TSC and other neurodevelopmental disorders, it would be valuable to investigate whether modulating SST signaling can restore network balance and improve cognitive and epileptogenic outcomes across different conditions.

In summary, our findings contribute to a better understanding of how SST and its receptors may influence cortical inhibitory circuits during development. In TSC, altered receptor expression could be one of several factors involved in the disrupted excitatory/inhibitory coordination and associated neurological features. While these data enrich our knowledge of SST-mediated functions and their potential role in network phenotypes of TSC, they likely represent one part of a complex, multifactorial process. Further physiological studies, ideally in more integrative models, are necessary to clarify the precise impact of SST signaling alterations and to evaluate the therapeutic potential of targeting these receptors.

Author contributions

E.A, J.D.M, G.R, E.P and M.S conceptualized the project. F.E.J, W.V. H, and A.M, helped with collection, selection and/or revision of human brain tissues and clinical data. M.S selected all scRNAseq samples and J.

J.A. prepared and cut all samples for sequencing. All processing and bioinformatical analysis was performed by M.S, under supervision of E.A and J.D.M. Electrophysiological experiments were performed by A.G., L. J.L, and G.R., under supervision of E.P. The original draft of the manuscript was prepared by M.S and A.G. with review and editing by J. D.M, E.A., D.S., and E.P. All authors read, refined and approved the final version of the manuscript.

CRediT authorship contribution statement

Eleonora Palma: Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Mirte Scheper:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Eleonora Aronica:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Alessandro Gaeta:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Gabriele Ruffolo:** Writing – review & editing, Supervision, Resources, Investigation, Conceptualization. **Lilian J. Lissner:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Wim van Hecke:** Resources. **Angelika Mühlebner:** Resources. **Dirk Schubert:** Writing – review & editing, Resources, Funding acquisition. **James D. Mills:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Marie Le Bihan:** Writing – review & editing, Resources. **Jasper J. Anink:** Resources. **Floor E. Jansen:** Resources.

Ethics approval

All procedures with human tissue were obtained with informed consent for the use in research and access to medical records in accordance with the Declaration of Helsinki and the Amsterdam UMC Research Code provided by the Medical Ethics Committee.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mirte Scheper reports financial support was provided by ZonMW. Eleonora Palma reports financial support was provided by Fondi Ateneo. Gabriele Ruffolo reports financial support was provided by Italian ministry of university and research. Gabriele Ruffolo reports financial support was provided by Italian ministry of health. Eleonora Aronica reports financial support was provided by ZonMW. Gabriele Ruffolo reports financial support was provided by Pasteur Institute-Cenci Bolognetti Foundation. Eleonora Aronica reports a relationship with UCB Pharma BV that includes: board membership. Eleonora Aronica reports a relationship with Danone Nutricia Research that includes: board membership. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pneurobio.2025.102864.

Data availability

The sequencing data underlying this study are currently available from the corresponding author upon reasonable request. These data will be deposited in a public repository and made publicly accessible prior to publication. The accession number will be provided in the final version of the manuscript.

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