

Role for serotonin_{2A} (5-HT_{2A}) and 2C (5-HT_{2C}) receptors in experimental absence seizures

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ARTICLE INFO

Article history:

Received 24 August 2015

Received in revised form

10 March 2016

Accepted 12 April 2016

Available online 13 April 2016

Keywords:

Absence epilepsy

Selective serotonin 2 receptor drugs

EEG

ABSTRACT

Absence seizures (ASs) are the hallmark of childhood/juvenile absence epilepsy. Monotherapy with first-line anti-absence drugs only controls ASs in 50% of patients, indicating the need for novel therapeutic targets. Since serotonin family-2 receptors (5-HT₂Rs) are known to modulate neuronal activity in the cortico-thalamo-cortical loop, the main network involved in AS generation, we investigated the effect of selective 5-HT_{2A}R and 5-HT_{2C}R ligands on ASs in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a well established polygenic rat model of these non-convulsive seizures. GAERS rats were implanted with fronto-parietal EEG electrodes under general anesthesia, and their ASs were later recorded under freely moving conditions before and after intraperitoneal administration of various 5-HT_{2A}R and 5-HT_{2C}R ligands. The 5-HT_{2A} agonist TCB-2 dose-dependently decreased the total time spent in ASs, an effect that was blocked by the selective 5-HT_{2A} antagonist MDL11,939. Both MDL11,939 and another selective 5-HT_{2A} antagonist (M100,907) increased the length of individual seizures when injected alone. The 5-HT_{2C} agonists lorcaserin and CP-809,101 dose-dependently suppressed ASs, an effect blocked by the selective 5-HT_{2C} antagonist SB 242984. In summary, 5-HT_{2A}Rs and 5-HT_{2C}Rs negatively control the expression of experimental ASs, indicating that selective agonists at these 5-HT₂R subtypes might be potential novel anti-absence drugs.

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Abbreviations: Serotonin, (5-HT); 5-HT receptor, (5-HTR); Knockout, (KO); Meta-chlorophenylpiperazine, (mCPP); Maximal dentate gyrus activation, (MDA); Absence seizures, (ASs); Genetic absence epilepsy rat from Strasbourg, (GAERS); Spike-and-wave discharges, (SWDs); Intraperitoneally, (i.p.); Two-way analysis of variance, (ANOVA); Wistar Albino Glaxo/Rij, (WAG/Rij); Thalamic reticular nucleus, (NRT); Rapid eye movement, (REM); Cannabinoid, (CB).

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1. Introduction

Since the original suggestion in the late 1950s' (Bonnycastle et al., 1957), many studies have supported the idea that the serotonin (5-HT) system might be implicated in both focal and generalized epilepsy. In particular, it has been shown that an increase in 5-HT tone is associated with an increased seizure threshold (and/or antiepileptic activity), whilst a reduced seizure threshold follows a decrease in 5-HT levels (reviewed in (Bagdy et al., 2007)). Moreover, many anti-epileptic drugs enhance brain extracellular 5-HT levels and many selective serotonin reuptake inhibitors (SSRIs) show an antiepileptic effect (Bagdy et al., 2007). Despite this large body of evidence, none of the currently available anti-epileptic drugs preferentially targets the 5-HT system, probably because of the lack of selective/specific ligands, the presence of harmful off-target effects and the complexity of the 5-HT receptor (5-HTR) system and its signaling pathways (Hannon and Hoyer, 2008; Stroth and Svenningsson, 2012).

The current classification of 5-HT₂Rs comprises up to 14 subtypes and the generation of selective pharmacological and genetic tools, i.e., knockout (KO) mice, to investigate the contribution of individual receptors is fairly recent (Hannon and Hoyer, 2008). Among the different 5-HT₂R subtypes (Hoyer et al., 2002), many lines of evidence suggest an involvement of 5-HT₂Rs in seizures (reviewed in (Di Giovanni and De Deurwaerdère, 2016; Guiard and Di Giovanni, 2015; Isaac, 2005; Jakus and Bagdy, 2011)). 5-HT_{2C}R KO mice display spontaneous tonic-clonic seizures which are occasionally lethal (Tecott et al., 1995), and a decreased threshold for various convulsing stimuli, e.g., kindling, pentylenetetrazol (PTZ), electroshock, audiogenic stimuli (Applegate and Tecott, 1998; Heisler et al., 1998). Further evidence of the protective role for 5-HT₂Rs against convulsive seizures comes from experiments using non-selective 5-HT_{2C} agonists which raise the threshold for PTZ- and electroshock-induced seizures (Upton et al., 1998). On the other hand, some 5-HT_{2C}R agonists with different pharmacological profiles, i.e., meta-chlorophenylpiperazine (mCPP) and lorcaserin, but not RO60-0175 (Martin et al., 1998) are able to stop the elongation of the electrically triggered hippocampal maximal dentate gyrus activation (MDA) in a limbic seizure model (Orban et al., 2014). As for 5-HT_{2A}Rs, fewer studies have investigated the role of these receptors in seizures, with most evidence showing that their activation has an antiepileptic effect (Gharedaghi et al., 2014; Guiard and Di Giovanni, 2015).

The evidence of a role for 5-HT₂Rs in generalized non-convulsive seizures is more limited and the interpretation of these studies is hampered by the use of relatively unselective drugs (Bagdy et al., 2007; Di Giovanni and De Deurwaerdère, 2016; Guiard and Di Giovanni, 2015). In the groggy model of absence seizures (ASs) (Tokuda et al., 2007), the 5-HT_{2A/2C} mixed agonist DOI dose-dependently reduces ASs, an effect that is blocked by the non-selective 5-HT₂R antagonist ritanserin (Ohno et al., 2010). In contrast, in the AY-9944 model of atypical ASs, mCPP has no effect, DOI dose-dependently decreases ASs and the moderately selective 5-HT_{2A}R antagonist ketanserin increases ASs in a non-dose-dependent manner (Bercovici et al., 2006). These authors concluded that 5-HT_{2A}Rs were responsible for this effect, although no 5-HT_{2A}R antagonist was tested against the anti-absence action of DOI. As far as typical ASs are concerned, experiments in the Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats, one of the best characterized rat models of these type of non-convulsive seizures (Coenen and Van Luijcklaar, 2003), have found that mCPP decreases ASs via 5-HT_{2C}Rs (Jakus et al., 2003). Moreover, SB-242084 a selective 5-HT_{2C}R antagonist, has no effect on ASs when administered alone, suggesting that 5-HT_{2C}Rs do not play a tonic modulatory role (Jakus and Bagdy, 2011; Jakus et al., 2003). In the other well characterized rat model of typical ASs, the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) (Danover et al., 1998) the contribution of the 5-HT system to ASs has only been partly investigated, probably because of the early negative results obtained with broad-spectrum first-generation 5-HT₂R agonists and antagonists or following modulation of the 5-HT tone by 5-HT uptake blockers and precursors (Marescaux et al., 1992a, 1992b) (reviewed in (Danover et al., 1998)).

In the present study, we evaluated the effects of pharmacological manipulation of 5-HT₂Rs on typical ASs and the interictal EEG in GAERS using drugs selective for 5-HT_{2A}Rs and 5-HT_{2C}Rs. The potent 5-HT_{2A}R agonist TCB-2 (McLean et al., 2006) was used in combination with the selective 5-HT_{2A}R antagonists MDL11,939 (Dudley et al., 1988) and M100,907 (Table 1) (Kehne et al., 1996). As far as 5-HT_{2C}Rs were concerned, we used CP-809,101, which shows ~1000 fold selectivity for the 5-HT_{2C}R over 5-HT_{2A}R and represents the most selective 5-HT_{2C}R drug currently available (Siuciak et al., 2007), and SB-242084, the most selective 5-HT_{2C}R antagonist

synthesized to date (Di Matteo et al., 2000; Kennett et al., 1997) (Table 1). Moreover, the anti-absence action of lorcaserin (Thomsen et al., 2008) was also investigated because, although it has only an approximately 10-fold higher affinity for 5-HT_{2C}Rs compared to 5-HT_{2A}Rs (Table 1), it is the first-in-class 5-HT_{2C}R agonist available for human use (FDA, 2012) and has shown an antiepileptic profile in an animal model of temporal lobe epilepsy (Orban et al., 2014). Our results show that both 5-HT_{2A}Rs and 5-HT_{2C}Rs negatively control the expression of experimental ASs, suggesting that selective agonists at these 5HT₂R subtypes might be potential novel anti-absence drugs.

