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BRIEF COMMUNICATION

Serotonin 2C receptor in a rat model of temporal lobe epilepsy: From brainstem expression to pharmacological blockade in relation to ventilatory function

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Abstract

Because of its involvement in breathing control and neuronal excitability, dysregulation of the serotonin (5-HT) 2C receptor (5-HT_{2C}) might play a key role in sudden unexpected death in epilepsy. Seizure-induced respiratory arrest is thus prevented by a 5-HT_{2B/C} agonist in different seizure model. However, the specific contribution of 5-HT_{2C} in chronic epilepsy-related respiratory dysfunction remains unknown. In a rat model of temporal lobe epilepsy (EPI rats), in which we previously reported interictal respiratory dysfunctions and a reduction of brainstem 5-HT tone, quantitative reverse transcriptase polymerase chain reaction showed overexpression of TPH2 (5-HT synthesis enzyme), SERT (5-HT reuptake transporter), and 5-HT_{2C} transcript levels in the brainstem of EPI rats, and of RNA-specific adenosine deaminase (ADAR1, ADAR2) involved in the production of 5-HT_{2C} isoforms. Interictal ventilation was assessed with whole-body plethysmography before and 2 h after administration of SB242084 (2 mg/kg), a specific antagonist of 5-HT_{2C}. As expected, SB242084 administration induced a progressive decrease in ventilatory parameters and an alteration of breathing stability in both control and EPI rats. However, the size of the SB242084 effect was lower in EPI rats than in controls. Increased 5-HT_{2C} gene expression in the brainstem of EPI rats could be part of a compensatory mechanism against epilepsy-related low 5-HT tone and expression of 5-HT_{2C} isoforms for which 5-HT affinity might be lower.

KEYWORDS

5-HT_{2C}, epilepsy, serotonin, SUDEP

1 | INTRODUCTION

Alteration of serotonin (5-HT) transmission in the brainstem might play a key role in the cascade of events that can result in seizure-related postictal apnea and sudden

unexpected death in epilepsy.¹ Among 5-HT receptor subtypes, dysregulation of the 5-HT_{2C} receptor (5-HT_{2C}) may strongly contribute to the impairment of respiratory control in epilepsy, by modulating the activity of neuronal groups involved in the central regulation of respiration.²

Laurent Bezin and Sylvain Rheims are co-last authors.

Hence, seizure-induced respiratory arrest is prevented by a 5-HT_{2B/C} agonist in DBA/2 mice but not in DBA/1 mice,³ likely due to interstrain difference in 5-HT_{2B} and 5-HT_{2C} expression.³ Moreover, 5-HT_{2C} knockout mice exhibit a reduced threshold for seizure induction and enhanced susceptibility to audiogenic-induced seizure that can lead to death.⁴ In contrast, although alterations in 5-HT are also associated with epilepsy-related chronic respiratory dysfunction,^{5–7} the specific contribution of 5-HT_{2C} to this process remains unknown.

Here, we investigated this issue in a rat model of temporal lobe epilepsy (TLE), which exhibits chronic interictal ventilatory dysfunction, including hypoventilation under basal conditions that is associated with a reduced brainstem 5-HT tone, yet has no mortality.^{5,6} Using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) quantification in control and epileptic (EPI) rats, we measured brainstem transcript levels of the 5-HT synthesis enzyme (TPH2), the 5-HT reuptake transporter (SERT), and 5-HT_{2C}, as well as those of 5-HT_{1A} and 5-HT_{3B}, which play a role in 5-HT-mediated modulation of neuronal excitability in epilepsy.⁸ We then assessed the involvement of 5-HT_{2C} on respiratory function in control and EPI rats using SB242084, a selective, brain-penetrant and highly specific 5-HT_{2C} antagonist.⁹

2 | MATERIALS AND METHODS

All details of the methods, especially those related to plethysmography, are described in Appendix S1. A flowchart of experiments detailing the sample sizes at each step is provided in Figure S1.

2.1 | Animals

Experimental procedures were approved by the ethical committee of the University Claude Bernard Lyon 1 (approval #32115-2021062414439857). A total of 44 male Sprague Dawley rats (Envigo) were used, and a protocol of animal care was followed until the end of experiment to reduce as much as possible the animal's discomfort.⁶

2.2 | Pilocarpine-induced status epilepticus and epilepsy onset

Pilocarpine-induced status epilepticus (Pilo-SE) was triggered in 31 rats at the age of 7 weeks, as previously described.⁶ Following Pilo-SE, a protocol of visual tracking of spontaneous recurrent seizures (SRSs) was applied⁶ during daylight period (between 9 a.m. and 6 p.m.) until

all Pilo-SE rats demonstrated the establishment of epilepsy (EPI) with SRSs.

2.3 | Experiment 1

At 18 weeks post-SE, total RNAs from the brainstem of three control and 10 EPI rats were used for qRT-PCR quantification of TPH2, SERT, 5-HT_{1A}, 5-HT_{2C}, 5-HT_{3B}, and ADAR (adenosine deaminase acting on RNA) 1 and 2 transcript levels, as previously described.⁶ Details about the primer pairs are provided in Table S1.

2.4 | Experiment 2

Ventilation and epileptic activity were evaluated after acute administration of SB242084 (2 mg kg⁻¹ sc), a 5-HT_{2C} antagonist.⁹ After electrode implantation, 22 rats underwent two video-electroencephalographic (video-EEG) recording sessions, including 14 EPI rats and eight control rats. Session 1 was performed at week 18 and lasted 90 min with a 30-min baseline evaluation and 60 min of video-EEG recording after injection of vehicle solution (10% dimethylsulfoxide in .9% NaCl sc). Session 2 was performed between weeks 19 and 22. During session 2, rats were placed individually in a plethysmography chamber to assess ventilation coupled with video-EEG recordings for 30 min under basal conditions, then for 2 h following subcutaneous injection of SB242084 solution.

