Thalamic distribution and effects of 5-HT2C receptors on tonic GABAA inhibition and absence seizures: implications for treatment

Anna Cavaccini[1,2], Marcello Venzi[1], Cristiano Bombardi[3], Vincenzo Crunelli[1,4]* and Giuseppe Di Giovanni[1,5,6]*

[1]Neuroscience Division, School of Bioscience, Cardiff University, Cardiff, United Kingdom, [2]Laboratory of Neural Circuit Assembly, Brain Research Institute, University of Zurich, Zurich, Switzerland, [3]Department of Veterinary Medical Science, University of Bologna, Bologna, Italy, [4]Institute of Pharmacology and Neurosciences, Faculty of Medicine, University of Lisbon, Lisbon, Portugal, [5]College of Medicine, Korea University, Seoul, Republic of Korea, [6]Department of Medical and Surgical Sciences, University of Magna Graecia, Catanzaro, Italy

Introduction: Childhood absence epilepsy (CAE) is associated with abnormal thalamocortical oscillations and enhanced GABAergic function in the ventrobasal (VB) thalamus, including increased extrasynaptic $GABA_A$ receptor−mediated tonic currents in thalamocortical (TC) neurons. Serotonin signaling modulates seizure activity in several epilepsy models, and activation of $5\text{-}HT_{2C}$ receptors ($5\text{-}HT_{2C}Rs$) has been reported to exert anti-absence seizure effects, although the underlying cellular mechanisms remain unclear. Here, we examined the thalamic distribution of $5\text{-}HT_{2C}Rs$ and their functional impact on tonic GABAA inhibition and absence seizures.

Methods: $5\text{-}HT_{2C}R$ expression in the nucleus reticularis thalami (NRT) and VB was assessed by immunohistochemistry in adult Wistar rats, Genetic Absence Epilepsy Rats from Strasbourg (GAERS), and their non-epileptic control strain (NEC). Whole-cell patch-clamp recordings were used to measure tonic GABAA currents in VB TC neurons in thalamic slices. In vivo EEG recordings in freely moving GAERS rats were performed to evaluate the effects of systemic administration of the 5-HT2CR agonist Ro 60-0175 on absence seizures.

Results: No differences in $5\text{-}HT_{2C}R$ expression were observed in the NRT across strains. In the VB, receptor expression was lowest in GAERS and highest in Wistar rats compared with NEC. Tonic GABAA currents in TC neurons were larger in GAERS than in Wistar or NEC rats. Activation of $5\text{-}HT_{2C}Rs$ with Ro 60-0175 reduced tonic GABAA currents in TC neurons in all strains. Systemic administration of Ro 60-0175 in adult GAERS produced a clear reduction in absence seizures.

Discussion: These findings indicate that $5\text{-}HT_{2C}Rs$ regulate thalamic extrasynaptic GABAA inhibition and that their activation reduces tonic inhibitory drive in TC neurons while exerting anti-absence effects in vivo. The lower expression of $5\text{-}HT_{2C}Rs$ in the GAERS VB suggests altered serotonergic control of thalamic inhibition in absence epilepsy. By reducing tonic GABAergic

currents, 5-HT$_{2C}$R activation may rebalance thalamocortical activity and suppress pathological oscillations, supporting these receptors as potential therapeutic targets for CAE.

1 Introduction

Childhood absence epilepsy (CAE) is characterized by brief non-convulsive seizures with spike-and-wave discharges (SWDs), arising from abnormal network oscillations (Crunelli and Leresche, 2002). Anatomical, electrophysiological, and computational studies indicate that these seizures emerge from dysfunctional interactions within the cortico–thalamo–cortical circuit, with additional contributions from basal ganglia networks (Vergnes et al., 1986; Hu and Wang, 2015; Depaulis et al., 2016; Hu et al., 2017; Jarre et al., 2017; Crunelli et al., 2020).

Thalamocortical (TC) neurons in the somatosensory ventrobasal thalamic complex (VB) exhibit phasic and tonic γ-aminobutyric acid type A (GABA$_A$) currents (Belelli et al., 2005; Cope et al., 2005; Cope et al., 2009). Phasic inhibition depends on synaptic GABA$_A$ receptors (GABA$_A$R) (Cope et al., 2005) activated by GABA released from neurons of the nucleus reticularis thalami (NRT) (Belelli et al., 2005; Cope et al., 2005). In contrast, tonic GABA$_A$ inhibition result from α4β2δ subunit-containing extrasynaptic GABA$_A$Rs binding ambient GABA (Cope et al., 2005; Jia et al., 2005; Cope et al., 2009). This tonic current magnitude primarily depends on extracellular GABA levels which are mainly regulated by astrocytic GABA uptake and GABA release via glial transporters and bestrophin-1 (Cope et al., 2009; Kwak et al., 2020), though with a contribution from GABA release from the NRT (Herd et al., 2013).

Aberrant tonic GABA$_A$ currents have been reported in different brain conditions including stress, stroke, traumatic brain injury, Parkinson's disease and focal and generalized seizures, including absence seizures (ASs) (Belelli et al., 2009; Cope et al., 2009; Crunelli et al., 2012; Walker et al., 2012; Errington et al., 2014; Heo et al., 2020). These are genetic non-convulsive seizures that are characterized by brief lapses of consciousness with EEG SWDs (Huguenard, 2019; Crunelli et al., 2020; Deidda et al., 2021). We previously showed that in rodent models of ASs there is an enhanced tonic GABA$_A$ current in VB TC neurons, due to a loss-of-function of the astrocytic GABA transporter 1 (GAT-1), which plays a key role in the expression of ASs (Cope et al., 2009; Errington et al., 2011; Crunelli et al., 2020; Deidda et al., 2021; Morais et al., 2021). This finding is supported by human data, showing that anti-seizure medicines (ASMs) that increase GABA levels can elicit and aggravate ASs (Perucca et al., 1998), and higher thalamic GABA levels were found in a child with ASs (Leal et al., 2016).

Thus, normalization of tonic GABA$_A$ inhibition might have potential therapeutic value for rescuing ASs. Unfortunately, no selective antagonists for α4β2δ subunit-containing GABA$_A$Rs have been synthesized yet. Thus, there is the need to modulate tonic GABA$_A$ tonic inhibition indirectly, particularly through

modulatory neurotransmitter systems. Among brainstem neurotransmitters modulating TC neuron excitability (McCormick, 1992; Di Giovanni et al., 2008; Crunelli and Di Giovanni, 2014; Deidda et al., 2021), serotonin (5-HT) is a key player, since it regulates cortico-thalamic activity (Varela and Sherman, 2009; Puig and Gulledge, 2011; Yang et al., 2015), impacting arousal, vigilance and cognition (Jacobs et al., 2000). The rodent thalamus receives dense and heterogeneous innervation from the raphe nuclei (Dahlstroem and Fuxe, 1964; Vertes et al., 1999; Rodriguez et al., 2011), with 5-HT receptors widely expressed in TC, NRT and thalamic interneurons in a nucleus-specific manner (Munsch et al., 2003; Li et al., 2004; Li et al., 2004; Bonnin et al., 2006; Coulon et al., 2010; Rodriguez et al., 2011). Additionally, 5-HT modulates thalamic GABA function (Bankson and Yamamoto, 2004; Crunelli and Di Giovanni, 2014; Crunelli and Di Giovanni, 2015; Goitia et al., 2016), and enhances GABA release (Deng and Lei, 2008).

5-HT modulates ASs in various rodent models (Jakus et al., 2003; Graf et al., 2004; Jakus et al., 2011; Russo et al., 2013; Deidda et al., 2021). Recently, we demonstrated altered 5-HT concentration in specific brain regions (De Deurwaerdère et al., 2022), and the anti-absence effect of activating 5-HT$_{2C}$Rs in Genetic Absence Epilepsy rats from Strasbourg (GAERS) (Venzi et al., 2016), a well-validated model of ASs (Vergnes et al., 1982; Depaulis et al., 2016). However, the alteration of thalamic 5-HT$_{2C}$R expression and the cellular mechanism responsible for the 5-HT$_{2C}$R modulation of ASs remains unknown. Notably, 5-HT modulates tonic GABA$_A$ currents in the visual thalamus (the dorsal lateral geniculate nucleus - dLGN) (Coulon et al., 2010; Crunelli and Di Giovanni, 2015), suggesting that the anti-absence effect of 5-HT$_{2C}$R activation may depend on the ability of these receptors to modulate tonic GABA$_A$ inhibition in VB thalamus.