2. Methods

Male GAERS rats (3–5 months old) were obtained from a colony bred at Cardiff University. Animals were housed in a 12:12 light cycle (lights on at 10.00 p.m. and off at 10.00 a.m.). All animal procedures were approved by the UK Home Office and carried out in accordance with Cardiff University ethical guidelines and in conformity with international law and policies (EU Directive, 2010/63/EU for animal experiments, ARRIVE guidelines and the Basel declaration including the 3R concept). All efforts were made to minimize animal suffering and to reduce the number of animals used (Lidster et al., 2015).

2.1. Surgery and EEG recordings

GAERS underwent chronic electrode implantation under general anesthesia (2% isoflurane). Epidural EEG electrodes (gold plated screws, Svenska Dentorama AB, Sweden) were implanted bilaterally over the frontal cortex, parietal cortex and in the cerebellum, as previously described (Cope et al., 2009). Animals were allowed to recover for at least 5 days. At 10.00 a.m. on the day of the experiment, the animals were placed into individual Plexiglas cages with access to food and water, and connected to a pre-amplifier (0.08 Hz high-pass filter, impedance 10 MΩ) and in turn to an analogue EEG amplifier (4-channel BioAmp, SuperTech Inc., Hungary) (1000 gain, low-pass filter at 500 Hz). The signal was digitized at 1000 Hz with a Cambridge Electronic Design (CED) Micro3 D.130 digitizer using CED Spike2 v7.3.

2.2. Experimental protocol

Once GAERS had been connected to the recording apparatus, they were left undisturbed for 1 h (habituation period). After that, video and EEG recordings commenced and continued for 40 min (control period). If the experiment involved pre-treatment of a 5-HT_{2A/2C} antagonist, the antagonist (or the corresponding vehicle) was intraperitoneally (i.p.) injected 10 min before the end of the control period. At the end of the control period, the animal was injected (i.p.) with the 5-HT drug of interest (or corresponding vehicle) and video and EEG recordings continued for the subsequent 2 h (treatment period). Drug injection order and doses were randomized in an incomplete crossover design and each drug-naïve animal received a maximum of three treatments in increasing dose protocols (vehicle, low dose, high dose) or four treatments when testing one dose of an agonist vs antagonist (e.g. vehicle + vehicle, vehicle + agonist, antagonist + vehicle, antagonist + agonist). The washout period was 5 days.

2.3. ASs detection and quantification

The detection of spike-and-wave discharges (SWDs) was semi-automatic, aided by the SeizureDetect script (kindly provided by Steve Clifford, CED) in Spike2 v7.3 (CED, UK), designed to

Table 1
Selectivity profile of 5-HT_{2A/2C} drugs used in the studyⁱ.

Compound	5-HT _{2A}			5-HT _{2B}			5-HT _{2C}			2C/2A
	Ki (nM) ^a	pEC ₅₀ ^b	Efficacy ^c	Ki (nM)	pEC ₅₀	Efficacy	Ki (nM)	pEC ₅₀	Efficacy	
5-HT _{2A} Rs										
TCB-2 ^d	0.73	6.8	—	—	—	—	—	—	—	—
MDL11,939 ^e	2.8	—	—	1419	—	—	853	—	—	—
M100,907 ^f	1.9 ^e	8.9	—	261 ^e	6	—	88 ^e	7.7	—	0.06
5-HT _{2C} Rs										
CP-809,101 ^g	6	6.8	0.67	64	7.2	0.57	1.6	10	0.93	1585
Lorcaserin ^h	159	6.7	1	190	6.0	1	29	7.9	1	16
SB242084 ^f	851	6.8	—	45	7.0	—	7.0	9	—	158

^a Ki: binding affinity.

^b pEC₅₀, potency: negative logarithm of the EC₅₀ (half-maximal effective concentration).

^c Efficacy relative to response to a supramaximal 5-HT concentration.

^d (McLean et al., 2006).

^e (Pehek et al., 2006).

^f (Bromidge et al., 1997).

^g (Siuciak et al., 2007).

^h (Thomsen et al., 2008).

ⁱ Modified from (Higgins et al., 2013a).

discriminate between sleep spindles and ASs. The data was high pass-filtered (DC remove, time constant 0.1 s) and the user selected manually a interictal segment of control, awake desynchronized EEG. The script automatically detected the crossings of a threshold defined as 5–9 SD above and below the mean voltage of the manually selected interictal period. Subsequently the crossings above threshold were grouped together in order to define putative SWDs according to 4 parameters. In order to define the start of the putative SWD, two crossings needed to be separated by less than the max onset interval (0.2 s). To be incorporated into the putative SWD the timing of the following crossings had to be lower than the maximum continuation interval (0.4 s). Putative SWDs less than 0.5 s apart were amalgamated. Putative SWDs with a duration of less than 1 s were discarded. The interval of consecutive crossings in each putative SWD was then used to define instantaneous frequencies (in the time domain). Only putative SWDs which had $\geq 75\%$ of their peaks in the 5–12 Hz frequency range were selected, resulting in the exclusion sleep/drowsiness epochs and artifacts. This semi-automatic SWD selection was further refined by visual inspection with a custom made Matlab script (Matlab R2013b, The Mathworks Inc., USA) and confirmed by the concomitant presence of behavioral arrest in the video recordings (Depaulis et al., 2016). Three parameters of ASs were quantified: total time spent in ASs, average duration of single ASs and total number of ASs (Marescaux et al., 1992a). For each animal and dose, the quantification of ASs was done in 20 min epochs and the value of each of the three parameters during the treatment period was normalized by expressing it as a percentage of the corresponding parameter in the control period. All statistical tests were performed on the data following this normalization (see section Statistical Analysis). For clarity, data are presented in the figures as percentage of the corresponding vehicle group (100% dotted line in figures).

2.4. Ictal EEG analysis

Spectral analysis of previously detected seizures was performed in order to determine the peak frequency of SWDs and to investigate possible changes induced by the drug treatment. Briefly, continuous wavelet transform (Morlet mother wavelet, $f_0 = 1$, range 5–14 Hz) was employed to determine the time-frequency profile of SWDs using a Matlab script kindly provided by Dr Dmytro Iatsenko (Iatsenko et al., 2013) (freely available at [http://www.](http://www.physics.lancs.ac.uk/research/nbmphysics/diats/tfr/)

[physics.lancs.ac.uk/research/nbmphysics/diats/tfr/](http://www.physics.lancs.ac.uk/research/nbmphysics/diats/tfr/)). The instantaneous frequencies corresponding to each previously detected SWD during the treatment period were extracted from the wavelet power maxima in the range 5–14 Hz. The mean frequency was calculated for each SWD and then averaged across all SWDs for each animal.

2.5. Interictal EEG analysis

Spectral analysis of the interictal EEG was performed on the raw data after preprocessing with Fieldtrip (Oostenveld et al., 2011). This involved resampling the data to 200 Hz after applying an anti-aliasing (low-pass) FIR filter. Each EEG recording was analyzed blind and artifacts and sleep epochs were marked manually with custom-made Matlab scripts. Time-frequency decomposition was performed with a script kindly provided by Dmytro Iatsenko (Leicester University, UK) performing short-time Fourier transform (binning 1–80 Hz, Gaussian window, $f_0 = 1$) on 20-min bins, matching those used for AS analysis. Samples corresponding to previously detected artifacts, SWDs and sleep periods were excluded from the analysis. Power spectra were then averaged over time for each 20-min epoch. Five EEG bands were defined for the analysis: delta (1–4 Hz), theta (5–8 Hz), alpha (9–12 Hz), beta (13–30 Hz) and gamma (31–80 Hz). Data within 48–52 Hz were excluded from the analysis of the gamma band to avoid contamination with the 50 Hz power line.

2.6. Statistical analysis

All statistical analyses were performed with Graphpad Prism version 5.00 (GraphPad Software, San Diego, USA). The effect of drug administration on the expression of absence seizures was analyzed via non-repeated measures two-way analysis of variance (ANOVA) with drug and time as factors. Dunnett's post-hoc testing was employed to test for the simple main effect of drug vs. vehicle for the full treatment period, whereas Sidak's multiple comparison test was applied to analyze the time-course of the drug effects for each 20 min bin compared to the corresponding time for vehicle. Statistical analysis on peak SWD frequency was performed using unpaired *t*-test. The effect of drug administration on the interictal EEG was analyzed via non-repeated measures two-way analysis of variance (ANOVA) with the frequency bands and time as factors.