During each session, seizure frequency, severity, and duration were evaluated using video-EEG recordings. Ventilatory response to acute SB242084 administration of each rat was evaluated as percentage of variation of the following ventilatory variables in comparison to values recorded prior its administration.

Ventilatory variables included minute ventilation (VE) as the product of respiratory frequency (FR) and an estimated tidal volume (VT). Calculation of apnea events (>2 breath cycles) were subdivided into postsigh and spontaneous subtypes whether or not apnea was preceded by a sigh event. Sigh was defined as breath amplitude 100% above the average of the preceding 20 s of breathing. Coefficient of variation is calculated as the ratio of SD to the mean frequency (methods detailed in Appendix S1).

2.5 | Data signal and statistical analysis

All data were analyzed using Spike-2 software (Cambridge Electronic Design). All data are expressed as mean ± SEM, and statistical analysis was performed using Prism 9.0.2 (GraphPad Software). Shapiro–Wilk test was used to

determine whether the data followed a normal distribution. For multiple group comparisons, we used unpaired *t*-test, two-way analysis of variance, and a mixed model of regression that took into account data repetition over sessions for each rat. Statistical significance was predefined as $p < .05$.

3 | RESULTS

3.1 | Increased level of 5-HT2C transcript within the brainstem of EPI rats

TPH2, SERT, 5-HT1A, 5-HT2C, and 5-HT3B transcript levels were measured in the brainstem (Table 1). A significant increase in 5-HT2C (+78%, $p = .045$), TPH2 (+73%, $p = .002$), and SERT (+50%, $p = .038$) mRNA levels was found in EPI rats compared to controls. No change was observed for 5-HT1A and 5-HT3B mRNA levels.

5-HT2C pre-mRNA can undergo adenosine-to-inosine (A-to-I) editing in humans, rats, and mice, resulting in the potential production of 24 5-HT2C isoforms to which 5-HT has a lower affinity, in comparison to 5-HT2C coded by the nonedited mRNA.¹⁰ Because the prevalence of A-to-I editing of a pre-mRNA appears to be correlated with transcript level of ADARs,¹¹ ADAR1 and ADAR2 mRNA levels were measured in EPI rats and were found to be significantly increased compared to control rats (+36% [$p = .01$] and +41% [$p = .003$], respectively; Table 1).

3.2 | Effect of SB242084 on ventilation during interictal period

Considering that the decrease of ventilation at rest in EPI rats was previously associated with the decrease of 5-HT

TABLE 1 Transcript levels of 5-HT-related genes within the brainstem of rats with active epilepsy.

| Transcript | cDNA copy number/ μ g total RNA | | EPI vs. CTRL | |
|------------|-------------------------------------|----------------------|--------------|-------------|
| | CTRL rats, $n = 3$ | EPI rats, $n = 10$ | % | p |
| TPH2 | 191 640 \pm 3084 | 332 402 \pm 17 835 | 173 | .002 |
| SERT | 170 805 \pm 15 710 | 256 396 \pm 18 877 | 150 | .038 |
| 5-HT1A | 124 427 \pm 7292 | 121 926 \pm 5375 | 98 | .819 |
| 5-HT2C | 167 305 \pm 11 501 | 297 607 \pm 30 419 | 178 | .045 |
| 5-HT3B | 500 \pm 83 | 792 \pm 83 | 158 | .931 |
| ADAR1 | 433 350 \pm 23 450 | 589 700 \pm 26 150 | 136 | .010 |
| ADAR2 | 698 850 \pm 20 200 | 981 900 \pm 41 000 | 131 | .003 |

Note: Bold type indicates statistical significance.

Abbreviations: 5-HT, serotonin; ADAR, adenosine deaminase acting on RNA; CTRL, control; EPI, epileptic; SERT, 5-HT reuptake transporter; TPH2, 5-HT synthesis enzyme.

tone⁶ and the role of 5-HT2C in breathing control,² we hypothesized that the increase in 5-HT2C transcript level in EPI rats might reflect an adaptive mechanism that aims to increase the binding of residual 5-HT with 5-HT2C to prevent more disturbance in breathing. In this context, we investigated whether administration of SB242084, a 5-HT2C antagonist, resulted in the aggravation of the epilepsy-related respiratory dysfunction (Figure 1).

Prior to SB242084 injection and under basal conditions, EPI rats showed alterations of ventilation compared to control rats, with $20 \pm 5\%$ ($p = .01$) and a $15 \pm 4\%$ ($p = .04$) lower VE and VT, respectively. No difference was observed in FR, the coefficient of variation, the frequency or duration of spontaneous and postsigh apneas, the ratio of postsigh and spontaneous apneas, the total frequency of sighs (with and without apneas), or the percentage of sighs without apneas between control rats and EPI rats (Table S2).

As expected, SB242084 administration induced a progressive decrease in VE, VT, and FR both in control rats ($p < .001$, $p = .047$, and $p < .001$, respectively) and in EPI rats ($p = .016$, $p = .025$, and $p = .006$, respectively; Figure 1 and Table S3A). However, although the evolution pattern of each of these variables after SB242084 administration was similar in both groups, the size of the SB242084 effect was lower in EPI rats than in control rats for both VE ($p < .001$) and FR ($p < .001$; Figure 1).

Compared to the baseline, SB242084 altered postsigh apnea frequency in both control ($p = .013$) and EPI ($p = .031$) rats (Figure 1 and Table S3B). SB242084 induced a similar change in frequency of sighs in both control ($p = .017$) and EPI ($p = .001$) rats (Figure 1 and Table S3C). In contrast, the duration of postsigh apneas was significantly higher after SB242084 administration in controls than in EPI rats ($p = .001$), whereas the frequency of spontaneous apneas increased significantly in EPI rats only (Figure 1).