Here, we directly tested this hypothesis by examining the distribution of 5-HT$_{2C}$Rs in the VB and NRT using immunohistochemistry and by assessing the effects of 5-HT$_{2C}$R activation on tonic GABA$_A$ current in VB TC neurons of GAERS rats. Compared to outbred Wistar rats and. Because recent studies have shown that inbred non-epileptic control (NEC) rats display anatomical, neurochemical, and behavioral alterations despite the absence of seizures (Neuparth-Sottomayor et al., 2023; Casarrubea et al., 2024), we complemented the traditional GAERS/NEC comparison by including outbred Wistar rats, from which both strains are derived (Vergnes et al., 1982; Depaulis et al., 2016), as an additional reference strain. We found that 5-HT$_{2C}$R expression was reduced in the GAERS VB compared to that of NEC and Wistar rats, while similar in the NRT among the three strains. Moreover, 5-HT$_{2C}$R activation decreased tonic GABA$_A$ inhibition *in vitro* and suppressed spontaneous ASs in freely moving GAERS rats, identifying these receptors as a promising therapeutic target.

2 Materials and methods

2.1 Experimental animals

All animal procedures were authorized by Cardiff University, in agreement with the United Kingdom Animals (Scientific Procedures) Act (1986) and international laws and policies (EU Directive, 2010/63/EU) for animal experiments. Male Wistar, GAERS and NEC rats were obtained from colonies bred at Cardiff University and housed at 21 °C under a 12 h light/dark cycle, with food and water available *ad libitum*. Immunohistochemistry and electrophysiological *in vitro* recordings were conducted on postnatal day (P) 25–30 rats, EEG recordings in P90-P120 rats. Every effort was made to minimize animal suffering and to reduce the number of animals used (Lidster et al., 2016).

2.2 Immunohistochemistry

Nine P25 rats (three NEC, three GAERS, three Wistar) were anesthetized (sodium pentobarbital 48 mg/kg, chloral hydrate 40 mg/kg, i.p.) and transcardially perfused with ice-cold saline (2 min, 30–35 mL/min) followed by 4% paraformaldehyde (30 min, 10 mL/min). Brains were post-fixed (3 h), cryoprotected in 30% sucrose (48 h, 4 °C), and sectioned coronally (30 μm) using a freezing microtome. Sections were stored in 30% sucrose/PBS (−20 °C) for immunohistochemistry or 10% formalin (RT) for thionin staining.

Free-floating coronal sections were washed in 0.02M PBS (3 × 10 min), treated with 1% H_2O_2 (15–30 min) to quench peroxidase activity, then rinsed (6×) in PBS. To block non-specific binding, sections were incubated (2 h, RT) in 10% normal goat serum (NGS, #CS 0922, Colorado Serum Co.) and 0.3% Triton X-100 in PBS. They were then incubated (48 h, 4 °C) with mouse anti-5-HT$_{2C}$R antibody (1:100, sc-17797, Santa Cruz) in 1% NGS and 0.3% Triton X-100. Sections were washed (3 × 10 min) in PBS +2% NGS, then incubated (1 h, RT) with biotinylated goat anti-mouse IgG (1:200, BA-9200, Vector) in 1% NGS and 0.3% Triton X-100. After washing (3 × 10 min, PBS +2% NGS), they were transferred to an avidin–biotin complex (ABC kit, PK-6100, Vector) for 45 min, and the immunoperoxidase reaction was developed using a DAB kit (SK-4100, Vector). Sections were mounted on gelatin-coated slides, dried (overnight, 37 °C), dehydrated in ethanol, cleared in xylene, and coverslipped with Entellan (Merck, Darmstadt, Germany). For enhancement, sections were defatted, treated with OsO_4 (0.005%, EMS #19130) and thiocarbohydrazide (0.05%, EMS #21900), then coverslipped with Entellan.

The mouse monoclonal anti-5-HT$_{2C}$R antibody targets amino acids 374-458 of the human 5-HT$_{2C}$R C-terminus. Its specificity was confirmed via Western blot and immunohistochemistry (Nocjar et al., 2015). In this study, control sections without primary antibodies showed no staining, and replacing or omitting secondary antibodies abolished immunostaining.

Adjacent sections were thionin-stained to delineate brain region boundaries. Sections were removed from 10% formaldehyde, mounted on gelatin-coated slides, and dried overnight at 37 °C. They were defatted in chloroform/ethanol (1:1) for 1 h, rehydrated through graded ethanol (100%, 96%, 70%, 50%; 2 min each), rinsed

in dH_2O (2 min), stained with 0.125% thionin (30 s), dehydrated, and coverslipped with Entellan (Merck, Darmstadt, Germany).

Sections were analyzed using a Leica DMRB microscope. Brightfield images were captured with a Polaroid DMC digital camera and DMC 2 software. Contrast and brightness were adjusted in Adobe Photoshop CS3. To assess 5-HT$_{2C}$R-immunoreactive (IR) neuron density in the NRT and VB, immunostained somata were plotted in every fifth section using Accustage 5.1. Regional boundaries were outlined from adjacent thionin-stained sections with a stereo microscope and overlaid on computer-generated plots in Corel Draw X3. Area measurements were performed using AxioVision Rel.4.8. Neuron density (somata/mm^2) was calculated separately for each section. Five sections per rat were analyzed, with counts expressed as mean ± SEM. Data were statistically analyzed using One-Way ANOVA with Tukey's multiple comparisons. The Bonferroni test yielded similar results. Statistical significance was set at $P < 0.05$.

2.3 *In vitro* electrophysiological recordings and analysis

2.3.1 Slice preparation

Acute horizontal brain slices containing the VB and the NRT were prepared as described previously (Cope et al., 2009). Briefly, acute horizontal brain slices (300 μm) containing the VB and the NRT were prepared from male P20-25 Wistar, GAERS and NEC rats, as described previously (Cope et al., 2005; Cope et al., 2009). During cutting, slices were maintained in a continuously oxygenated (95% O_2: 5% CO_2) ice-cold artificial cerebrospinal fluid (aCSF) containing (mM): 126 NaCl, 26 NaHCO$_3$, 2.5 KCl, 2 MgCl$_2$, 2 CaCl$_2$, 1.25 NaH$_2$PO$_4$, 10 glucose, 0.045 indomethacin and 3 kynurenic acid. After cutting, slices were incubated in a chamber containing oxygenated aCSF of the above composition without indomethacin and kynurenic acid, for at least 1 h, before being transferred to the recording chamber. In the recording chamber, slices were continuously perfused (~2 mL/min) with warmed (32 °C ± 1 °C) oxygenated recording aCSF of above composition, but with a lower concentration of MgCl$_2$ (1 mM) and without indomethacin. Kynurenic acid was used in the cutting solution to increase slice viability and in the recording solution to block ionotropic glutamate receptors and therefore isolate GABA$_A$R currents. Experiments were performed on only a single neuron for each slice.

2.3.2 Electrophysiology

TC neurons of the VB were visualized using a Nikon (Tokyo, Japan) Eclipse E600FN microscope equipped with a 40x immersion lens and a video camera (Hamamatsu, Hamamatsu City, Japan). Whole-cell patch clamp recordings were made from VB TC neurons held at −70 mV using pipettes (resistance, 2.5–5 MΩ) containing (mM): 130 CsCl, 2 MgCl$_2$, 4 Mg-ATP, 0.3 Na-GTP, 10 Na-HEPES and 0.1 EGTA, pH 7.25-7.30, (290–295 mOsm). Data were acquired using an Axopatch 200B amplifier controlled by pClamp 9.0 software (Molecular Device), filtered at 2 kHz and sampled at 20 kHz (Digidata 1322A; Molecular Device). Series resistance and whole-cell capacitance were determined from responses to 5 mV voltage steps. Series resistance was compensated by ~80% and was continuously monitored during recordings. If the access resistance changed >20%, data were excluded from the analysis.

2.3.3 Data collection, analysis and statistics

The tonic GABA$_A$ current was measured as the outward change in baseline current observed following focal (via a pipette) application onto each slice of the GABA$_A$ antagonist gabazine (100 μM, GBZ) (holding potential: −70 mV), as previously described (Rosenberg et al., 2017). In particular, the baseline current was measured as the averaged 20 s current before GBZ application. The shift in baseline current, measured as the averaged 20 s current after GBZ application, was compared to the baseline current. The average membrane capacitance of recorded VB TC neurons at P25–P30 was ~100–120 pF, consistent with previous electrophysiological studies in juvenile GAERS and control rats (Cope et al., 2005; Cope et al., 2009). Tonic GABA$_A$ current amplitude was normalized to whole-cell capacitance for each neuron. 5-HT$_{2C}$R ligands were applied in the recording solution, either alone or in combination. Only one TC neuron was recorded in each slice, with each animal contributing two to three cells in total.

2.4 *In vivo* EEG recordings and analysis

EEG recordings were conducted on P90-P120 old male GAERS rats. The surgical procedure for chronic EEG implantation is described in detail elsewhere (Cope et al., 2009). Briefly, animals were anaesthetized with isoflurane and the body temperature maintained at 37 °C via a homoeothermic heat blanket (Harvard Apparatus, United States). Gold-plated EEG screw electrodes (Svenska Dentorama, Sweden) were implanted bilaterally over frontal and parietal cortices, and two ground electrodes were placed over the cerebellum. All electrodes were secured with acrylic cement. Rats were administered 0.5 mg/kg meloxicam post-operatively and were allowed to recover for at least 5 days before the start of the experiments.