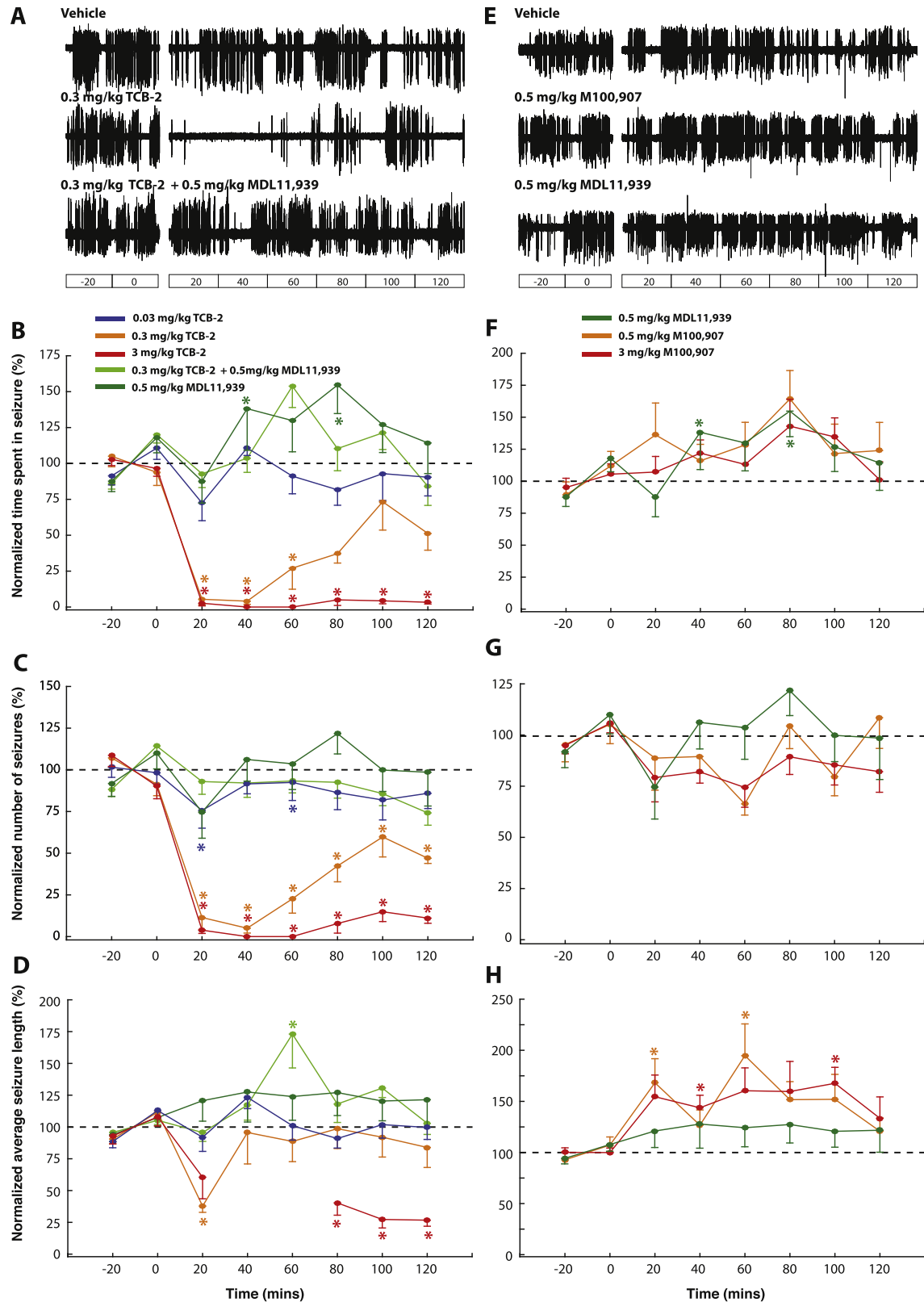


Fig. 1. Effects of the 5-HT_{2A} agonist TCB-2 and the 5-HT_{2A} antagonists MDL11,939 and M100,907 on ASs. (A) Representative EEG traces for GAERS injected i.p. with vehicle, 0.3 mg/kg TCB-2 and 0.3 mg/kg TCB-2 following pre-treatment with MDL11,939 (0.5 mg/kg, i.p.) (interruption in each trace indicates time of injection). Dose response curves for TCB-2 effects on normalized total time spent in seizures (B), number of seizures (C) and seizure length (D). Pre-treatment with the selective 5-HT_{2A} antagonist MDL11,939 (0.5 mg/kg) blocked the effect of TCB-2 (3 mg/kg) on the three seizure parameters. (E) Representative EEG traces for GAERS injected i.p. with vehicle, 0.5 mg/kg MDL11,939 and 0.5 mg/kg M100,907 (interruption in each trace indicates time of injection). Effect of MDL11,939 (0.5 mg/kg) and M100,907 (0.5–3 mg/kg) on normalized total time spent in seizures (F), seizure length (G) and number of seizures (H). Although no single time point reached significance after post-hoc testing, both 5-HT_{2A} antagonists significantly increased the time spent in seizures and seizure length when considering the overall treatment time (see text for details). All values are normalized to the control period (–40 to 0 min), and are

Sidak's multiple comparison test was applied to analyze the time-course of the drug effect on the interictal EEG for each 20 min bin compared to the corresponding time for vehicle. All quantitative data are reported in the text and figures as mean \pm SEM (unless otherwise stated).

2.7. Drugs

The selective 5-HT_{2A}R antagonist M100907 (Kehne et al., 1996) was purchased from Sigma-Aldrich (UK). The selective 5-HT_{2C}R antagonist SB-242084 (Kennett et al., 1997), the potent 5-HT_{2A}R agonist TCB-2 (McLean et al., 2006), the selective 5-HT_{2A}R antagonist MDL11,939 (Dudley et al., 1988) and the selective 5-HT_{2C}R agonist CP-809,101 (Siuciak et al., 2007) were purchased from Tocris Biosciences (UK). The 5-HT_{2C}R agonist Lorcaserin (Thomsen et al., 2008) was a kind gift from Arena Pharmaceuticals Inc. (USA). All common laboratory reagents were purchased from Sigma-Aldrich (UK). TCB-2 and lorcaserin were dissolved in 0.9% saline. CP-809,101 was dissolved in 2% Tween 80. SB-242084 and M100907 were dissolved in 25 mM citric acid, 8% (2-Hydroxypropyl)- β -cyclodextrin (w/v) in 0.9% saline. MDL11,939 was dissolved in 0.9% saline and 5% glacial acetic acid and the pH adjusted to 7.0 with NaOH. Drugs were selected for being the most selective 5-HT_{2A}R/5-HT_{2C}R agents available to date for research use (Table 1) and, in the case of Lorcaserin, in view of its clinical use (FDA, 2012). TCB-2 was selected for its high affinity for 5-HT_{2A}R because, to the best of our knowledge, no selective 5-HT_{2A}R agonist has been synthesized to date.

3. Results

The behavioral and EEG features of ASs recorded in vehicle-treated, freely moving GAERS were similar to those previously reported for this experimental model under similar experimental conditions (Cope et al., 2009; Danober et al., 1998). These included behavioral arrest, with occasional head and vibrissae twitching, and SWDs of 16.1 ± 14.3 s duration and 6.90 ± 0.72 Hz peak frequency ($n = 8914$ seizures, mean \pm standard deviation) (see Fig. 3A).