3.3 | Effect of SB242084 on seizure activity of rats with chronic epilepsy

SB242084 provoked no seizure in control rats and did not significantly aggravate seizure activity in EPI rats ($p = .07$), in line with previous studies in rats using absence epilepsy or TLE models (results detailed in Appendix S1).^{12,13}

4 | DISCUSSION

We investigated the involvement of 5-HT2C in a rat model of chronic epilepsy with reduced 5-HT tone in the brainstem and long-term interictal respiratory alterations.^{5,6}

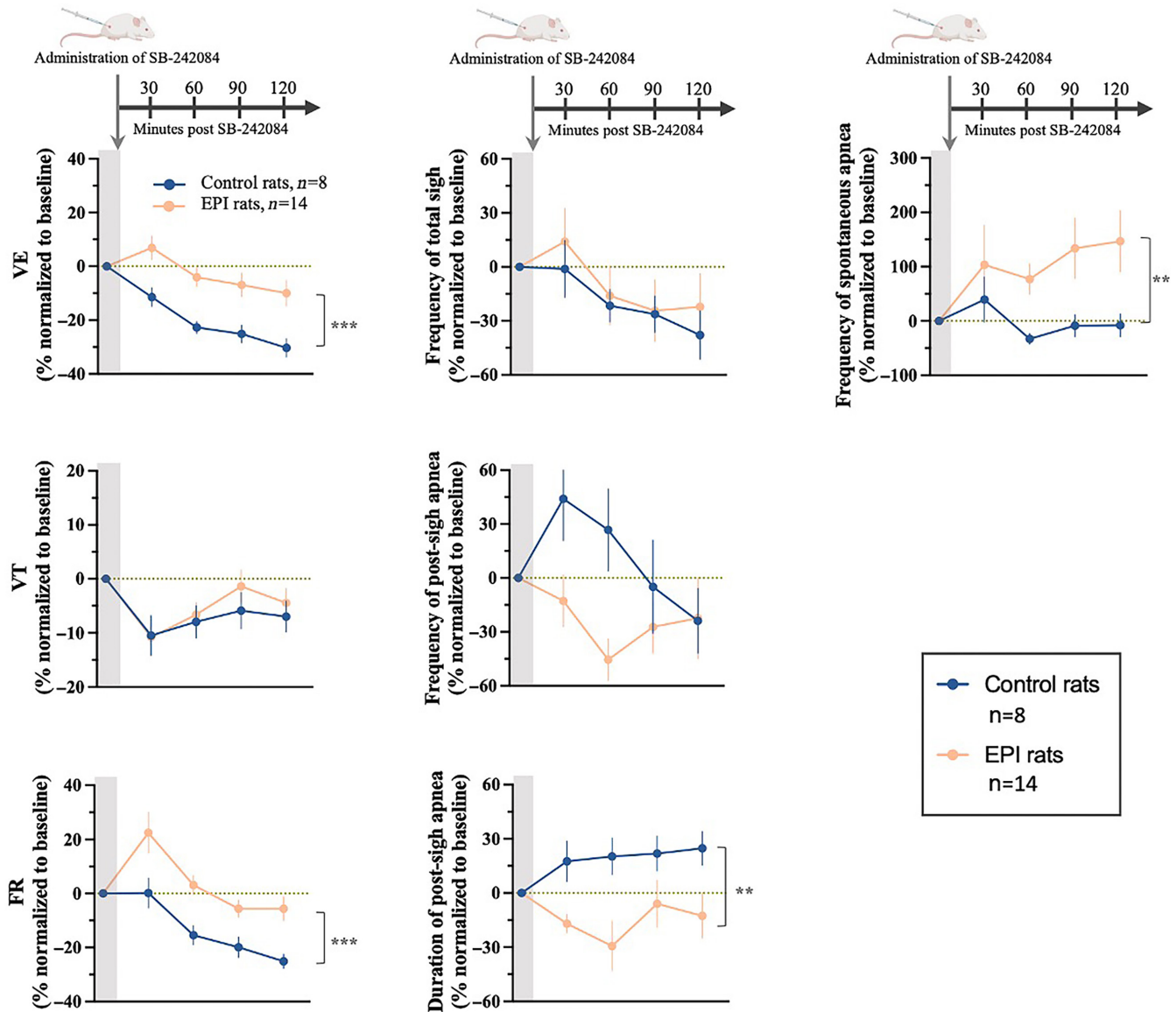
Ventilatory variablesSigh and apnea pattern

FIGURE 1 Effect of SB242084 on ventilation and apnea/sigh pattern. At 19–22 weeks after status epilepticus, after 30-min measurement of ventilation under basal conditions, the effect of a specific 5-HT_{2C} antagonist on ventilation was evaluated by injecting SB242084 into control ($n=8$) and epileptic (EPI; $n=14$) rats. Signal was analyzed for each 30-min breathing period over 2 h of recording, corresponding to the four time points after drug administration in the graphs. Gray bars represent values under basal conditions. Each time point represents ventilation variables in EPI (in orange) and control (in blue) rats, and is expressed as the mean \pm SEM of the percentage of variation compared to baseline values. Ventilatory variables include minute ventilation (VE), tidal volume (VT), and frequency (FR). Apnea and sigh pattern include only those with statistical significance (** $p < .01$, *** $p < .001$). Mixed model of regression analysis was used for the comparison between groups after administration of SB242084.

This study demonstrated that (1) 5-HT_{2C} was involved in 5-HT excitatory drive toward ventilatory function in both healthy and epileptic rats and (2) increased 5-HT_{2C} gene expression in the brainstem of EPI rats could be part of a compensatory mechanism against epilepsy-related low 5-HT tone and expression of 5-HT_{2C} isoforms for which 5-HT affinity might be lower.