On the day of the experiment the following drug administration protocol was used: rats were connected to the recording apparatus and left undisturbed for 1 h (habituation period), then the EEG and video recordings were started and a 40-min control period was recorded (control period). Afterwards, GAERS rats were randomly assigned to receive an intraperitoneal injection of either the selective 5-HT$_{2C}$R agonist Ro 60-0175 (3 mg/kg, i. p.) (Orban et al., 2014; Di Giovanni and De Deurwaerdere, 2016; Silenieks et al., 2019) or its vehicle (0.1% of DMSO in saline), and the EEG and video recordings continued for an additional 2 h period (treatment period).

Data were acquired with a SBA4-v6 BioAmp amplifier (SuperTech Inc., Hungary), sampled at 1,000 Hz (Cambridge Electronic Design, CED, Micro3 D.130) and analyzed with CED Spike2 v7.3 and Matlab (R2014b, The Mathworks Inc., United States). Visual inspection and video confirmation were performed without knowledge of treatment condition. Detection of SWDs was performed as previously described (Cope et al., 2009). Briefly, the EEG data were DC removed and SWDs were detected semi-automatically using a custom-made Spike2 (SeizureDetect, Spike2, CED, Cambridge, United Kingdom). Detection was further refined by visual inspection of the data that was also used to confirm that the presence of motor arrest accompanied the EEG SWD, i.e., that an AS had occurred. Seizures were quantified using three parameters: total time spent in seizure, number of seizures and average seizure length (Marescaux et al., 1992). Values were normalized by expressing them as a percentage of the corresponding parameter in the control period (−40 to 0 min).

2.4.1 Drugs

The 5-HT$_{2C}$R agonist (2S)-1-(6-chloro-5-fluoroindol-1-yl) propan-2-amine (Ro 60-0175), the 5-HT$_{2C}$R antagonist 6-chloro-5-methyl-N-(6-(2-methylpyridin-3-yl)oxypyridin-3-yl)-2 (SB242084) and gabazine (SR 95531 hydrobromide) were purchased from Tocris Bioscience (United Kingdom). The doses of Ro 60-0175 were chosen according to previous published works on epilepsy animal models and its general pharmacology to avoid off-target effects (Di Giovanni and De Deurwaerdere, 2016). General laboratory salts, indomethacin and kynurenic acid were purchased from Sigma Aldrich (United Kingdom).

2.4.2 Statistics

Electrophysiological datawere analysed by one-way ANOVA (GraphPad Instat 3 software) followed by *post hoc* analyses (Dunnett's and Dunn's multiple comparison tests) if ANOVA yielded significant main effects. For comparisons of two groups, an independent two-sample t-test was used (GraphPad Instat 3 software). Significance was set at $p < 0.05$ for all statistical tests. All quantitative data are presented in the text and figures as mean ± SEM.

The anti-absence effects of the 5-HT$_{2C}$R agonist in freely moving adult GAERS were assessed using a two-way repeated measures ANOVA. Treatment (drug vs. control) was included as the between-subjects factor, while time (1st vs. 2nd h) served as the within-subjects factor. To assess main effects and interactions on seizure frequency, Tukey's *post hoc* test was applied.

3 Results

3.1 5-HT$_{2C}$R-immunostaining in the NRT and VB of NEC, GAERS and Wistar rats

5-HT$_{2C}$Rs are expressed in TC neurons across different thalamic nuclei with a nucleus-specific distribution (Munsch et al., 2003; Li et al., 2004; Coulon et al., 2010). In line with this evidence, 5-HT$_{2C}$R immunostaining was detected in the somata and neuropil of the NRT and VB neurons in NEC, Wistar and GAERS. Neuropil immunoreactivity was diffuse, not associated with identifiable neuronal structure, and consistent among the three strains (Figures 1A,D,G). In the NRT, the density of 5-HT$_{2C}$R-immunopositive (5-HT$_{2C}$R$^+$) neurons was unchanged across the three strains (Figures 1B,E,H–J). However, a one-way ANOVA revealed a significantly higher density of 5-HT$_{2C}$R$^+$ neurons in the VB of Wistar than NEC and GAERS rats (n = 3 for each strain, $P < 0.05$), with GAERS showing the lowest number of 5-HT$_{2C}$R$^+$ neurons ($P < 0.05$) (Figures 1C,F,I,K).

3.2 5-HT$_{2C}$R modulation of the tonic GABA$_A$ current in GAERS and NEC rats

To assess the potential translational significance of the above data for ASs, we next investigated whether the 5-HT$_{2C}$R agonist Ro 60-175 was capable of reducing the aberrant tonic GABA$_A$ current in VB TC neurons of GAERS (Venzi et al., 2016). We confirmed the presence of an enhanced tonic GABA$_A$ current in epileptic GAERS (GAERS$_{CTL}$: 2 ± 0.5 pA/pF, n = 6) compared to non-epileptic NEC
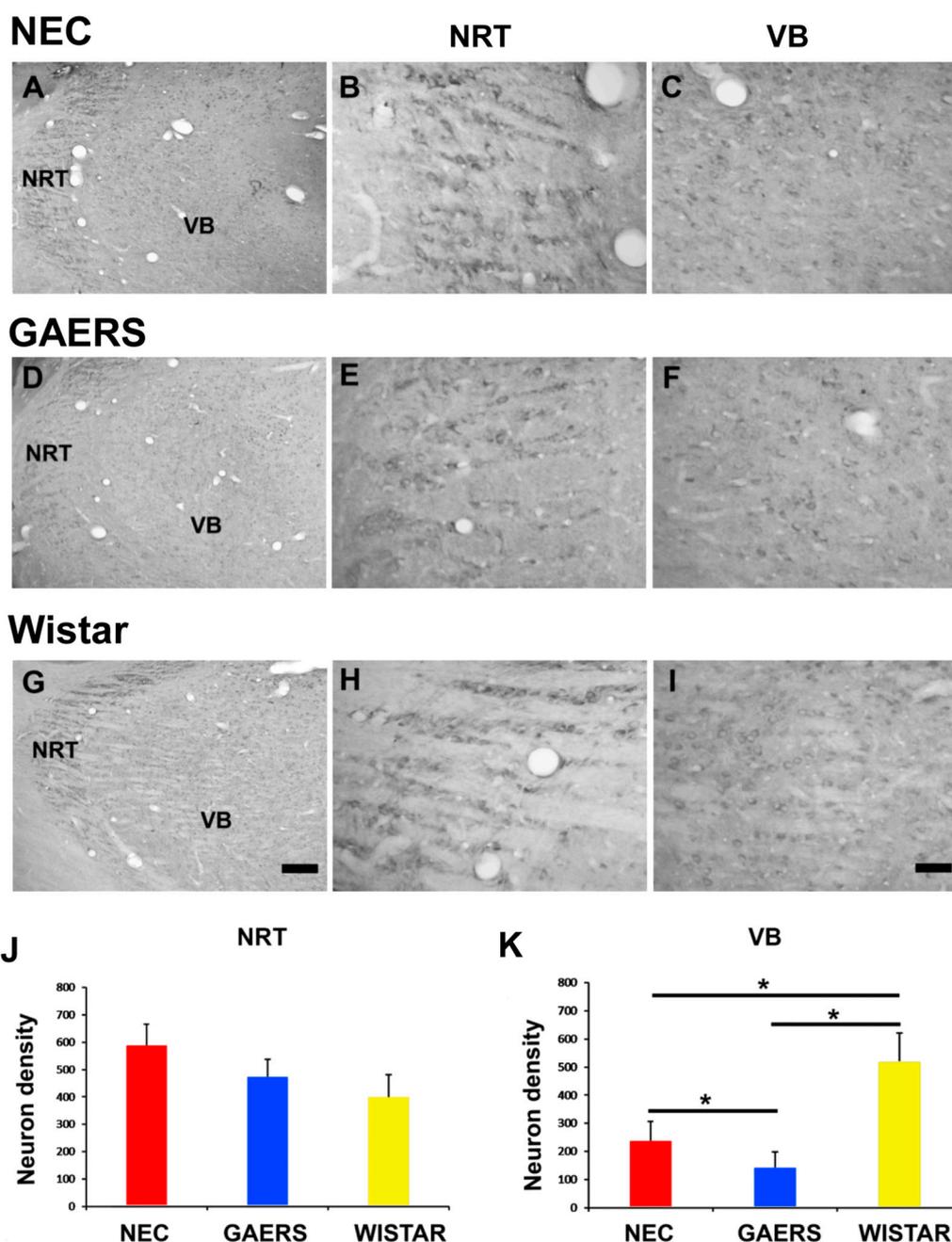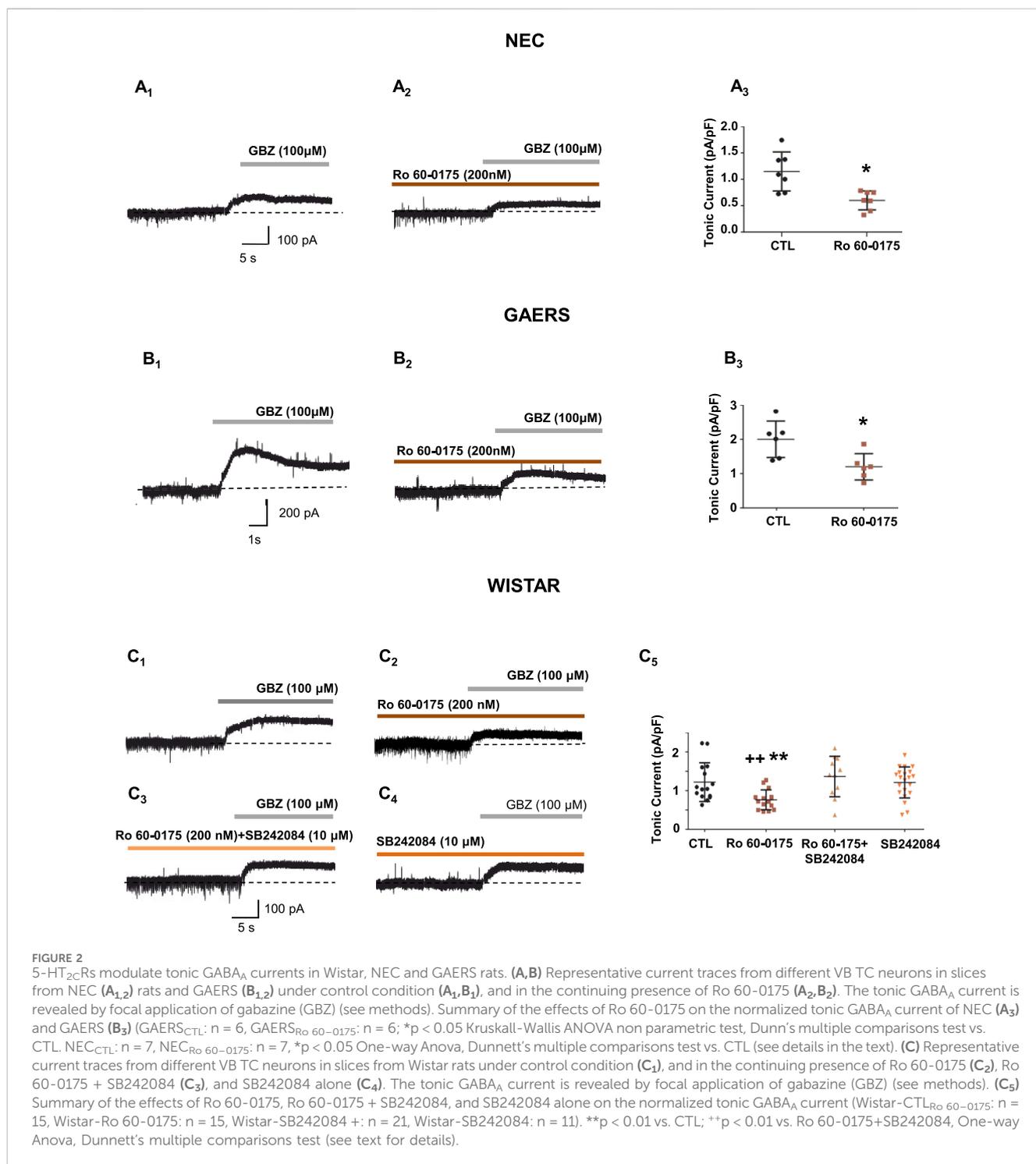
FIGURE 1
Distribution of 5-HT$_{2C}$Rs in the VB and NRT. Brightfield photomicrographs showing the distribution of 5-HT$_{2C}$ receptor immunoreactivity in the VB and NRT **(A,D,G)**, and enlarged sections of the NRT **(B,E,H)** and VB **(C,F,I)** of P25-30 NEC, GAERS and Wistar rats, as indicated. **(J,K)** Quantification of 5-HT$_{2C}$R$^+$ neuron density in the NRT and VB of the three strains. No significant differences were observed in the NRT. In the VB, Wistar rats displayed a significantly higher 5-HT$_{2C}$R$^+$ neuron density than NEC and GAERS ($P < 0.05$), with GAERS showing the lowest count ($P < 0.05$). Scale bar = 200 µm in **(G)** (applies to **(A,D,G)**) and = 50 µm in **(I)** (applies to **(B,C,E,F,H,I)**). One-Way ANOVA with Tukey's multiple post-test comparison (*$P < 0.05$).