3.1. Effect of 5-HT_{2A}R modulation on spontaneous ASs

GAERS were injected i.p. with vehicle ($n = 9$) or TCB-2 (0.03, 0.3, 3 mg/kg; $n = 6$ –9 for each dose) in a randomized order, and the resulting effects (normalized to the effect of the vehicle group) are shown in Fig. 1A–D. As revealed by post-hoc testing for the simple main effect of the drug, TCB-2 decreased the total time spent in seizure compared to vehicle (0.03 mg/kg: $10.1 \pm 4.6\%$ overall decrease, $p < 0.05$; 0.3 mg/kg: $69.4 \pm 9.2\%$ overall decrease, $p < 0.001$; 3 mg/kg: $97.4 \pm 1.5\%$ overall decrease, $p < 0.001$). This effect was dose-dependent (0.03 vs 0.3 mg/kg: $p < 0.001$; 0.3 vs 3 mg/kg: $p < 0.001$) (Fig. 1B). The average number of seizures was also significantly decreased by all doses of TCB-2 (0.03 mg/kg: $14.4 \pm 9.7\%$ overall decrease, $p < 0.001$; 0.3 mg/kg: $68.6 \pm 7.2\%$ overall decrease, $p < 0.001$; 3 mg/kg: $93.7 \pm 2.8\%$ overall decrease, $p < 0.001$), an effect that was dose-dependent (0.03 vs 0.3 mg/kg: $p < 0.001$; 0.3 vs 3 mg/kg: $p < 0.001$) (Fig. 1C). There was no effect on the length of individual ASs at 0.03, while 0.3 mg/kg TCB-2 elicited a reduction in seizure length at 20 min post-injection

($62.2 \pm 4.9\%$ decrease, $p < 0.001$). Moreover 3 mg/kg TCB-2 elicited a drastic reduction of seizure length ($81.1 \pm 7.0\%$ decrease at 80–120 min, $p < 0.001$) (Fig. 1D). Note that during the 40 and 60 min post-injection time-bins of 3 mg/kg TCB-2 no seizures were observed, and thus no estimation of seizure length was possible (gaps in Fig. 1D). Finally, an increase of the SWD peak frequency was observed only at the highest dose of TCB-2 (vehicle: 7.1 ± 0.1 Hz; 3 mg/kg TCB-2: 7.6 ± 0.2 Hz; $p < 0.05$) (Fig. 3B).

Pre-treatment with the 5-HT_{2A} antagonist MDL11,939 (0.5 mg/kg, i.p., $n = 7$) blocked the effect of 0.3 mg/kg TCB-2 on the total time spent in seizure (Fig. 1A, B) and seizure length (Fig. 1D) (simple main effect of 0.5 mg/kg MDL11,939 + 0.3 mg/kg TCB-2 vs vehicle, ns), and greatly attenuated the effect of the agonist on the number of seizures (Fig. 1C) (simple main effect of MDL11,939 + TCB-2 vs vehicle, $11.5 \pm 7.9\%$ overall decrease, $p < 0.05$; compared to $68.6 \pm 7.2\%$ overall decrease for 0.3 mg/kg TCB-2 vs vehicle). Interestingly, MDL11,939 (0.5 mg/kg, $n = 11$) on its own significantly increased the total time spent in ASs compared to vehicle (overall $25.5 \pm 21.1\%$ increase, $p < 0.05$). Moreover, a significant effect of this drug was observed on seizure length (overall $23.5 \pm 18.9\%$ increase, $p < 0.05$), but not on seizure number. To further confirm these effects of MDL11,939, another selective 5-HT_{2A}R antagonist, M100,907, was injected in a naïve group of GAERS (Fig. 1E–H). Post-hoc testing on the simple main effect of the drug showed that M100,907 induced a significant increase in the total time spent in ASs both at 0.5 mg/kg ($31.7 \pm 20.4\%$ overall increase, $p < 0.001$, $n = 11$) and at 3 mg/kg ($20.1 \pm 14.6\%$ overall increase, $p < 0.05$, $n = 9$). This effect was driven by a significant increase in the seizure length, both at 0.5 mg/kg ($52.3 \pm 20.7\%$ overall increase, $p < 0.001$) and at 3 mg/kg ($53.2 \pm 20.3\%$ overall increase, $p < 0.001$). In addition, M100,907 induced a small, but significant, decrease in the number of ASs both at 0.5 mg/kg ($10.4 \pm 10.7\%$ overall decrease, $p < 0.01$) and at 3 mg/kg ($17.9 \pm 9.2\%$ overall decrease, $p < 0.01$). Moreover, while no significant change of the peak SWD frequency was found upon treatment with MDL11,939, both doses of M100,907 induced a modest, but significant decrease in the SWD peak frequency (vehicle: 7.1 ± 0.1 Hz vs 0.5 mg/kg: 6.85 ± 0.05 Hz; $p < 0.05$; vs 3 mg/kg: 6.9 ± 0.05 ; $p < 0.05$) (Fig. 3B).

The block of ASs by TCB-2 was accompanied by behavioral components typical of 5-HT_{2A}R activation in rodents (e.g., wet dog shakes, head twitches) (Bedard and Pycock, 1977), that were short-lasting compared to its antiabsence effect. Importantly, the effect of TCB-2 on both ASs and the animal behavior was blocked by pre-treatment with the 5-HT_{2A} antagonist MDL11,939, confirming that the effect was indeed driven by the activation of 5-HT_{2A}Rs. In summary, these results indicate that selective activation of 5-HT_{2A}Rs markedly decreases spontaneous, genetically determined ASs and that this 5-HTR subtype exerts a negative tonic modulation of these non-convulsive seizures.

3.2. Effect of 5-HT_{2C}R modulation on spontaneous ASs

We next investigated the action of the selective 5-HT_{2C}R agonist CP-809,101 in different groups of GAERS at doses (0.3, 3 and 10 mg/kg, $n = 6$ –10 per dose) similar to those used in previous studies (Higgins et al., 2013b; Siuciak et al., 2007). No effect of 0.3 mg/kg was found on the total time spent in ASs, seizure length and number of seizures (Fig. 2A–D). However CP-809,101 induced a