5-HT_{2C}, like other 5-HT₂ receptor subtypes,^{2,14} has an excitatory effect on the activity of inspiratory neurons of the pre-Bötzinger complex and on ventilatory motor output from phrenic and hypoglossal nerves in brainstem–spinal cord preparation of juvenile rats.² The present study shares converging conclusions at the in vivo level, as inactivation of 5-HT_{2C} by acute

administration of SB242084 induced a decrease of VE, resulting in a decrease of both FR and VT. Furthermore, our results indicated that 5-HT_{2C} has a major role in the generation of sighs. According to the literature, sigh is a breathing behavior that enables the central respiratory control to be reset and breathing stability to be improved.¹⁵ Although the lower VE at rest in EPI rats than in controls observed in the present study was a confirmation of the results we reported using a more comprehensive experimental setup,⁶ it is important to note that the VT was not directly measured but estimated (see Appendix S1). Furthermore, in the absence of concomitant measurement, the effect of acute administration of SB242084 on oxygen consumption rate could not be assessed. Similarly, we could not exclude that chronic administration could result in a different effect, including aggravation of seizures or more adverse effects on ventilation.

However, the effect of SB242084 was lower than expected in EPI rats, in which the epilepsy-related increased level of 5-HT_{2C} transcript has directed our expectation toward a very severe respiratory phenotype after acute pharmacologic inactivation of 5-HT_{2C}. The exact physiological explanation of this observation remains to be determined. We cannot exclude that the epilepsy-related overexpression of 5-HT_{2C} transcript was ancillary to other adaptive mechanisms, which would not have been affected by SB242084, including 5-HT₄ modulation.^{5,16} However, according to the increase of ADAR1 and ADAR2 transcript levels observed in EPI rats, another hypothesis might be an increase in 5-HT_{2C} pre-mRNA editing mechanisms in EPI rats, leading to 5-HT_{2C} isoforms with reduced coupling to G proteins and/or with reduced affinity for 5-HT.¹⁷ It has been reported that affinity of 5-HT can be >10 times lower with edited 5-HT_{2C} isoforms than that with nonedited 5-HT_{2C} forms.^{17,18} In support of this hypothesis, it has been shown that increased 5-HT_{2C} gene expression in EPI rats in the same pilocarpine model is associated with decreased 5-HT affinity for 5-HT_{2C}.¹⁹ If the affinity to 5-HT for 5-HT_{2C} is decreased, a similar decrease in SB242084 affinity for 5-HT_{2C} is expected, which may partially explain the slight ventilatory effect of SB242084 in EPI rats. However, this hypothesis will need to be specifically investigated in dedicated studies, including the comparative effects of different agonists and antagonists of 5-HT_{2C} as well as the impact of the modulation of the ADAR1 and ADAR2 activity. Importantly, as 5-HT_{2C} mRNA editing involves five adenosines spread over a 13-nucleotide sequence, it is impossible to design primers to reliably detect and quantify the different possible isoforms using RT-PCR. Consequently, future studies will have to include high-throughput sequencing to detect and quantify 5-HT_{2C} mRNA isoforms subjected to A-to-I editing.

AUTHOR CONTRIBUTIONS

Hayet Kouchi, Laurent Bezin, and Sylvain Rheims designed the study. Hayet Kouchi collected and analyzed all respiratory data. Béatrice Georges and Laurent Bezin performed the qRT-PCR analyses. Hayet Kouchi wrote the first draft of the manuscript. All authors contributed to the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

S.R. has received speaker or consulting fees, or both, from UCB Pharma, Eisai, GW Pharma, Jazz Pharmaceuticals, LivaNova, Zogenix, and Angelini Therapeutics. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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1 Supplementary methods

2
3 ***Pilocarpine induced-SE.*** Epilepsy was triggered following pilocarpine induced *status*
4 *epilepticus* (Pilo-SE) in 180-200 g Sprague-Dawley (Envigo) male rats at the age of 7 weeks, as
5 previously described ¹. Starting by the administration of scopolamine methylnitrate (1 mg×kg-
6 1, s.c.; S-2250, Sigma-Aldrich) 30 min prior to pilocarpine hydrochloride (350 mg×kg-1, i.p.; P-
7 6503, Sigma-Aldrich). The onset of SE was characterized by continuous tonic-clonic activity of
8 the trunk and the limbs. Two hours later, to stop SE, rats received a first injection of diazepam
9 (Diazepam TVM[®], 10 mg×kg-1, i.p.; TVM lab) and a second half dose 30 min later. Rats were
10 then hydrated with 2 mL of saline solution (0.9% NaCl; s.c.) and housed in groups of 5 to avoid
11 any stress that may result from social isolation. During the recovery period, animals were
12 weighted, nourished and hydrated individually until their body-weight curve reached the one
13 of control rats.

14
15 ***Visual-tracking of spontaneous recurrent seizures after Pilo-SE.*** Visual tracking of behavioral
16 spontaneous recurrent seizures (SRSs) in rats aims to make sure that all Pilo-SE rats had
17 become epileptic (EPI rats). The visual-tracking of SRSs was fully described in a previous
18 published work ⁽¹⁾. It requires a daylight surveillance (between 9 a.m. and 5 p.m.) during
19 usually the 2-3 weeks following Pilo-SE. Animals that were identified as EPI rats developed at
20 least two spontaneous seizures during SRSs tracking. All rats that underwent Pilo-SE had
21 chronic epilepsy before they were included into experiments 1 and 2.

22
23 ***RT-qPCR quantification of serotonergic transcripts level within the brainstem.*** At 18 weeks
24 post-SE, epileptic and control rats were anesthetized with 4% of isoflurane, injected with lethal
25 dose of Euthasol (150 mg x kg⁻¹) and transcardially perfused with cold saline solution (30 mL
26 x min⁻¹) for 3 minutes to wash-out the blood from cerebral vessels. Blocs including medulla
27 oblongata and pons were dissected rostrally from the mesencephalon and caudally from
28 spinal-cord and frozen with liquid nitrogen. The quantification of transcript levels coding for
29 the key enzyme of 5-HT synthesis (TPH2), 5-HT reuptake (SERT), full-length 5-HT2C receptor
30 (5-HT2CR), and 2 ADARS isoforms (adenosine deaminase acting on RNA: ADAR1 and ADAR 2)
31 was performed using RT-PCR as previously reported (Kouchi, 2022). After extraction, total
32 RNAs were reverse transcribed to complementary DNA (cDNA) using both oligo dT and
33 random primers with PrimeScript RT Reagent Kit (Takara, #RR037A) according to
34 manufacturer's instructions in the presence of a synthetic external non-homologous poly(A)
35 standard messenger RNA (SmRNA; A. Morales and L. Bezin, patent WO2004.092414) to
36 normalize the RT step. Each cDNA of interest was amplified using the Rotor-Gene Q
37 thermocycler (Qiagen), the SYBR Green PCR kit (Qiagen, #208052) and oligonucleotide
38 primers (Eurogentec) specific to the targeted cDNA. cDNA copy number detected was
39 determined using a calibration curve, and results were expressed as cDNA copy number/μg
40 tot RNA. Sequences of the primer pairs used for PCR amplification are listed in supplementary
41 table S1.