(NEC$_{CTL}$: 1.1 ± 0.4 pA/pF, n = 7, p < 0.01) (Figures 2A$_{1,3}$, B$_{1,3}$). Perfusion of slices with Ro 60–0175 significantly decreased (40% reduction) the tonic GABA$_A$ current of GAERS (GAERS$_{Ro\ 60-0175}$: 1.2 ± 0.4 pA/pF, n = 6, p < 0.05 vs. GAERS$_{CTL}$) compared to that observed under control condition (Figures 2B$_{2,3}$). Moreover, Ro 60–0175 significantly decreased (48% reduction) tonic GABA$_A$ current in NEC rats (NEC$_{Ro\ 60-0175}$: 0.6 ± 0.2 pA/pF, n = 7, p < 0.05 vs. NEC$_{CTL}$) (Figures 2A$_{2,3}$).

3.3 5-HT$_{2C}$Rs modulate the tonic GABA$_A$ current in Wistar rats

The selective 5-HT$_{2C}$R agonist Ro 60–0175 significantly decreased (33% reduction) tonic GABA$_A$ current of VB TC neurons (Wistar-RO 60-0175: 0.8 ± 0.3 pA/pF, n = 15) compared to control (Wistar-CTL$_{RO\ 60-0175}$: 1.2 ± 0.5 pA/pF, n = 15; p < 0.01) (Figures 2C$_{1,2,5}$). This inhibitory effect of Ro

FIGURE 2
5-HT$_{2C}$Rs modulate tonic GABA$_A$ currents in Wistar, NEC and GAERS rats. **(A,B)** Representative current traces from different VB TC neurons in slices from NEC **(A$_{1,2}$)** rats and GAERS **(B$_{1,2}$)** under control condition **(A$_1$,B$_1$)**, and in the continuing presence of Ro 60-0175 **(A$_2$,B$_2$)**. The tonic GABA$_A$ current is revealed by focal application of gabazine (GBZ) (see methods). Summary of the effects of Ro 60-0175 on the normalized tonic GABA$_A$ current of NEC **(A$_3$)** and GAERS **(B$_3$)** (GAERS$_{CTL}$: n = 6, GAERS$_{Ro\ 60-0175}$: n = 6; *p < 0.05 Kruskall-Wallis ANOVA non parametric test, Dunn's multiple comparisons test vs. CTL. NEC$_{CTL}$: n = 7, NEC$_{Ro\ 60-0175}$: n = 7, *p < 0.05 One-way Anova, Dunnett's multiple comparisons test vs. CTL (see details in the text). **(C)** Representative current traces from different VB TC neurons in slices from Wistar rats under control condition **(C$_1$)**, and in the continuing presence of Ro 60-0175 **(C$_2$)**, Ro 60-0175 + SB242084 **(C$_3$)**, and SB242084 alone **(C$_4$)**. The tonic GABA$_A$ current is revealed by focal application of gabazine (GBZ) (see methods). **(C$_5$)** Summary of the effects of Ro 60-0175, Ro 60-0175 + SB242084, and SB242084 alone on the normalized tonic GABA$_A$ current (Wistar-CTL$_{Ro\ 60-0175}$: n = 15, Wistar-Ro 60-0175: n = 15, Wistar-SB242084 +: n = 21, Wistar-SB242084: n = 11). **p < 0.01 vs. CTL; $^{++}$p < 0.01 vs. Ro 60-0175+SB242084, One-way Anova, Dunnett's multiple comparisons test (see text for details).

60-0175 on the tonic GABA$_A$ current was mediated by 5-HT$_{2C}$Rs, since it was not present when the selective 5-HT$_{2C}$R antagonist SB242084 (10 μM) was co-applied with Ro 60-175 in the recording solution (Wistar-SB242084 + Ro 60-0175: 1.2 ± 0.4 pA/pF, n = 21, vs. Wistar-Ro 60-0175, p < 0.01) (Figures 2C$_{3,5}$). Moreover, perfusion of thalamic slices with SB242084 (10 μM) alone did not affect tonic GABA$_A$ current compared to control (Wistar-SB242084: 1.4 ± 0.5 pA/pF, n = 11, vs. Wistar-CTL$_{RO\ 60-0175}$, p > 0.05) (Figures 2C$_{4,5}$), suggesting either the absence of a detectable basal 5-HT$_{2C}$R tone on

extrasynaptic GABA$_A$R function or insufficient endogenous 5-HT levels *ex vivo* under our experimental conditions.