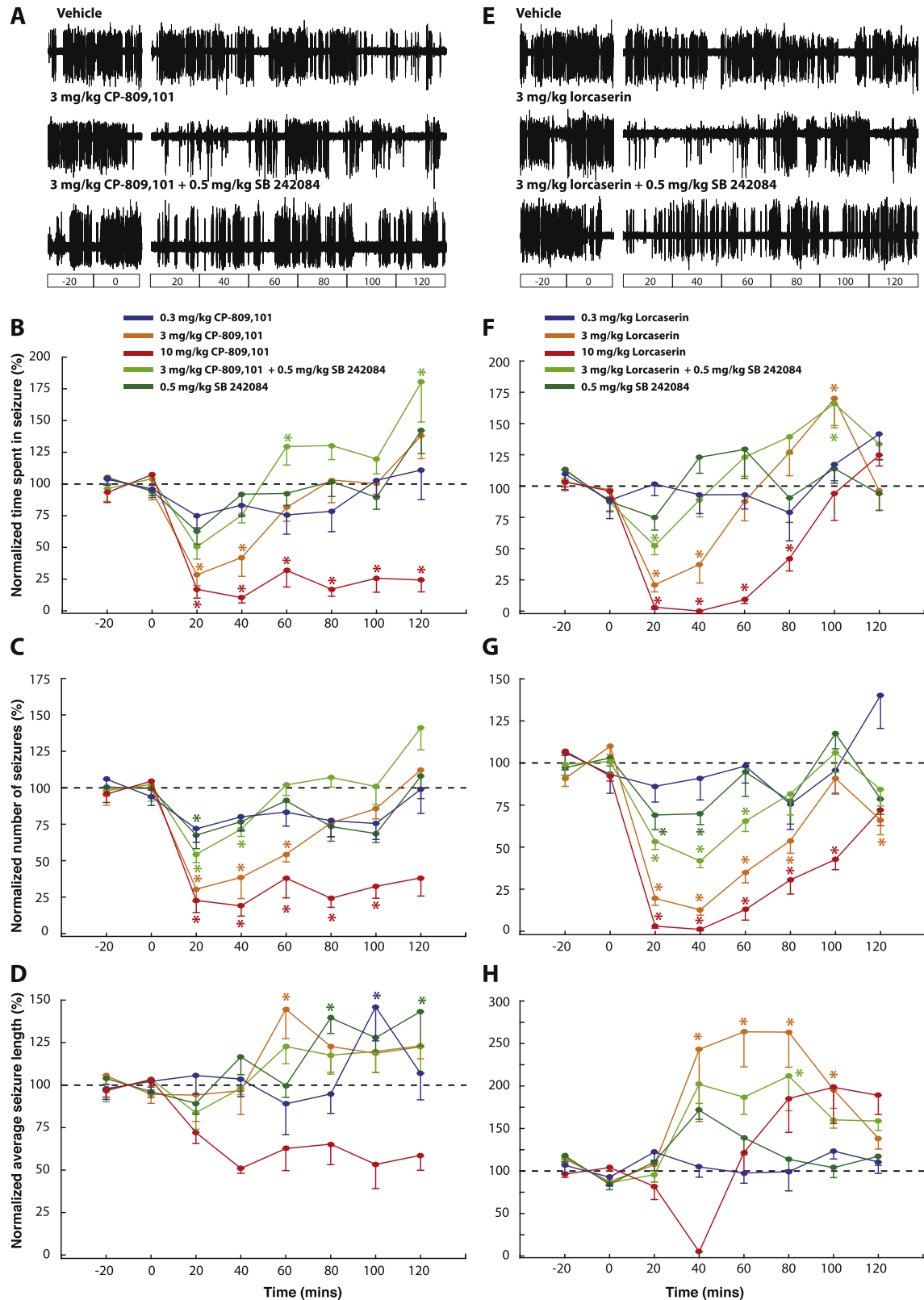


Fig. 2. The 5-HT_{2c}R agonists lorcaserin and CP-809,101 dose-dependently decrease ASS. (A) Representative EEG traces for GAERS injected i.p. with vehicle, 3 mg/kg CP-809,101 and 3 mg/kg CP-809,101 following pre-treatment with SB242084 (0.5 mg/kg, i.p.) (interruption in each trace indicates time of injection). Dose response curve of CP-809,101 at 0.3–3–10 mg/kg for normalized total time spent in seizure (B), number of seizures (C) and seizure length (D). Pre-treatment with the selective 5-HT_{2c}R antagonist SB242084 (0.5 mg/kg) partially blocked the effect of CP-809,101 (3 mg/kg) on the three seizures parameters. (E) Representative EEG traces for GAERS injected i.p. with vehicle, 3 mg/kg lorcaserin and 3 mg/kg lorcaserin following pre-treatment with SB242084 (0.5 mg/kg, i.p.) (interruption in each trace indicates time of injection). Dose response curves of lorcaserin (right side of the figure) at 0.3–3–10 mg/kg for normalized total time spent in seizure (F), number of seizures (G) and seizure length (H). Pre-treatment with the selective 5-HT_{2c}R antagonist SB242084 (0.5 mg/kg) partially blocked the effect of CP-809,101 (3 mg/kg) on the three seizures parameters. All values are normalized to the control period (–40 to 0 min), and are

decrease in the total time spent in seizure at 3 mg/kg ($17.7 \pm 13.1\%$ overall reduction, $p < 0.001$) and 10 mg/kg ($78.9 \pm 8.3\%$ overall reduction, $p < 0.001$). The effect of 3 mg/kg was strong but of short duration, with post-hoc testing showing a significant effect for the first 40 min post-injection ($64.7 \pm 11.7\%$ decrease, $p < 0.05$), whereas for 10 mg/kg the total time spent in ASs was drastically reduced throughout the 2 h treatment period (Fig. 2B). Moreover, whereas 3 mg/kg CP-809,101 increased seizures length ($21.4 \pm 14.4\%$ overall increase, $p < 0.05$), 10 mg/kg elicited a significant reduction in seizure length ($39.5 \pm 9.5\%$ overall reduction, $p < 0.001$) (Fig. 2D). The number of seizures was reduced for both 3 and 10 mg/kg CP-809,101, an effect that was significant for 60 min ($58.7 \pm 9.5\%$ mean reduction, $p < 0.05$) and 100 min post-injection ($72.7 \pm 8.6\%$ mean reduction, $p < 0.05$) (Fig. 2C). Pre-treatment with 0.5 mg/kg SB-242084 ($n = 10$) blocked the effect of CP-809,101 (3 mg/kg) on the total time spent in ASs (simple main effect of 0.5 mg/kg SB-242084 + 3 mg/kg CP-809,101 vs vehicle, ns), but only partially blocked the effect of this agonist on seizure length and number of seizures (Fig. 2A–D). Moreover, a significant increase of the SWD peak frequency was observed following the highest dose of CP-809,101 (vehicle: 7.1 ± 0.1 Hz, 10 mg/kg: 7.5 ± 0.1 Hz; $p < 0.05$) (Fig. 3B).

The action of the less selective 5-HT_{2C} agonist lorcaserin (Thomsen et al., 2008) (0.3, 3, 10 mg/kg; $n = 6$ –10 per dose) was more complex than that observed following CP-809,101 treatment. At 0.3 mg/kg lorcaserin had no significant effect on total time spent in ASs, seizure length and number of seizures (Fig. 2F, G, H). Although the overall effect of 3 mg/kg lorcaserin was only a small but significant decrease in the total time spent in ASs ($10.1 \pm 15.2\%$, $p < 0.05$), post-hoc testing showed that this action was due to clear time-dependent biphasic effect, i.e. a marked decrease in total AS time during the first 40 min post-injection (20 min: $78.9 \pm 5.7\%$, $p < 0.001$; 40 min: $62.6 \pm 14.9\%$, $p < 0.01$), which was followed by an increase 100 min post-injection (69.8 ± 21.5 ; $p < 0.01$) (Fig. 2F). This complex action of 3 mg/kg lorcaserin on total AS time could be explained by the different effect that this dose had on the number of seizures and seizure length, with the former markedly decreasing for almost all time points post-injection (overall reduction: $53.6 \pm 6.5\%$, $p < 0.001$) (Fig. 2G) while the latter was increased during the same observation period (overall enhancement: $201 \pm 30\%$, $p < 0.001$) (Fig. 2H). The higher dose of lorcaserin (10 mg/kg) elicited an overall more potent reduction in the total time spent in ASs (overall decrease: $54.5 \pm 7.5\%$, $p < 0.001$) which could also be explained by a marked reduction in the number of seizures. A trend for an increase in seizure length was evident in the second hour of the recording although no individual time bin reached significance in the post-hoc testing (Fig. 2H). Moreover, we observed no change in the SWD peak frequency following any doses of lorcaserin (Fig. 3B).

Pre-treatment with the selective 5-HT_{2C} antagonist SB 242084 (Di Matteo et al., 2000) (0.5 mg/kg, $n = 9$) almost fully abolished the effect of lorcaserin (3 mg/kg) on the total time spent in ASs (simple main effect of SB 242084 + lorcaserin vs vehicle, ns), but only partially blocked the effect of the agonist on seizure length and number of seizures (Fig. 2F–H). SB 242084 injected alone ($n = 10$) had no significant effects on the total time spent in seizures and on seizure length. Surprisingly, SB 242084 decreased the number of seizures in the first 20 and 40 min post-injection (20 min: $30.1 \pm 8.7\%$, $p < 0.01$; 40 min: $30.1 \pm 6.4\%$, $p < 0.05$) (Fig. 2F–H). No

change in the SWD peak frequency was observed following treatment with SB 242084 (Fig. 3B).

Both CP-809,101 and lorcaserin produced 5-HT_{2C} behavioral effects consistent with those reported previously (Di Giovanni and De Deurwaerdere, 2016). In particular penile grooming was observed at all doses of both 5-HT_{2C} agonists, and hypolocomotion was evident especially at high doses (i.e., 10 mg/kg lorcaserin and CP-809,101). These effects were short-lasting compared to their antiabsence effect and antagonized by pre-treatment with the selective 5-HT_{2C} antagonist SB 242084, which had no behavioral effect on his own, consistent with previous reports (Higgins et al., 2001; Kennett et al., 1997). In summary, these results indicate that selective activation of 5-HT_{2C}Rs decreases spontaneous, genetically determined ASs and that this 5-HTR subtype does not appear to exert a tonic modulation on these non-convulsive seizures. Of note, SB 242084 showed an antiabsence effect at some time-points, confirming the complexity of the 5-HT_{2C}R system (see (Di Giovanni and De Deurwaerdere, 2016)).

3.3. Effects of 5-HT_{2A}R and 5-HT_{2C}R agonists on interictal EEG

In addition to modifying ASs, both at the EEG and behavioral level, the highest doses of TCB-2 (3 mg/kg) and CP-809,101 (10 mg/kg) were also able to produce significant modifications of the power of different frequency bands in the interictal EEG (Fig. 4). TCB-2 induced a long-lasting decrease in the power of the gamma ($26.4 \pm 4.4\%$ mean reduction, $p < 0.001$) and alpha ($28.0 \pm 8.5\%$ mean reduction, $p < 0.001$ vs vehicle) frequency bands. CP-809,101 elicited a reduction in the gamma band power ($26.1 \pm 21.5\%$, mean decrease, $p < 0.05$) and a drastic increase in the delta band ($64.5 \pm 23.0\%$ mean increase, $p < 0.001$). Finally, a trend for an increase in the delta band power and a decrease in the alpha band was apparent in the interictal EEG following the injection of 10 mg/kg lorcaserin although no individual time epoch reached statistical significance.

4. Discussion

The main conclusions of this study are twofold i) selective activation of both 5-HT_{2A}Rs and 5-HT_{2C}Rs decreases spontaneous, genetically determined ASs, and ii) only 5-HT_{2A}Rs exert a negative tonic modulation on these non-convulsive seizures.