42
43 ***Implantation of cerebral electrodes and wireless EEG recording.*** At 5-7 weeks post-SE, rats
44 were anesthetized with a mix of ketamine and xylazine (80/10 mg x kg⁻¹). Animals were placed
45 on a stereotaxic set-up and their head was fixed with ear-bars. After incision on the scalp, 4

46 cranial drills were made and stainless-steel screw electrodes were implanted bilaterally on
47 above the partial cortex, the right hippocampus and then the cerebellum as a reference. In
48 addition, each rat had an intra-cerebral electrode into the left hippocampus at Bregma
49 coordinates: AP: 0.38, ML: 0.25 and DV:0.32 according to the rat brain atlas. The distal part of
50 each electrode was plugged into a head connector and consolidated with dental cement.
51 During 2 weeks of recovery period, protocol of animal care was applied with 3 days of
52 analgesia (Carprofen, i.p., 5mg/kg), daily body-weight measurement, humidified food. Seizure
53 frequency, severity and duration were evaluated using video-EEG recordings coupled to a
54 wireless head-connector (EMKA technologies) that displayed EEG activity from 4 channels
55 corresponding to bilateral parietal and hippocampal regions. Telemetric signal was recorded
56 with an acquisition box and a home-made python program.

57

58 **Whole-body plethysmography.** Under BTPS (body temperature and pressure, saturated)
59 conditions, unrestrained rats were placed into a customized plethysmography chamber
60 (diameter: 20cm, height: 30cm) with two compartments, one where animal is placed and the
61 second as reference chamber (EMKA technologies, France). Breath-signal was acquired using
62 differential pressor captor (EMKA technologies, France) between 9:00 am and 5:00 pm and
63 home-made python program software ^(2,3). A constant airflow was supplied to animal with
64 inflow (from the top, 2000 mL x min⁻¹) and outflow (from the bottom port, 2000 mL x min⁻¹).
65 Note that the customization of high volume of plethysmography chamber and air-flow
66 supplying was thought to allow head-implanted animal to stand and move freely and avoid
67 important variation in temperature and accumulation of humidity within plethysmography
68 chambe. Experiments were conducted within animal facility where temperature of
69 experiment room is maintained at 22-24C° and relative humidity at 40-60%.

70 Before each animal recording, animal is weighted and the calibration was performed by
71 injecting three times 1 mL air-pulse inside the recording chamber. The mean value is therefore
72 used for milliliters conversion and the calculation of breathing volumes. During each
73 recording-day, we alternated assessment of ventilation between EPI and control groups in
74 order to reduce daytime bias and have close conditions between experimental groups,
75 including variation of body temperature, and arousal state. Hence, for morning and afternoon
76 sessions, at least one EPI rats and one control rats were assessed, respectively. Note that we
77 have previously reported that over the nycthemeral cycle (24-hours), body-temperature was
78 similar between control and EPI rats ⁽¹⁾.

79 Using spike-2 (Cambridge Electronic Design Limited, UK), respiratory frequency (FR) is
80 calculated from the deflation curve that results from rat's air inhalation, and which is
81 calculated from time (second) between two consecutive inspiratory events. Note that for
82 better accuracy, breath analysis is based on inspiratory events for the calculation of ventilatory
83 parameters. The estimation of tidal volume (VT) is defined by the inhaled volume, which is
84 calculated as the integral of each inspiratory curve that we assumed to occur under saturated
85 vapor pressior at body-temperature and normal barometric pressure (sea level, 760 mmHg)
86 conditions ^(4;5). Note that based on previous work, we also assumed that both EPI and control
87 rats had during the same period of the day, similar body-temperatures ⁽¹⁾. VT values are
88 corrected using calibration and 100 grammes of body-weight of rats. Minute ventilation (VE)
89 is calculated as product of instantaneous FR and VT. Coefficient of variation is calculated for
90 each rat as ratio of standard deviation divided and mean frequency. Apnea number and

91 duration were calculated as event that was greater than duration of two breath cycles within
92 a previous 20 sec of breathing. We subdivided apnea into spontaneous apnea and post-sigh
93 apnea, which is an apnea that occurs directly after a sigh event or separated by two respiratory
94 cycles. Sigh is each breath amplitude above at least 100% of the average of previous 20 sec of
95 breathing. Numbers of apneas and sighs are expressed as frequency of event per min of
96 breathing.

97 ***Paradigm of ventilation coupled to video-EEG recordings after acute administration of***
98 ***SB242084 (Figure S1).***

99 Session 1. At 18 weeks post-SE, to evaluate the effect of handling and injection on seizure
100 occurrence, head-connector was plugged to cortical electrodes of EPI rats to record video-EEG
101 for 30 min then rats are injected subcutaneously with vehicle solution (s.c., 10% DMSO in
102 NaCl) and recorded for one more hour. Control rats underwent the same protocol.

103 Session 2. At 19-22 weeks post-SE, to evaluate the effect of 5-HT_{2C} antagonism on epileptic
104 activity and ventilatory function, head-connected rats were placed into a plethysmography
105 chamber to perform coupled ventilation/video-EEG assessments under basal conditions for
106 30 min before SB242084 (diluted into 10% DMSO-NaCl solution) was injected (s.c., 2mg/kg).
107 After administration of SB242084, coupled ventilation/video-EEG was performed for 2 hours.
108 Note that video-EEG also allowed to define whether animal is awake or asleep during the
109 recording.