3.4 Systemic administration of Ro 60-0175 decreases ASs in freely moving adult GAERS rats

We have previously shown that lorcaserin and CP-809,101, two 5-HT$_{2C}$R agonists, reduce spontaneous ASs in GAERS (Venzi et al.,
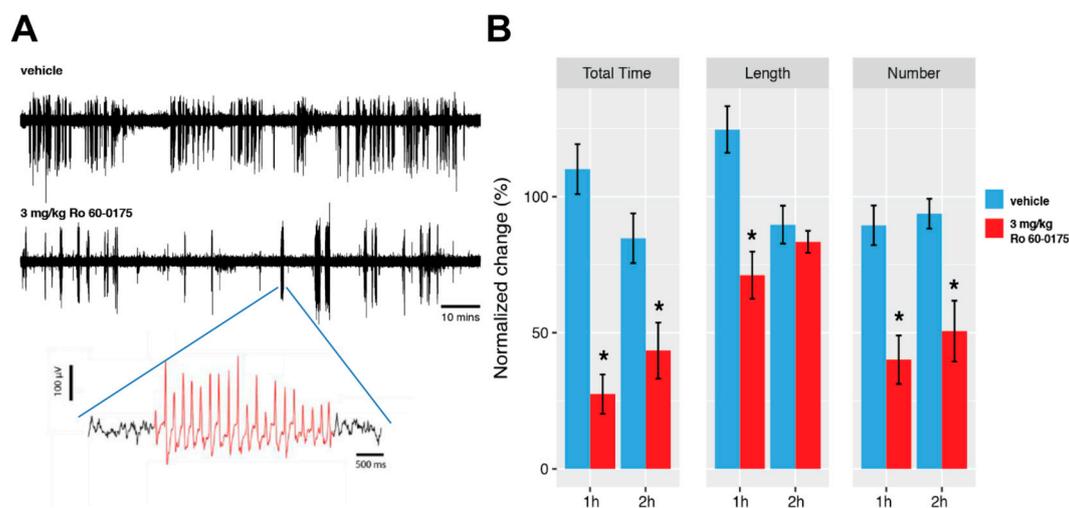
FIGURE 3
Effects of Ro 60−0175 on spontaneous ASs in GAERS rats. **(A)** Representative EEG traces from a GAERS rat injected i. p. either with vehicle (top trace) or 3 mg/kg Ro 60-0175 (bottom trace). An enlarged trace of a spike-and-wave discharge (SWDs) (red) is shown below. **(B)** Effect of Ro 60-0175 on total time spent in seizures, number of seizures and seizure length during the first and second h post-Ro 60-0175 treatment. All values are normalized to the control period (−40 to 0 min). Values represent mean $\pm$ SEM. Asterisks indicate $P < 0.05$ for Ro 60-0175 treatment vs. the corresponding time-bin in the vehicle group (two-way ANOVA, Sidak's multiple comparison test) (n = 8 rats).

2016). Since, i) a decrease in tonic current has an anti-absence effect (Cope et al., 2009), and ii) Ro 60-0175 reduces the tonic current in GAERS (Figure 2B$_3$), we tested the effect of this 5-HT$_{2C}$R agonist on spontaneous ASs recorded in freely moving adult GAERS. The systemic injection of Ro 60-0175 (3 mg/kg/i.p.) in eight GAERS rats reduced the total time spent in seizures (Figures 3A,B) (repeated measure two-way ANOVA on the time spent in seizure: $P < 0.001$; 62.8% reduction over the 2-h window). This anti-absence effect was driven by a 50.5% decrease in the number of seizures (Figure 3B) (p < 0.0001). The average seizure length, however, remained unchanged in the 2nd hour of treatment (Figure 3B). To control for potential arousal or motor effects of systemic 5-HT$_{2C}$R agonism (Di Giovanni et al., 2006; Di Giovanni and De Deurwaerdere, 2016), all seizure events were verified by simultaneous EEG and video recordings. These findings indicate that activation of 5-HT$_{2C}$Rs by Ro 60-0175 exerts an anti-absence effect similar to that observed in the same rat model with lorcaserin and CP-809,101 (Venzi et al., 2016).

4 Discussion

In this study, we identified three key findings: 1) 5-HT$_{2C}$R expression is lower in the VB, but similar in the NRT, of GAERS compared to Wistar and NEC rats; 2) 5-HT$_{2C}$R activation reduces tonic GABA$_A$ currents in TC neurons in Wistar, NEC and GAERS rats; 3) activation of 5-HT$_{2C}$R by systemic Ro 60-0175 administration reduces ASs in freely moving GAERS, highlighting 5-HT$_{2C}$Rs as potential therapeutic targets. Thus, our results provide *in vivo* and *in vitro* electrophysiological evidence that 5-HT$_{2C}$Rs modulate tonic GABA$_A$ inhibition in the somatosensory thalamus of both the healthy brain and those prone to the expression of spontaneous ASs. Moreover, our findings suggest that a strain-dependent differences in 5-HT$_{2C}$R expression may contribute to altered thalamocortical inhibition in ASs, potentially underlying this seizure phenotype.

Inbred NEC rats have traditionally been used as a control strain for GAERS in studies of CAE (Depaulis et al., 2016; Crunelli et al., 2020), however, recent anatomical (Bombardi et al., 2018), neurochemical (De Deurwaerdère et al., 2022) and behavioral (Neuparth-Sottomayor et al., 2023; Casarrubea et al., 2024; Neuparth-Sottomayor et al., 2025) studies have revealed abnormalities in NEC rats, raising concerns about their suitability for comparative studies. For this reason, we have extended our invistigation to outbred Wistar rats. We previously found no functional differences in δ subunit-containing GABA$_A$Rs among GAERS, NEC and Wistar rats, as the selective agonist THIP similarly increased tonic inhibition in all three strains (Cope et al., 2009). Despite GAERS rats exhibiting abnormally high tonic inhibition, attributed to the loss-of-function of GAT-1, its expression is similar in VB and cortex of GAERS and NEC (Cope et al., 2009). Accordingly, in this study we found that the tonic GABA$_A$ current was higher in GAERS VB TC neurons and comparably lower in Wistar and NEC rats. Therefore, Wistar rats are comparable to NEC rats and may serve as controls for electrophysiological studies of the tonic GABA$_A$ inhibition in GAERS.

Consistent with prior studies (Munsch et al., 2003; Li et al., 2004; Coulon et al., 2010), we observed that 5-HT$_{2C}$Rs had a nucleus-specific distribution within the thalamus. No differences were observed in 5-HT$_{2C}$R$^+$ neuronal density within the NRT of the three strains. In contrast, GAERS rats exhibited a fivefold lower density of 5-HT$_{2C}$R$^+$ neurons in the VB compared to Wistar rats, with NEC rats significantly higher than GAERS but lower than Wistar. In line with an anticonvulsant 5-HT$_{2C}$R role, a reduction 5-HT$_{2C}$R expression has been associated with increased neuronal excitability in experimental temporal lobe epilepsy (Orban et al., 2014), and 5-HT$_{2C}$R KO mice exhibit a higher susceptibility to convulsive seizures and occasionally die from epilepsy (Applegate and Tecott, 1998).

As expected, Ro 60-0175, reduced tonic $GABA_A$ currents in Wistar, NEC and GAERS rats. The effect of $5\text{-}HT_{2C}R$ activation in Wistar rats was blocked by co-application of SB242084, a selective $5\text{-}HT_{2C}R$ antagonist (Di Matteo et al., 2000). This aligns with findings in visual dLGN, where mCPP, another $5\text{-}HT_{2C}R$ agonist, decreased the tonic $GABA_A$ current in an SB242084-dependent manner (Crunelli and Di Giovanni, 2015). Of note, SB242084 alone did not affect the tonic $GABA_A$ current of VB TC neurons, suggesting either low endogenous 5-HT levels in our thalamic slices or the lack of basal $5\text{-}HT_{2C}R$ modulation.

To test our hypothesis that the anti-absence effect of $5\text{-}HT_{2C}R$ agonists observed in vivo (Venzi et al., 2016) is mediated by the normalization of the high tonic $GABA_A$ inhibition in the thalamus, we used Ro 60-0175, a $5\text{-}HT_{2C}R$ preferring agonist (Martin et al., 1998; Di Giovanni and De Deurwaerdere, 2016). Ro 60–0175 showed an anti-absence effect in freely moving adult GAERS, which was comparable to those of other $5\text{-}HT_{2C}R$ agonists, such as CP-809,101 and lorcaserin in GAERS (Venzi et al., 2016) or mCPP in WAG/Rij (Jakus et al., 2003), another well-validated AS model (Coenen et al., 1992).