4.1. Effect of 5-HT_{2A}R ligands on ASs

5-HT_{2A}Rs have not previously been implicated in the pathogenesis or modulation of ASs, except from the indirect evidence provided by the block of ASs by DOI, a mixed 5-HT_{2A/2C} agonist, in the groggy rats, a model of these non-convulsive seizures that still remains to be fully characterized (Ohno et al., 2010). Regardless of the current lack of highly selective 5-HT_{2A} agonists (Nichols, 2004; Roth, 2011), the solidity of our results is supported by our approach of using, as in previous studies by other groups (Fox et al., 2010; Goda et al., 2013), a potent (i.e., nM affinity) 5-HT_{2A}R agonist (TCB-2) in conjunction with a selective 5-HT_{2A} antagonists (MDL11,939) at a concentration that is known to block 5-HT_{2A}- but not 5-HT_{2C}-mediated behaviors (Fletcher et al., 2002).

The presence of an inhibitory tone of 5-HT_{2A}Rs on ASs, as indicated by a significant increase in seizure length following

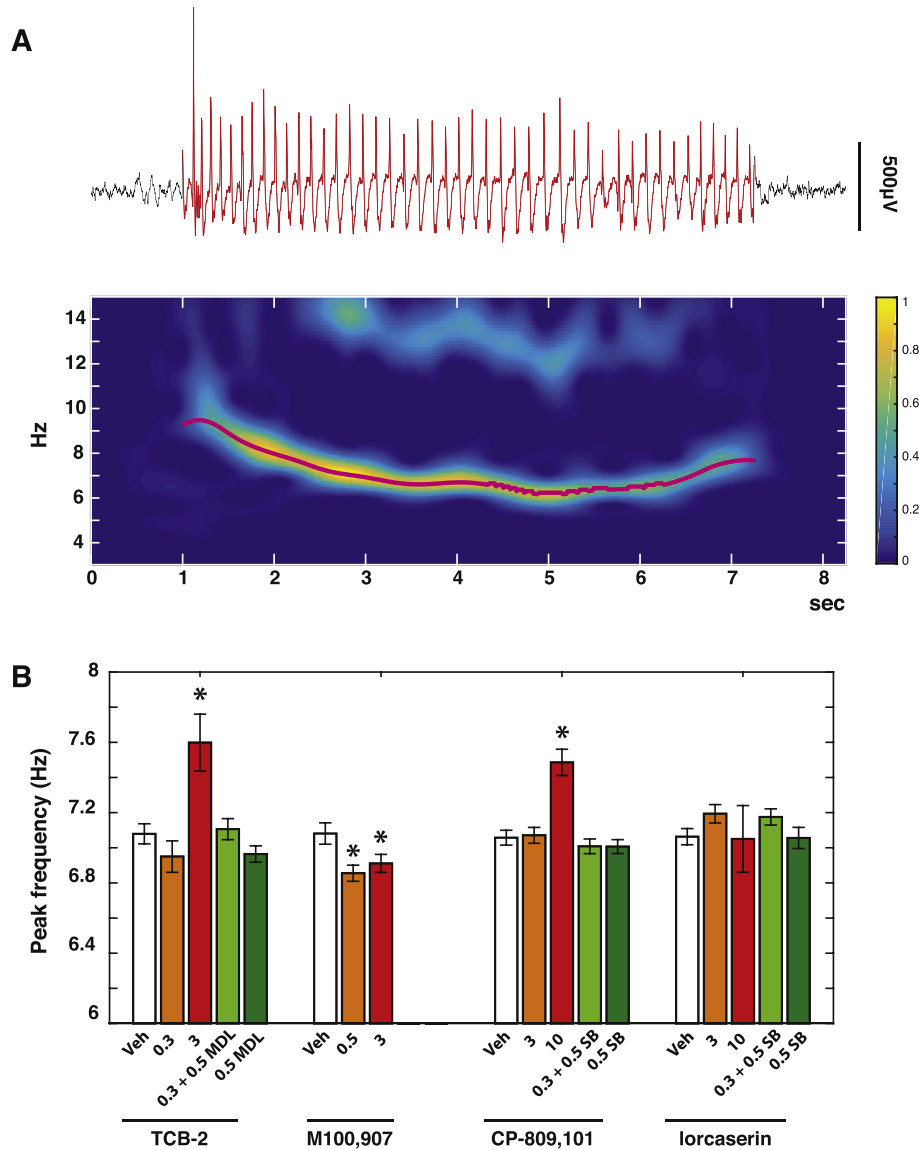


Fig. 3. Effect 5-HT_{2A/C}Rs modulation on SWDs peak frequency. (A) Representative spike and wave discharge (SWD) (red, top) and corresponding wavelet power spectrum on a time-frequency representation (bottom). The peak frequency of the SWD was identified by extracting the instantaneous frequency of the power maximum in the range 5–14 Hz at each time point of the SWD (magenta line in time-frequency plot) and subsequently calculating its mean. (B) Changes (mean \pm SEM) in SWD peak frequency induced by administration of 5-HT_{2A}R and 5-HT_{2C}R agonists and antagonists (bottom row) isolated or in combination with another drug (doses in mg/kg are reported at the bottom of the graph). Asterisks indicate $p < 0.05$ (independent samples t -test for drug vs vehicle).

administration of either of the two 5-HT_{2A}R antagonists used in this study (MDL11,939 and M100,907), supports the idea that these receptors tonically affect the duration of seizures in GAERS. The conclusion that drugs blocking 5-HT_{2A}Rs might increase AS duration is in line with the findings in WAG/Rij rats treated with atypical antipsychotics where risperidone but not quetiapine was found to increase AS duration (Citraro et al., 2015). Indeed, risperidone has a ~100 fold higher affinity than quetiapine for 5-HT_{2A}Rs (Richtand et al., 2007). However, it should be noted that atypical antipsychotics affect multiple neurotransmitter systems which may also underlie their effects on seizures.

4.2. Effects of 5-HT_{2C}R ligands on ASs

Of the two 5-HT_{2C}R agonists employed in this study, CP-809,101 displays a high (>1000 fold) selectivity for 5-HT_{2C}Rs over 5-HT_{2A}Rs whereas lorcaserin has a lower (~10 fold) selectivity, though its

pharmacokinetics and pharmacodynamics have been thoroughly characterized. The selected doses of both substances are comparable to those that elicit typical 5-HT_{2C}-mediated behaviors in rats, such as hypophagia and nicotine self-administration (Higgins et al., 2013b; Thomsen et al., 2008). These two 5-HT_{2C}R agonists induced a dose-dependent, marked and SB 242084-sensitive decrease of ASs up to 80 min post-injection. These results are consistent with, and extend, those obtained in WAG/Rij rats with the non-selective 5-HT_{2/1B}R agonist mCPP (Jakus et al., 2003). The lack of pro-convulsant effect of the selective 5-HT_{2C} antagonist SB 242084 on ASs in GAERS, and in WAG/Rij rats (Jakus et al., 2003), might be surprising since 5-HT_{2C}R KO mice show convulsive seizures and a higher susceptibility to different convulsive agents (Tecott et al., 1995). However, it is well known that the pathophysiological mechanisms leading to the expression of ASs are drastically different from those of convulsive seizures (Crunelli and Leresche, 2002). Conversely, in our hands SB 242084 had at some time-