110 Data analysis. For the detection of epileptic activity in EPI rats, two experimenters analyzed
111 the video-EEG recordings. To evaluate the effect of SB242084 on seizure activity, we compared
112 seizure frequency, duration and severity over one-hour periods between session 1 and session
113 2. The one-hour period in session 1 included 60 min of video-EEG signal after vehicle
114 administration. In session 2, we recorded 2 hours after administration of SB242084, hence we
115 analyzed video-EEG signal for each hour, separately. Seizure severity was classified according
116 to the Racine's scale, defined as follows: stage 0, immobility; stage 1, mouth and facial
117 movements; stage 2, head nodding, clonic head movements; stage 3, forelimb clonus, clonic
118 forelimb movements; stage 4, rearing with bilateral forelimb clonus; stage 5, rearing and
119 falling with bilateral forelimb clonus.

120 To evaluate the ventilatory effect of SB242084, basal ventilation was assessed during 30 min
121 preceding SB242084 injection. Analysis of ventilation variables after SB242084 administration
122 was divided into 4 epochs of 30 min each as follow: 30 min (segment between injection time
123 0 to 30 min), 60 min (segment between injection time 31 to 60 min), 90 min (segment
124 between injection time 61 to 90 min), and 120 min (segment between injection time 91 to
125 120 min). It is noteworthy that to reduce bias of injection, the first 10 min of ventilatory signal
126 following injection was excluded. Moreover, ictal ventilation and the 10 min breath signal
127 following seizures was also removed from data presented for each time point. For the
128 comparison of ventilatory effect of SB242084 in EPI vs control rats, we considered each rat as
129 its own control and calculated the percentage of variation in ventilation after the
130 administration of SB242084 normalized to the mean value recorded under basal conditions.
131 This approach allowed to reduce both internal and external confounded factors and to obtain
132 more accuracy on the effect of SB242084 for the group comparison.

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135 **Supplementary results.**

136 **Effect of SB242084 on epileptic activity.**

137 We observed a trend toward an increased risk of seizure after SB242084 administration in EPI
138 rats ($p=0.07$), while no seizures were observed in controls. During session 1, only one of the
139 14 EPI rats (7%) showed a stage 4 seizure of 67.55 sec duration. SB242084 effect on seizure
140 activity was observed during the second hour post-administration. Indeed, during the first
141 hour post-SB242084 administration (from 0 to 60 min), 21% of EPI rats (3 of 14 rats) had
142 seizures, whose severity ranged from stages 2-3 ($n=2$) to stage 4 ($n=1$) with an average
143 duration of 36 ± 9 sec. Conversely, during the second hour after SB242084 administration (60
144 to 120 min), six EPI rats (43%) showed one seizure, ranging from stage 2-3 ($n=3$) to stage 4
145 ($n=3$) according to Racine scale. The mean \pm sem seizure duration was 47.2 ± 15.3 sec. No
146 seizure-related death was observed during both sessions or over experiments corroborating
147 previous studies that showed that mortality in pilocarpine induced-SE in rat is only reported
148 during SE and not during chronic phase of epilepsy^(7; 8).

149 Analysis of video-EEG showed that during the period of recordings (90 min and 180 min in
150 session 1 and 2, respectively), none of rats were asleep. This could therefore be linked to the
151 short period of recording and the fact that animal's rest during recording is interrupted when
152 rats received an injection with saline or SB242084 after 30 min of baseline.

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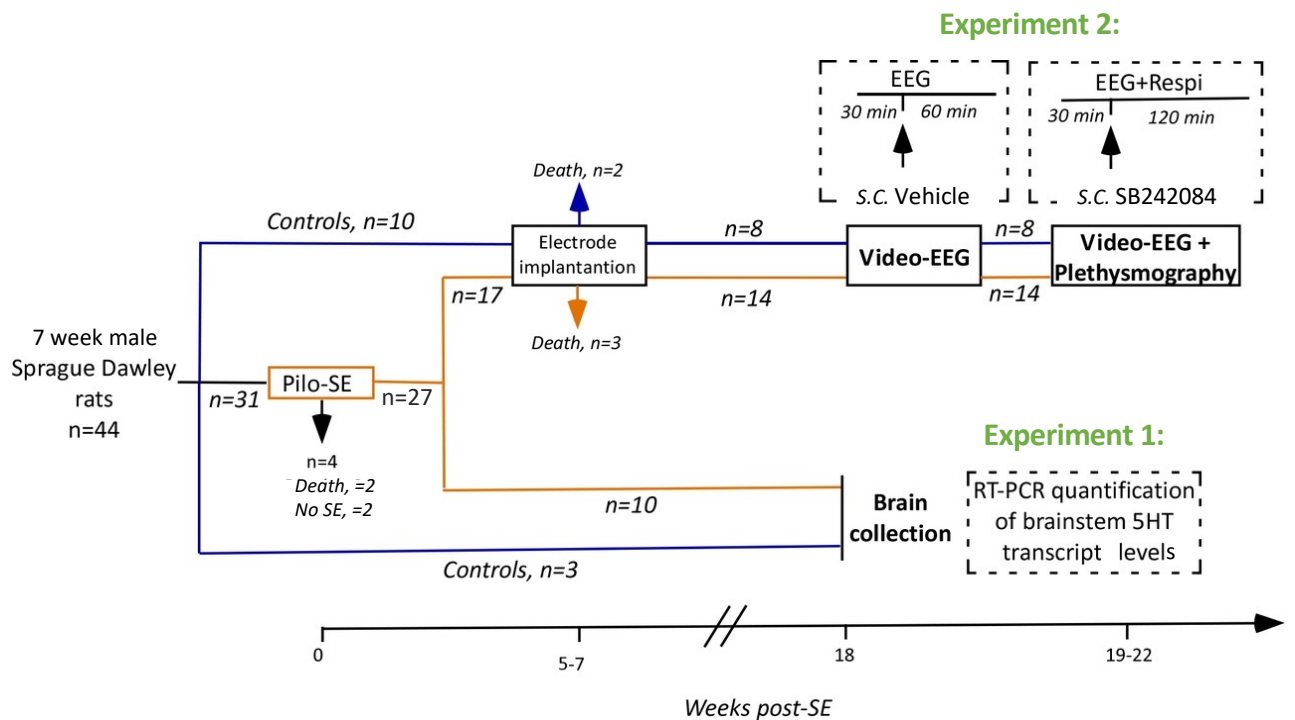
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172 **Supplementary tables/figures.**