5-HT control of the tonic $GABA_A$ inhibition varies by brain region and receptor subtype (Crunelli and Di Giovanni, 2015; Meis et al., 2018), but its intracellular pathways remain largely unexplored, except for its reduction in the visual cortex via a $5\text{-}HT_{1A}$/PKA mechanism (Jang et al., 2015). This effect is likely mediated by PKC phosphorylation of $GABA_A$Rs (Connelly et al., 2013; Crunelli and Di Giovanni, 2014; Deidda et al., 2021). Indeed, inhibitors of PKC have been shown to block both the $5\text{-}HT_2R$-mediated suppression (in the prefrontal cortex (Feng et al., 2001) and enhancement (in the spinal cord (Xu et al., 1998; Li et al., 2000)) of phasic $GABA_A$ currents. As shown in Xenopus oocytes expressing both $5\text{-}HT_{2C}Rs$ and $GABA_A$Rs (Huidobro-Toro et al., 1996), other calcium-dependent pathways, independent of protein kinases or phosphatases, may contribute to the inhibitory effects of $5\text{-}HT_{2C}Rs$ on $GABA_A$Rs seen in our study.

$5\text{-}HT_{2C}Rs$ are expressed on both NRT and VB TC neurons, therefore, their inhibitory modulation of the GABAergic transmission may involve pre and/or postsynaptic receptors on TC neurons. This completes a scenario in which 5-HT activation of $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}Rs$ on presynaptic NRT terminals decreases and enhances VB GABA release, respectively (Goitia et al., 2016), while $5\text{-}HT_{2C}Rs$ reduce GABAergic inhibition, highlighting the complexity of 5-HT modulation of thalamic functions. Our findings suggest that reduced VB $5\text{-}HT_{2C}R$ levels, possibly due to increased levels of 5-HT, may lead to excessive tonic $GABA_A$ current, promoting hypersynchronous oscillations in ASs. The Ro 60–0175 modulation of $GABA_A R$ inhibition likely underlies the anticonvulsant effect of the $5\text{-}HT_{2C}R$ agonists in vivo in ASs (present evidence and (Venzi et al., 2016). Although the reduction of tonic $GABA_A$ current by Ro 60-0175 is not disease-specific per se, its functional relevance is state-dependent, as only GAERS display an abnormally elevated tonic inhibition that can be normalized by $5\text{-}HT_{2C}R$ receptor activation. Given the role of thalamocortical circuits in ASs (Cope et al., 2009; McCafferty et al., 2018) these findings highlight the serotonergic system's role in thalamic excitability and AS ictogenesis. Furthermore, our study supports Wistar rats as a suitable control strain for GAERS studies (Neuparth-Sottomayor et al., 2023; Casarrubea et al., 2024).

Together with existing clinical evidence from Dravet syndrome and Lennox-Gastaut syndrome demonstrating antiseizure efficacy of serotonergic agents such as the 5-HT releaser fenfluramine (Sourbron and Lagae, 2023), $5\text{-}HT_{2C}R$ agonist lorcaserin (Bialer and Perucca, 2022) and the selective $5\text{-}HT_{2C}R$ superagonist LP352 (Ren et al., 2025), our mechanistic findings provide a strong rationale to evaluate serotonergic agents with $5\text{-}HT_{2C}R$ engagement, in CAE, ideally in parallel with more selective $5\text{-}HT_{2C}R$ agonists to disentangle target-specific effects. One limitation of our study, shared with much of the existing literature, is that in vitro patch-clamp experiments were performed in juvenile slices, as reliable whole-cell recordings from adult (P90–P120) thalamocortical neurons are technically unfeasible in standard acute slices and are therefore routinely conducted in P25–P30 (Cope et al., 2009). Notably, the pathological changes in the GAT1 transporter, that underlie the generation of ASs start from P17 and are maintained throughout adult life in GAERS rats (Morais et al., 2021). In contrast, robust expression of ASs in GAERS requires adult animals (≥P90) (Vergnes et al., 1986; Depaulis et al., 2016; Crunelli et al., 2020).

The study has some limitations, including modestly powered IHC datasets and reliance on prior antibody validation. Moreover, the dissociation between receptor density and function suggests that $5\text{-}HT_{2C}R$ efficacy depends on localization, coupling, and circuit-level mechanisms rather than absolute receptor abundance.

In conclusion, a deeper understanding of the altered $5\text{-}HT_{2C}R$ modulation of GABAergic inhibition in the thalamocortical loop and its underlying mechanisms could refine therapeutic strategies targeting $5\text{-}HT_{2C}R$ pathways to restore inhibitory control and reduce ASs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

All animal procedures were authorized by Cardiff University, in agreement with the UK Animals (Scientific Procedures) Act (1986) and international laws and policies (EU Directive, 2010/63/EU) for animal experiments. Male Wistar, GAERS and NEC rats were obtained from colonies bred at Cardiff University and housed at 21°C under a 12 h light/dark cycle, with food and water available ad libitum. Immunohistochemistry and electrophysiological in vitro recordings were conducted on postnatal day (P) 25-30 rats, EEG recordings in P90-P120 rats. Every effort was made to minimize animal suffering and to reduce the number of animals used (Lidster et al. 2016).

Author contributions

AC: Data curation, Formal Analysis, Investigation, Methodology, Writing – review and editing. MV: Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – review and editing. CB: Formal Analysis, Investigation, Methodology, Resources, Visualization,

Writing – original draft. VC: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing. GDG: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

Conflict of interest

The authors declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author GDG declared that they were an editorial board member of Frontiers at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Applegate, C. D., and Tecott, L. H. (1998). Global increases in seizure susceptibility in mice lacking 5-HT2C receptors: a behavioral analysis. *Exp. Neurol.* 154 (2), 522–530. doi:10.1006/exnr.1998.6901

Bankson, M. G., and Yamamoto, B. K. (2004). Serotonin-GABA interactions modulate MDMA-induced mesolimbic dopamine release. *J. Neurochem.* 91 (4), 852–859. doi:10.1111/j.1471-4159.2004.02763.x

Belelli, D., Peden, D. R., Rosahl, T. W., Wafford, K. A., and Lambert, J. J. (2005). Extrasynaptic GABAA receptors of thalamocortical neurons: a molecular target for hypnotics. *J. Neurosci.* 25 (50), 11513–11520. doi:10.1523/JNEUROSCI.2679-05.2005

Belelli, D., Harrison, N. L., Maguire, J., Macdonald, R. L., Walker, M. C., and Cope, D. W. (2009). Extrasynaptic GABAA receptors: form, pharmacology, and function. *J. Neurosci.* 29 (41), 12757–12763. doi:10.1523/JNEUROSCI.3340-09.2009

Bialer, M., and Perucca, E. (2022). Lorcaserin for Dravet syndrome: a potential advance over fenfluramine? *CNS Drugs* 36 (2), 113–122. doi:10.1007/s40263-022-00896-3

Bombardi, C., Venzi, M., Crunelli, V., and Di Giovanni, G. (2018). Developmental changes of GABA immunoreactivity in cortico-thalamic networks of an absence seizure model. *Neuropharmacology* 136 (Pt A), 56–67. doi:10.1016/j.neuropharm.2018.01.047

Bonnin, A., Peng, W., Hewlett, W., and Levitt, P. (2006). Expression mapping of 5-HT1 serotonin receptor subtypes during fetal and early postnatal mouse forebrain development. *Neuroscience* 141 (2), 781–794. doi:10.1016/j.neuroscience.2006.04.036

Casarrubea, M., Radic, M., Morais, T. P., Mifsud, E., Cuboni, E., Aiello, S., et al. (2024). A quantitative and T-pattern analysis of anxiety-like behavior in male GAERS, NEC, and Wistar rats bred under the same conditions, against a commercially available Wistar control group in the hole board and elevated plus maze tests. *CNS Neurosci. Ther.* 30 (3), e14443. doi:10.1111/cns.14443

Coenen, A. M., Drinkenburg, W. H., Inoue, M., and van Luijtelaar, E. L. (1992). Genetic models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. *Epilepsy Res.* 12 (2), 75–86. doi:10.1016/0920-1211(92)90029-s

Connelly, W. M., Errington, A. C., Di Giovanni, G., and Crunelli, V. (2013). Metabotropic regulation of extrasynaptic GABA(A) receptors. *Front. Neural Circuits* 7, 171. doi:10.3389/fncir.2013.00171

Cope, D. W., Hughes, S. W., and Crunelli, V. (2005). GABAA receptor-mediated tonic inhibition in thalamic neurons. *J. Neurosci.* 25 (50), 11553–11563. doi:10.1523/JNEUROSCI.3362-05.2005

Cope, D. W., Di Giovanni, G., Fyson, S. J., Orban, G., Errington, A. C., Lorincz, M. L., et al. (2009). Enhanced tonic GABAA inhibition in typical absence epilepsy. *Nat. Med.* 15 (12), 1392–1398. doi:10.1038/nm.2058

Coulon, P., Kanyshkova, T., Broicher, T., Munsch, T., Wettschureck, N., Seidenbecher, T., et al. (2010). Activity modes in thalamocortical relay neurons are modulated by G(q)/G(11) family G-proteins - serotonergic and glutamatergic signaling. *Front. Cell Neurosci.* 4, 132. doi:10.3389/fncel.2010.00132

Crunelli, V., and Di Giovanni, G. (2014). Monoamine modulation of tonic GABAA inhibition. *Rev. Neurosci.* 25 (2), 195–206. doi:10.1515/revneuro-2013-0059

Crunelli, V., and Di Giovanni, G. (2015). Differential control by 5-HT and 5-HT1A, 2A, 2C receptors of phasic and tonic GABAA inhibition in the visual thalamus. *CNS Neurosci. Ther.* 21 (12), 967–970. doi:10.1111/cns.12480

Crunelli, V., and Leresche, N. (2002). Childhood absence epilepsy: genes, channels, neurons and networks. *Nat. Rev. Neurosci.* 3 (5), 371–382. doi:10.1038/nrn811

Crunelli, V., Leresche, N., and Cope, D. W. (2012). "GABA-A receptor function in typical absence seizures," in *Jasper's basic mechanisms of the epilepsies.*

Crunelli, V., Lőrincz, M. L., McCafferty, C., Lambert, R. C., Leresche, N., Di Giovanni, G., et al. (2020). Clinical and experimental insight into pathophysiology, comorbidity and therapy of absence seizures. *Brain* 143 (8), 2341–2368. doi:10.1093/brain/awaa072

Dahlstroem, A., and Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand. Suppl. Suppl.* 232, 231–255.