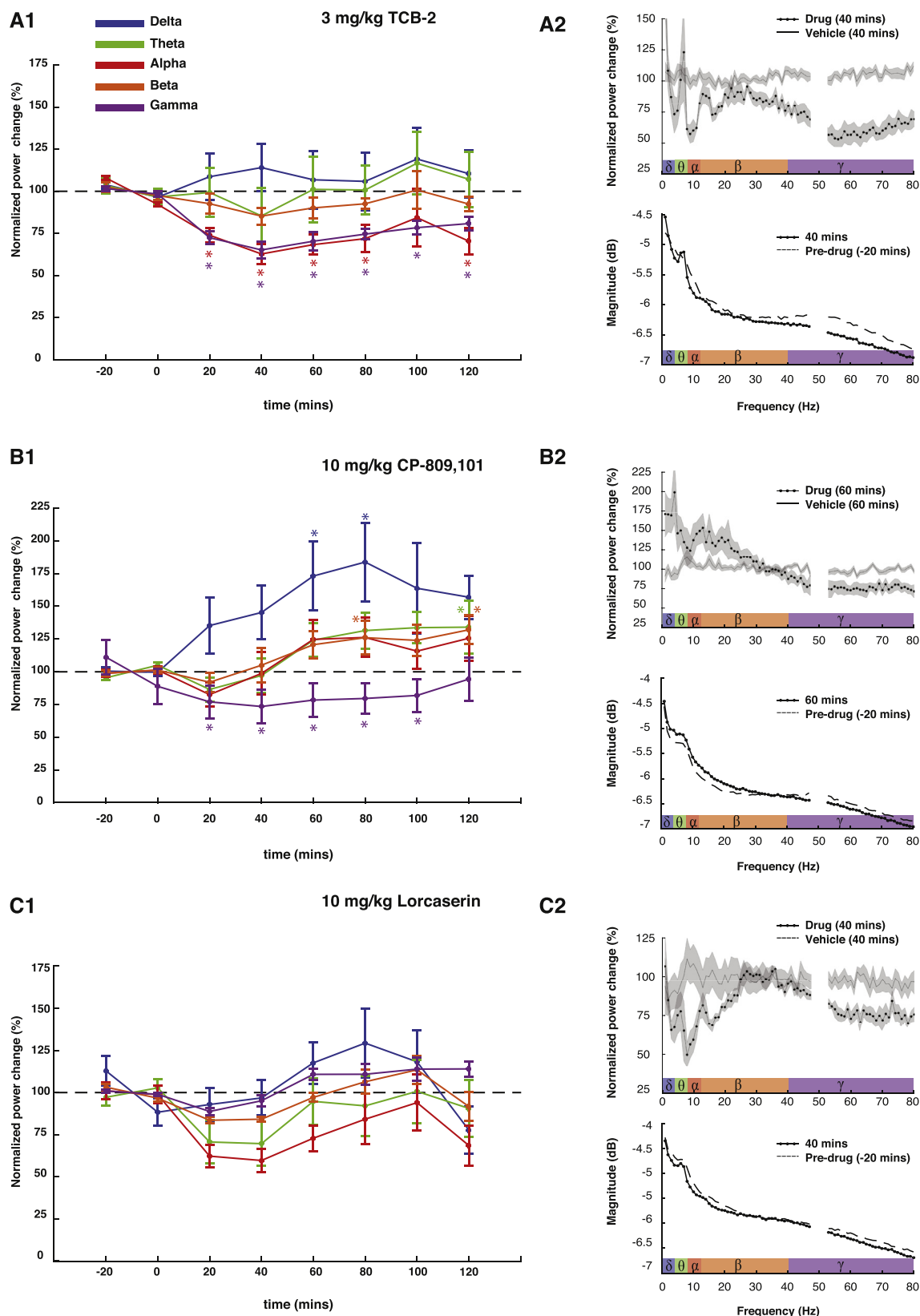


Fig. 4. Effect of TCB-2, CP-809,101 and lorcaserin on interictal EEG. (A1) TCB-2 (3 mg/kg, $n = 6$) significantly reduced EEG power in the alpha (8–12 Hz) and gamma (30–80 Hz) bands compared to vehicle ($n = 7$). (A2) Mean normalized power change at 40 min compared to the relative time point in the vehicle injected animals (top) and raw EEG spectrum at 40 min compared to pre-drug (bottom). (B1) CP-809,101 (10 mg/kg, $n = 6$) significantly increased EEG power in the delta (1–4 Hz) and decreased power in the gamma band (30–80 Hz) compared to vehicle ($n = 8$). (B2) Mean normalized power change at 60 min compared to equivalent time point in the vehicle injected animals (top) and raw EEG spectrum at 60 min compared to pre-drug (bottom). (C1) In the lorcaserin treated animals (10 mg/kg) no individual point reached statistical significance after post-hoc testing, although a trend for an effect in the delta and gamma bands is visible at 40 min (C2). All values are normalized to the control period (–40 to 0 min), and are expressed as a percentage of their respective vehicle group. Values represent mean \pm SEM. Asterisks indicate $p < 0.05$ for a given time bin in the treatment group vs the corresponding time bin in the vehicle group (two-way ANOVA, Sidak's multiple comparison test).

points a significant antiepileptic effect (limited to the total time spent in seizures). This convergence of anti-absence effects by 5-HT₂CR agonists and antagonists, although surprising, has already been reported for their antidepressant effects (Di Giovanni and De Deurwaerdère, 2016; Millan, 2005).

The marked reduction in ASs elicited by intermediate doses of CP-809,101 and lorcaserin did not last for the entire 2 h observation period. This may be due to pharmacokinetics/pharmacodynamics features of the drugs, although the half-life of lorcaserin (>3 h) (Higgins et al., 2013b) makes this possibility unlikely (no data is available on CP-809,101 pharmacokinetics). Alternatively, the well-known, rapid (within minutes) 5-HT₂CR desensitization, which however has been observed only *in vitro* however (Stout et al., 2002), might explain the relatively short duration of the anti-absence effect. Moreover, lorcaserin, but not CP-809,101, induced a drastic increase in seizure length. It is difficult at present to understand whether this contrasting effect may depend on the 5-HT_{2C/2A} selectivity of the two drugs, off-target effects and/or differences in their functional selectivity (Urban et al., 2007; Stroth and Svenningsson, 2012; Canal et al., 2013). Interestingly, the 5-HT₂CR agonist RO60-0175 (3 mg/kg, i.p.) produces a similar anti-absence effect to that induced by CP-809,101, i.e., block of ASs without an increase in seizure length (unpublished observation).

4.3. Pathophysiological significance of 5-HT_{2A}R- and 5-HT_{2C}R-elicited block of ASs

5-HT fibers, originating from both dorsal and medial raphe nuclei provide a diffuse distribution in the cortico-thalamo-cortical circuit (Descarries et al., 2010), the key neuronal network responsible for AS generation (Crunelli and Leresche, 2002), with a preferential innervations of GABAergic cells in both brain regions (Hornung, 2010). In particular, 5-HT_{2A}R levels are relatively high in the GABAergic neurons of the nucleus reticularis thalami (NRT) in rats (Bonnin et al., 2006; Rodriguez et al., 2011), particularly on their fine and medium-size dendrites (Aznar et al., 2003; Li et al., 2004; Rodriguez et al., 2011), but are also present to a lower level in sensory thalamic nuclei (Li et al., 2004), though apparently absent in mice dorsal lateral geniculate nucleus (dLGN) (Coulon et al., 2010). Moreover, 5-HT_{2A}R and 5-HT_{2C}R mRNA is detected in GABAergic interneurons isolated from the dLGN of young rats (Munsch et al., 2003). 5-HT_{2A}Rs and 5-HT_{2C}Rs are present in cortical GABAergic interneurons, and to a lesser extent in pyramidal neurons both in rats (Nocjar et al., 2015; Santana et al., 2004) and in primates (De Almeida and Mengod, 2007).

In view of this complex distribution of 5-HT_{2A}Rs and 5-HT_{2C}Rs in the cortico-thalamo-cortical system, and because of the lack of data on the cellular action of selective 5-HT_{2A}R and 5-HT_{2C}R agonists and antagonists on the neuronal components of this network, it is difficult to precisely relate the present findings on the modulation of ASs induced by systemic injection of 5-HT_{2A}Rs and 5-HT_{2C}Rs to known physiological effects of these ligands on different thalamic and cortical neuronal populations. Nevertheless, one might speculate that the putative 5-HT_{2A}R-dependent i) decrease in firing of pyramidal cells *in vivo* (Ashby et al., 1990), ii) increase in IPSCs *in vitro* in pyramidal cells (Zhou and Hablitz, 1999) and iii) depolarization of fast-spiking interneurons *in vitro* (Weber and Andrade, 2010) may all be contributing to a reduced firing activity in the putative cortical “initiation site” from where SWDs firstly originate (Polack et al., 2007), thus explaining the reduction of ASs observed in the present study following 5-HT_{2A}R activation. However, it should be noted that the putative 5-HT_{2A}R-dependent increase in firing rate (Zhang and Arsenault, 2005) and burst discharges which have been reported in layer 5 pyramidal neurons *in vivo* (Celada et al., 2008; Spindle and Thomas, 2014) could be favoring, and

not reducing, AS expression. At the thalamic level, 5-HT and putative 5-HT_{2C}R agonists depolarize TC neurons (Chapin and Andrade, 2001; Meuth et al., 2006; Pape and McCormick, 1989). However, the α -methyl-5-HT-elicited excitation of TC neurons (Coulon et al., 2010) could be mediated by this drug activating 5-HT₇Rs which are known to control the excitability of this brain region (Chapin and Andrade, 2001), and some TC neurons in higher order thalamic nuclei are hyperpolarized by 5-HT (Monckton and McCormick, 2002; Varela and Sherman, 2009). The depolarizing action on TC neurons, together with the putative 5-HT_{2A/2C}R-mediated inhibition of NRT neuron burst firing (McCormick and Pape, 1990; McCormick and Wang, 1991), might counteract the increased tonic GABA_A inhibition of TC neurons in different absence models (Cope et al., 2009), thus contributing to the reduction in ASs by 5-HT_{2A}R and 5-HT_{2C}R agonists reported in the present study.