173 **Supplementary figure S1.** Experimental design of the study. At 7 weeks of age, 31 rats
 174 underwent pilocarpine induced *status epilepticus* (SE) and 13 rats were used as controls.
 175 Subsequently to SE, 4 rats either died (n=2) or did not develop SE (n=2) and 28 rats became
 176 epileptic (EPI rats) at 2-3 weeks post-SE. At 5-7 weeks post-SE, 10 control and 17 EPI rats were
 177 implanted with cerebral electrodes to record seizures. Two control rats and 3 EPI rats did not
 178 survive to surgery. Experiment 1: at 18 weeks post-SE, 3 control rats and 10 EPI rats, which
 179 were not subjected to electrode implantations, have been used to quantify by RT-qPCR
 180 transcripts of the serotonin system from the brainstem. Experiment 2: at 18 weeks post-SE
 181 (session 1 of video-EEG), each rat (8 control rats, 14 EPI rats) was video-EEG recorded for 30
 182 min under baseline conditions, then subcutaneously injected with vehicle solution (s.c.; 10%
 183 of DMSO in 0.9% NaCl) and recorded for one additional hour. The aim of session 1 was to
 184 control the effect of injection and manipulation on epileptic activity, in order to later assess
 185 the specific effect of SB242084 on the same variables. Between 19- and 22- week post-SE
 186 (session 2 of video-EEG coupled with assessment of ventilatory function), each rat was
 187 individually placed into a plethysmography chamber coupled with video-EEG for 30 min under
 188 baseline conditions, then injected s.c. with SB242084 (specific 5-HT_{2C} antagonist, 2 mg/kg
 189 diluted in 10% of DMSO in NaCl solution) and recorded thereafter for 2 hours. The aim of
 190 session 2 was to evaluate the effect of SB242084 on epileptic activity and ventilatory function.

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195 **Supplementary table S1. Pair of primers**

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| Rat | Forward primer sequence (5'→3') | Reverse primer sequence (5'→3') |
|----------------------------------|---------------------------------|-----------------------------------|
| TPH2 (NM_173839.2) | TAC GGC ACC GAG CTT GAC | TGG CCA CAT CCA CAA AAT AC |
| SERT (NM_013034.3) | ATC ACC TGG ACG CTG CAT | TGG ATC TGC AGG ACA TGG |
| 5-HTR1a (NM_012585.1) | GGC ACC TTC ATC CTC TGC T | GTG GCA GCT GCT TTC ACA G |
| 5-HTR2c (NM_012765.3) | CCG AGT CCG TTT CTC GTC TA | CTA TGC TTG CAG GTA ATA GTT GAC A |
| 5-HTR3b (NM_022189.1) | CTC CTG GTG GCT GTC GTA G | AGA GGA ATT CCC AGG CTG A |
| ADAR1 (NM_031006.1) | CCT CTA CAT CAG CAC GGC AC | GGT ACG GAG TCT TTC CCC AA |
| ADAR2 (NM_001111056.1) | GTC TGC CTT GGC TAC TGT CT | AGC ACT TTT CTT CGT GCG TG |

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Supplementary table S2. Ventilation of rat with chronic epilepsy. Between weeks 19 and 22 post-SE, ventilation of 14 EPI and 8 control rats was recorded for 30 min. Values are expressed as mean ± SEM. Significance defined as P value <0.05 and resulted from unpaired t-test comparison between experimental groups.

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| Ventilation under basal conditions | Groups | | P value |
|--|--------------------|-----------------|-------------|
| | Control rats (n=8) | EPI rats (n=14) | Ctr vs EPI |
| Ventilation | | | |
| Ventilatory frequency (FR), breath cycle/min | 83 ± 3 | 79 ± 3 | 0.39 |
| Tidal volume (VT), mL/100g | 0.61 ± 0.04 | 0.52 ± 0.03 | 0.04 |
| Minute ventilation (VE), breath cycle/min/100g | 50 ± 3 | 40 ± 2 | 0.01 |
| Coefficient of variation | 0.38 ± 0.04 | 0.33 ± 0.04 | 0.46 |
| Apneas | | | |
| Frequency of spontaneous apnea, counts/min | 0.12 ± 0.03 | 0.11 ± 0.02 | 0.68 |
| Duration of spontaneous apnea, s | 1.91 ± 0.16 | 1.61 ± 0.09 | 0.10 |
| Frequency of post-sigh apnea, counts/min | 0.30 ± 0.05 | 0.23 ± 0.04 | 0.27 |
| Duration of post-sigh apnea, s | 1.68 ± 0.13 | 1.81 ± 0.10 | 0.46 |
| Total time in apnea, s/min | 0.75 ± 0.14 | 0.57 ± 0.08 | 0.24 |
| Sighs | | | |
| Frequency of total sighs, counts/min | 0.67 ± 0.09 | 0.49 ± 0.05 | 0.09 |
| Percentage of sighs without apnea | 53 ± 7 | 57 ± 7 | 0.69 |

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Supplementary table S3. Time course of ventilatory variables after the administration of SB242084. Between weeks 19 and 22 post-SE, ventilatory function of 8 control and 14 EPI rats was recorded over 120 min to evaluate the effect of SB242084 (specific 5-HT_{2C} antagonist).