De Deurwaerdère, P., Casarrubea, M., Cassar, D., Radic, M., Puginier, E., Chagraoui, A., et al. (2022). Cannabinoid 1/2 receptor activation induces strain-dependent behavioral and neurochemical changes in genetic absence epilepsy rats from strasbourg and non-epileptic control rats. *Front. Cell Neurosci.* 16, 886033. doi:10.3389/fncel.2022.886033

Deidda, G., Crunelli, V., and Di Giovanni, G. (2021). 5-HT/GABA interaction in epilepsy. *Prog. Brain Res.* 259, 265–286. doi:10.1016/bs.pbr.2021.01.008

Deng, P. Y., and Lei, S. (2008). Serotonin increases GABA release in rat entorhinal cortex by inhibiting interneuron TASK-3 K+ channels. *Mol. Cell Neurosci.* 39 (2), 273–284. doi:10.1016/j.mcn.2008.07.005

Depaulis, A., David, O., and Charpier, S. (2016). The genetic absence epilepsy rat from strasbourg as a model to decipher the neuronal and network mechanisms of generalized idiopathic epilepsies. *J. Neurosci. Methods* 260, 159–174. doi:10.1016/j.jneumeth.2015.05.022

Di Giovanni, G., and De Deurwaerdere, P. (2016). New therapeutic opportunities for 5-HT2C receptor ligands in neuropsychiatric disorders. *Pharmacol. Ther.* 157, 125–162. doi:10.1016/j.pharmthera.2015.11.009

Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A., and Esposito, E. (2006). Central serotonin2C receptor: from physiology to pathology. *Curr. Topics Medicinal Chemistry* 6 (18), 1909–1925. doi:10.2174/156802606778522113

Di Giovanni, G., Cope, D. W., and Crunelli, V. (2008). Cholinergic and monoaminergic modulation of tonic GABAA inhibition in the rat dorsal lateral geniculate nucleus. *Soc. Neurosci.* 531, 532–D533.

Di Matteo, V., Di Giovanni, G., and Esposito, E. (2000). SB 242084: a selective 5-HT2C receptor antagonist. *Cns Drug Rev.* 6 (3), 195–205. doi:10.1111/j.1527-3458.2000.tb00147.x

Errington, A. C., Gibson, K. M., Crunelli, V., and Cope, D. W. (2011). Aberrant GABA(A) receptor-mediated inhibition in cortico-thalamic networks of succinic semialdehyde dehydrogenase deficient mice. *PLoS One* 6 (4), e19021. doi:10.1371/journal.pone.0019021

Errington, A. C., Di Giovanni, G., and Crunelli, V. (2014). *Extrasynapitic GABAA receptors. The receptors.* New York: Springer.

Feng, J., Cai, X., Zhao, J., and Yan, Z. (2001). Serotonin receptors modulate GABA(A) receptor channels through activation of anchored protein kinase C in prefrontal cortical neurons. *J. Neurosci.* 21 (17), 6502–6511. doi:10.1523/JNEUROSCI.21-17-06502.2001

Goitia, B., Rivero-Echeto, M. C., Weisstaub, N. V., Gingrich, J. A., Garcia-Rill, E., Bisagno, V., et al. (2016). Modulation of GABA release from the thalamic reticular nucleus by cocaine and caffeine: role of serotonin receptors. *J. Neurochem.* 136 (3), 526–535. doi:10.1111/jnc.13398

Graf, M., Jakus, R., Kantor, S., Levay, G., and Bagdy, G. (2004). Selective 5-HT1A and 5-HT7 antagonists decrease epileptic activity in the WAG/Rij rat model of absence epilepsy. *Neurosci. Lett.* 359 (1-2), 45–48. doi:10.1016/j.neulet.2004.01.072

Heo, J. Y., Nam, M.-H., Yoon, H. H., Kim, J., Hwang, Y. J., Won, W., et al. (2020). Aberrant tonic inhibition of dopaminergic neuronal activity causes motor symptoms in animal models of parkinson's disease. *Curr. Biol.* 30 (2), 276–291. doi:10.1016/j.cub.2019.11.079

Herd, M. B., Brown, A. R., Lambert, J. J., and Belelli, D. (2013). Extrasynaptic GABAA receptors couple presynaptic activity to postsynaptic inhibition in the somatosensory thalamus. *J. Neurosci.* 33 (37), 14850–14868. doi:10.1523/JNEUROSCI.1174-13.2013

Hu, B., and Wang, Q. (2015). Controlling absence seizures by deep brain stimulus applied on substantia nigra pars reticulata and cortex. *Chaos, Solit. and Fractals* 80, 13–23. doi:10.1016/j.chaos.2015.02.014

Hu, B., Chen, S., Chi, H., Chen, J., Yuan, P., Lai, H., et al. (2017). Controlling absence seizures by tuning activation level of the thalamus and striatum. *Chaos, Solit. and Fractals* 95, 65–76. doi:10.1016/j.chaos.2016.12.009

Huguenard, J. (2019). Current controversy: spikes, bursts, and synchrony in generalized absence epilepsy: unresolved questions regarding thalamocortical synchrony in absence epilepsy. *Epilepsy Curr.* 19 (2), 105–111. doi:10.1177/1535759719835355

Huidobro-Toro, J. P., Valenzuela, C. F., and Harris, R. A. (1996). Modulation of GABAA receptor function by G protein-coupled 5-HT2C receptors. *Neuropharmacology* 35 (9-10), 1355–1363. doi:10.1016/s0028-3908(96)00084-6

Jacobs, B. L., and Fornal, C. A. (2000). *Physiology and pharmacology of brain serotoninergic neurons. Serotoninergic neurons and 5-HT receptors in the CNS.* Berlin, Heidelberg: Springer Berlin Heidelberg, 91–116.

Jakus, R., Graf, M., Juhasz, G., Gerber, K., Levay, G., Halasz, P., et al. (2003). 5-HT2C receptors inhibit and 5-HT1A receptors activate the generation of spike-wave discharges in a genetic rat model of absence epilepsy. *Exp. Neurol.* 184 (2), 964–972. doi:10.1016/S0014-4886(03)00352-2

Jakus, R., Bagdy, G., Di Giovanni, G., Esposito, E., and Di Matteo, V. (2011). "The role of 5-HT2C receptor in epilepsy," in *5-HT2C receptors in the pathophysiology of CNS disease. 5-HT2C receptors in the pathophysiology of CNS disease* (Wien: Springer-Verlag), 429–444.