The possibility cannot be discarded, however, that the reduction in ASs observed in the present study following systemic injection of 5-HT_{2A}R and 5-HT_{2C}R agonists might result from an indirect action on other brain areas and/or physiological control systems. Firstly, since both 5-HT₂R subtypes are well expressed in the basal ganglia (Li et al., 2004), which indirectly modulate AS generation (Deransart and Depaulis, 2002), the 5-HT_{2A}R- and 5-HT_{2C}R-induced reduction in ASs reported in the present study could be a consequence of changes in firing rate in these brain regions. Secondly, since ASs are less common during active wakefulness and non-REM sleep (Crunelli and Leresche, 2002; Depaulis and van Luijckelaar, 2006; Dewolfe et al., 2013), the 5-HT_{2A}R- and 5-HT_{2C}R-elicited decrease in ASs might be due to changes in wake/sleep states elicited by activation of these 5-HT receptor subtypes. Our study design did not allow us to robustly record circadian sleep which would have required acquiring a stable sleep baseline in GAERS before drug injection and a different habituation protocol. However we note that the available data on vigilance state alterations elicited by 5-HT_{2A}R and 5-HT_{2C}R activation are contradictory. 5-HT_{2A}R and 5-HT_{2C}R KO mice show increased waking and reduced non-REM sleep (Spindle and Thomas, 2014). On the other hand, systemic administration of DOI (Monti and Jantos, 2006) and RO60-0175 (Martin et al., 1998; Monti and Jantos, 2015) increases waking and reduces non-(rapid eye movement) REM and REM sleep. Opposite results have been obtained with selective 5-HT_{2A}R agonist, and 5-HT_{2C}R antagonists or non-selective 5-HT_{2A/2C}R antagonists that increased drowsiness and non-REM sleep and reduced REM sleep (Morairty et al., 2008; Popa et al., 2005). Nevertheless, our analysis of the interictal EEG in the same GAERS rats where ASs were investigated indicate a decrease in gamma waves by TCB-2 and CP-809,101, a decrease in alpha waves by TCB-2 and lorcaserin, and an increase of delta waves by CP-809,101 and lorcaserin. Overall, therefore, these results provide indirect evidence that these drugs decrease vigilance and increase non-REM sleep, effects which might contribute to a reduction in ASs. Indeed, it is interesting to note that 5-HT_{2A}R and 5-HT_{2C}R activation produces similar actions on vigilance states and ASs whereas these two classes of 5-HTRs produce opposite effects on a plethora of other behaviors (Boulougouris and Robbins, 2010; Cunningham et al., 2013; Di Giovanni and De Deurwaerdère, 2016; Di Giovanni et al., 1999; Di Giovanni et al., 2008; Di Matteo et al., 2008; Fletcher et al., 2012; Halberstadt et al., 2009; Winstanley et al., 2004).

Another possibility that could be considered is that the stereotypic behaviors induced by 5-HT_{2A}R and 5-HT_{2C}R activation might contribute to the reduction in absence seizures observed in this study. Although a causal relationship between wet dog shakes, head twitches and penile grooming cannot be fully ruled out, these behaviours decrease and mostly disappear (our observation and (Halberstadt and Geyer, 2013)) 30 min after the 5-HT_{2A/2C}R agonist

administration while their antiabsence effect is of much longer duration (>1.5 h).

Finally, it is worth to note that the highest doses of the 5-HT_{2A}R agonist TCB-2 and the 5-HT_{2C}R agonist CP809101 produced a small increase in the peak SWD frequency, while both doses of the 5-HT_{2A}R antagonist M100907 induced a small, but significant decrease in the peak SWD frequency. Although the mechanism that pace the rhythm of SWDs in not completely understood, it is known that the peak SWD frequency differs in various experimental models. In fact, gamma-hydroxybutyrate (GHB)- and PTZ-induced SWDs are generally in the range of 4–6 Hz in the rat (McLean et al., 2004; Venzi et al., 2015), while SWDs in genetic absence models, as GAERS, WAG/Rij and Long Evans rats, generally appear at frequencies of 7 Hz or higher (Coenen and Van Luijtelaa, 2003; Crunelli and Leresche, 2002; Shaw, 2004). Therefore, it is reasonable to assume that the rodent thalamocortical network has the intrinsic ability of producing SWD oscillations at various frequencies and pharmacological interventions are capable of modulating the pace of this oscillation. Although there are few examples of this phenomenon, it was reported that systemic administration of carbamazepine reduced the frequency of PTZ-elicited SWDs by ~0.5 Hz (McLean et al., 2004). Moreover, application of lidocaine on the perioral region of the somatosensory cortex was shown to shift the peak frequency of SWDs towards slower frequencies, although this effect was less pronounced when recorded in brain regions further away from the injection site (Sitnikova and van Luijtelaa, 2004). Clearly, the current lack of knowledge of the electrophysiological effects of 5-HT_{2A}R and 5-HT_{2C}R agonists and antagonist in cortical and thalamic neurons limits our ability to pinpoint a mechanism on the action of these compounds on SWD frequency. Future studies where local administration of drugs is coupled to single units extracellular recordings *in vivo* at the site of administration (Taylor et al., 2014) may help to elucidate this question.

4.4. Therapeutic potential of 5-HT_{2A}R and 5-HT_{2C}R ligands in ASs

The results reported here suggest that 5-HT_{2A}Rs and 5-HT_{2C}Rs might be potential targets for novel anti-absence drugs. However, the potential hallucinogenic activity of 5-HT_{2A}R agonists must be taken into account, though 5-hydroxytryptophan, 5-HT_{1A}R antagonists, benzodiazepines and cannabinoid (CB) antagonists/inverse agonists elicit head-twitch behavior, but lacks hallucinogenic effects in humans (Fantegrossi et al., 2005). Moreover, the activation of 5-HT_{2A}R heteroreceptor complexes with mGluR2 (Gonzalez-Maeso et al., 2008) and D2Rs (Fuxe et al., 2014), CB1Rs (Vinals et al., 2015) and 5-HT_{2C}Rs (Herrick-Davis et al., 2005) might be important for the effects observed here and for potential targets for drug development. Of note, our results with 5-HT_{2A}R antagonists warn that atypical antipsychotics, which exert their therapeutic action, at least in part, by blocking 5-HT_{2A}R-mediated responses (Meltzer, 1995) and some 5-HT_{2A}R antagonists, which are being developed for the treatment of insomnia or anxiety, could potentially induce an increase in seizure length in patients with ASs. 5-HT_{2C}R-targeting drugs, therefore, appear at present a safer and more promising avenue for novel anti-absence medicines, especially in view of the fact that lorcaserin has already been approved for human use. Moreover, since depression/anxiety-like symptoms are common comorbid psychiatric disorders both in pediatric epileptic patients (Vega et al., 2011) and animal models of absence epilepsy (Jones et al., 2008; Sarkisova and van Luijtelaa, 2012), the 5-HT_{2C}R agonist antidepressant properties (Di Giovanni and De Deurwaerdere, 2016; Millan, 2005) make this receptor even a more attractive target for treatment of absence epilepsy. Nevertheless, the ability of some 5-HT_{2C}R agonists to increase seizure length as shown in this study suggests caution.

Conflict of interest

None.

Author contribution

VC and GDiG designed research; MV, FD, VC and GDiG designed experiments; MV, FD, CB, JB and AC performed experiments and analyzed data; MV, VC and GDiG wrote the paper.

Acknowledgments

This work was supported by the ERUK (grant P1202 to VC and GDiG), the Wellcome Trust (grant 91882 to VC), the Malta Council for Science and Technology (grant R&I-2013-14 to GDiG and VC) and EU COST Action CM1103. We wish also to thank Mr. Timothy Gould for technical assistance.

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