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211 Ventilatory response to SB242084 was calculated for each experimental group and divided
212 into 4 epochs of 30-min period each. The time course started at the time of injection (time
213 point =0). Values are expressed as mean \pm SEM of each group. **(a)** Ventilatory function was
214 evaluated by minute ventilation (VE), which is the product of respiratory frequency (FR) and
215 estimated tidal volume (VT) that was reported to 100g of body-weight. **(B)** Apnea was defined
216 as cessation of breathing that was longer than previous two breath cycles. Spontaneous apnea
217 subtype was each apnea that was not preceded by a sigh event. Apnea post-sigh was each
218 ventilatory pause that occurred within the 2 breaths succeeding to a sigh. Frequencies are
219 expressed as numbers of events per time unit (min; sec). **(C)** Sigh was each breath amplitude
220 above at least 100% of the previous 20 sec of beathing, the frequency of total sighs included
221 number of sighs followed or not by apnea and reported per min of breathing. SB242084 effect
222 on control and EPI rats was statistically tested after comparison of baseline and SB242084
223 values, by using a one-way ANOVA with repeated measures, with the level of significance
224 defined by a P value <0.05. Dunnett post-hoc multiple comparison allowed to compare each
225 time point of the postSB242084 period to the baseline value. *<0.05, ** <0.01 and ***<0.001.

A.

| Ventilatory variables | Time course after SB242084 (s.c. 2mg/kg) | | | | Versus baseline (P value) |
|--|--|---------------|-------------|-------------|---------------------------|
| | 30 min | 60 min | 90 min | 120 min | |
| Controls rats (n=8) | | | | | |
| Ventilatory frequency (FR), cycle/min | 83 ± 6 | 70 ± 4* | 66 ± 4** | 62 ± 2*** | <0.0001 |
| Tidal volume (VT), mL/100g | 0.55 ± 0.04 | 0.56 ± 0.03 | 0.57 ± 0.03 | 0.56 ± 0.03 | 0.047 |
| Minute ventilation (VE), breath cycle/min/100g | 44 ± 2 | 39 ± 2*** | 37 ± 2** | 35 ± 2*** | <0.0001 |
| Coefficient of variation | 0.38 ± 0.03 | 0.30 ± 0.04 | 0.30 ± 0.05 | 0.29 ± 0.04 | 0.079 |
| Epileptic rats (n=14) | | | | | |
| Ventilatory frequency (FR), cycle/min | 96 ± 6 * | 80 ± 3 | 74 ± 3 | 74 ± 5 | 0.006 |
| Tidal volume (VT), mL/100g | 0.46 ± 0.03 * | 0.48 ± 0.03 * | 0.51 ± 0.02 | 0.49 ± 0.02 | 0.025 |
| Minute ventilation (VE), breath cycle/min/100g | 42 ± 2 | 38 ± 2 | 37 ± 2 | 36 ± 2 | 0.016 |
| Coefficient of variation | 0.38 ± 0.04 | 0.36 ± 0.04 | 0.30 ± 0.03 | 0.27 ± 0.02 | 0.034 |

B.

| Apnea pattern | Time course after SB242084 (s.c. 2mg/kg) | | | | versus baseline (P value) |
|--|--|---------------|--------------|-------------|---------------------------|
| | 30 min | 60 min | 90 min | 120 min | |
| Controls rats (n=8) | | | | | |
| Frequency of spontaneous apnea, counts/min | 0.12 ± 0.02 | 0.08 ± 0.02 | 0.10 ± 0.03 | 0.09 ± 0.02 | 0.280 |
| Frequency of post-sigh apnea, counts/min | 0.38 ± 0.06 | 0.33 ± 0.04 | 0.24 ± 0.05 | 0.19 ± 0.05 | 0.013 |
| Duration of spontaneous apnea, s | 1.40 ± 0.41 | 1.55 ± 0.29 | 1.79 ± 0.34 | 1.75 ± 0.32 | 0.389 |
| Duration of post-sigh apnea, s | 2.01 ± 0.27 | 2.03 ± 0.22 | 2.05 ± 0.19 | 2.10 ± 0.21 | 0.086 |
| Epileptic rats (n=14) | | | | | |
| Frequency of spontaneous apnea, counts/min | 0.15 ± 0.04 | 0.16 ± 0.03 | 0.16 ± 0.03 | 0.18 ± 0.03 | 0.400 |
| Frequency of post-sigh apnea, counts/min | 0.19 ± 0.05 | 0.12 ± 0.04** | 0.13 ± 0.02* | 0.15 ± 0.03 | 0.031 |
| Duration of spontaneous apnea, s | 1.04 ± 0.21* | 1.84 ± 0.20 | 1.76 ± 0.13 | 1.90 ± 0.12 | 0.013 |
| Duration of post-sigh apnea, s | 1.47 ± 0.09 | 1.24 ± 0.24 | 1.62 ± 0.24 | 1.52 ± 0.23 | 0.241 |

C.

| Sigh pattern | Time course after SB242084 (s.c. 2mg/kg) | | | | versus baseline (P value) |
|--------------------------------------|--|--------------|----------------|--------------|---------------------------|
| | 30 min | 60 min | 90 min | 120 min | |
| Controls rats (n=8) | | | | | |
| Frequency of total sighs, counts/min | 0.60 ± 0.06 | 0.52 ± 0.08 | 0.47 ± 0.07 | 0.40 ± 0.09* | 0.017 |
| Percentage of sighs without apnea, % | 37 ± 7 | 32 ± 7 | 52 ± 6 | 46 ± 11 | 0.155 |
| Epileptic rats (n=14) | | | | | |
| Frequency of total sighs, counts/min | 0.46 ± 0.05 | 0.34 ± 0.04* | 0.29 ± 0.04 ** | 0.31 ± 0.05* | 0.001 |
| Percentage of sighs without apnea, % | 62 ± 7 | 73 ± 7 | 64 ± 8 | 59 ± 8 | 0.349 |

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