Jang, H. J., Cho, K. H., Joo, K., Kim, M. J., and Rhie, D. J. (2015). Differential modulation of phasic and tonic inhibition underlies serotonergic suppression of long-term potentiation in the rat visual cortex. *Neuroscience* 301, 351–362. doi:10.1016/j.neuroscience.2015.06.018

Jarre, G., Altwegg-Boussac, T., Williams, M. S., Studer, F., Chipaux, M., David, O., et al. (2017). Building up absence seizures in the somatosensory cortex: from network to cellular epileptogenic processes. *Cereb. Cortex* 27 (9), 4607–4623. doi:10.1093/cercor/bhx174

Jia, F., Pignataro, L., Schofield, C. M., Yue, M., Harrison, N. L., and Goldstein, P. A. (2005). An extrasynaptic GABAA receptor mediates tonic inhibition in thalamic VB neurons. *J. Neurophysiol.* 94 (6), 4491–4501. doi:10.1152/jn.00421.2005

Kwak, H., Koh, W., Kim, S., Song, K., Shin, J.-I., Lee, J. M., et al. (2020). Astrocytes control sensory acuity *via* tonic inhibition in the thalamus. *Neuron* 108 (4), 691–706. doi:10.1016/j.neuron.2020.08.013

Leal, A., Vieira, J. P., Lopes, R., Nunes, R. G., Goncalves, S. I., Lopes da Silva, F., et al. (2016). Dynamics of epileptic activity in a peculiar case of childhood absence epilepsy and correlation with thalamic levels of GABA. *Epilepsy Behav. Case Rep.* 5, 57–65. doi:10.1016/j.ebcr.2016.03.004

Li, H., Lang, B., Kang, J. F., and Li, Y. Q. (2000). Serotonin potentiates the response of neurons of the superficial laminae of the rat spinal dorsal horn to gamma-aminobutyric acid. *Brain Res. Bull.* 52 (6), 559–565. doi:10.1016/s0361-9230(00)00297-5

Li, Q. H., Nakadate, K., Tanaka-Nakadate, S., Nakatsuka, D., Cui, Y., and Watanabe, Y. (2004). Unique expression patterns of 5-HT2A and 5-HT2C receptors in the rat brain during postnatal development: western blot and immunohistochemical analyses. *J. Comp. Neurol.* 469 (1), 128–140. doi:10.1002/cne.11004

Lidster, K., Jefferys, J. G., Blümcke, I., Crunelli, V., Flecknell, P., Frenguelli, B. G., et al. (2016). Opportunities for improving animal welfare in rodent models of epilepsy and seizures. *J. Neurosci. Methods* 260, 2–25. doi:10.1016/j.jneumeth.2015.09.007

Marescaux, C., Vergnes, M., and Depaulis, A. (1992). Genetic absence epilepsy in rats from Strasbourg--a review. *J. Neural Transm. Suppl.* 35, 37–69. doi:10.1007/978-3-7091-9206-1_4

Martin, J. R., Bos, M., Jenck, F., Moreau, J. L., Mutel, V., Sleight, A. J., et al. (1998). 5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential. *J. Pharmacol. Exp. Ther.* 286 (2), 913–924. doi:10.1016/S0022-3565(24)37669-4

McCafferty, C., David, F., Venzi, M., Lőrincz, M. L., Delicata, F., Atherton, Z., et al. (2018). Cortical drive and thalamic feed-forward inhibition control thalamic output synchrony during absence seizures. *Nat. Neuroscience* 21 (5), 744–756. doi:10.1038/s41593-018-0130-4

McCormick, D. A. (1992). Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog. Neurobiol.* 39 (4), 337–388. doi:10.1016/0301-0082(92)90012-4

Meis, S., Endres, T., Munsch, T., and Lessmann, V. (2018). Presynaptic regulation of tonic inhibition by neuromodulatory transmitters in the basal amygdala. *Mol. Neurobiol.* 55 (11), 8509–8521. doi:10.1007/s12035-018-0984-1

Morais, T., Pina, C., Sottomayor, M., Sebastião, A., Di Giovanni, G., Vaz, S., et al. (2021). *GAT1 uptake dysfunction in absence seizures.* Chicago. Society for Neuroscience.

Munsch, T., Freichel, M., Flockerzi, V., and Pape, H. C. (2003). Contribution of transient receptor potential channels to the control of GABA release from dendrites. *Proc. Natl. Acad. Sci. U. S. A.* 100 (26), 16065–16070. doi:10.1073/pnas.2535311100

Neuparth-Sottomayor, M., Pina, C. C., Morais, T. P., Farinha-Ferreira, M., Abreu, D. S., Solano, F., et al. (2023). Cognitive comorbidities of experimental absence seizures are independent of anxiety. *Neurobiol. Dis.* 186, 106275. doi:10.1016/j.nbd.2023.106275

Neuparth-Sottomayor, M., Morais, T. P., Good, M., Sebastião, A. M., Di Giovanni, G., Crunelli, V., et al. (2025). Impairment of spatial working memory but preservation of recognition memory in female rats with spontaneous absence seizures. *Neurochem. Res.* 50 (4), 236. doi:10.1007/s11064-025-04485-w

Nocjar, C., Alex, K., Sonneborn, A., Abbas, A., Roth, B., and Pehek, E. (2015). Serotonin-2C and-2a receptor co-expression on cells in the rat medial prefrontal cortex. *Neuroscience* 297, 22–37. doi:10.1016/j.neuroscience.2015.03.050

Orban, G., Bombardi, C., Marino Gammazza, A., Colangeli, R., Pierucci, M., Pomara, C., et al. (2014). Role(s) of the 5-HT2C receptor in the development of maximal dentate activation in the hippocampus of anesthetized rats. *CNS Neurosci. Ther.* 20 (7), 651–661. doi:10.1111/cns.12285

Perucca, E., Gram, L., Avanzini, G., and Dulac, O. (1998). Antiepileptic drugs as a cause of worsening seizures. *Epilepsia* 39 (1), 5–17. doi:10.1111/j.1528-1157.1998.tb01268.x

Puig, M. V., and Gulledge, A. T. (2011). Serotonin and prefrontal cortex function: neurons, networks, and circuits. *Mol. Neurobiol.* 44 (3), 449–464. doi:10.1007/s12035-011-8214-0

Ren, A., Zhu, X., Lehmann, J., Kasem, M., Schrader, T. O., Dang, H., et al. (2025). Diazepine agonists of the 5-HT2C receptor with unprecedented selectivity: discovery of Bexicaserin (LP352). *J. Med. Chem.* 68 (11), 10599–10618. doi:10.1021/acs.jmedchem.4c02923

Rodriguez, J. J., Noristani, H. N., Hoover, W. B., Linley, S. B., and Vertes, R. P. (2011). Serotonergic projections and serotonin receptor expression in the reticular nucleus of the thalamus in the rat. *Synapse* 65 (9), 919–928. doi:10.1002/syn.20920

Rosenberg, E. C., Patra, P. H., and Whalley, B. J. (2017). Therapeutic effects of cannabinoids in animal models of seizures, epilepsy, epileptogenesis, and epilepsy-related neuroprotection. *Epilepsy Behav.* 70 (Pt B), 319–327. doi:10.1016/j.yebeh.2016.11.006

Russo, E., Citraro, R., Davoli, A., Gallelli, L., Donato Di Paola, E., and De Sarro, G. (2013). Ameliorating effects of aripiprazole on cognitive functions and depressive-like behavior in a genetic rat model of absence epilepsy and mild-depression comorbidity. *Neuropharmacology* 64, 371–379. doi:10.1016/j.neuropharm.2012.06.039

Silenieks, L. B., Carroll, N. K., Van Niekerk, A., Van Niekerk, E., Taylor, C., Upton, N., et al. (2019). Evaluation of selective 5-HT(2C) agonists in acute seizure models. *ACS Chem. Neurosci.* 10 (7), 3284–3295. doi:10.1021/acschemneuro.8b00739

Sourbron, J., and Lagae, L. (2023). Fenfluramine: a plethora of mechanisms? *Front. Pharmacology* 14, 1192022. doi:10.3389/fphar.2023.1192022

Varela, C., and Sherman, S. M. (2009). Differences in response to serotonergic activation between first and higher order thalamic nuclei. *Cereb. Cortex* 19 (8), 1776–1786. doi:10.1093/cercor/bhn208

Venzi, M., David, F., Bellet, J., Cavaccini, A., Bombardi, C., Crunelli, V., et al. (2016). Role for serotonin2A (5-HT2A) and 2C (5-HT2C) receptors in experimental absence seizures. *Neuropharmacology* 108, 292–304. doi:10.1016/j.neuropharm.2016.04.016

Vergnes, M., Marescaux, C., Micheletti, G., Reis, J., Depaulis, A., Rumbach, L., et al. (1982). Spontaneous paroxysmal electroclinical patterns in rat: a model of generalized non-convulsive epilepsy. *Neurosci. Lett.* 33 (1), 97–101. doi:10.1016/0304-3940(82)90136-7

Vergnes, M., Marescaux, C., Depaulis, A., Micheletti, G., and Warter, J.-M. (1986). Ontogeny of spontaneous petit mal-like seizures in Wistar rats. *Dev. Brain Res.* 30 (1), 85–87. doi:10.1016/s0006-8993(86)80011-7

Vertes, R. P., Fortin, W. J., and Crane, A. M. (1999). Projections of the median raphe nucleus in the rat. *J. Comp. Neurol.* 407 (4), 555–582.

Walker, M. C., and Kullmann, D. K. (2012). "Tonic GABAA receptor-mediated signaling in epilepsy," in *Jasper's basic mechanisms of the epilepsies.* 4th edition (Bethesda (MD): National Center for Biotechnology Information US).

Xu, T. L., Pang, Z. P., Li, J. S., and Akaike, N. (1998). 5-HT potentiation of the GABA(A) response in the rat sacral dorsal commissural neurones. *Br. J. Pharmacol.* 124 (4), 779–787. doi:10.1038/sj.bjp.0701896

Yang, Y. C., Hu, C. C., and Lai, Y. C. (2015). Non-additive modulation of synaptic transmission by serotonin, adenosine, and cholinergic modulators in the sensory thalamus. *Front. Cell Neurosci.* 9, 60. doi:10.3389/fncel.2015.00060