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ORIGINAL ARTICLE

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Role(s) of the 5-HT2C Receptor in the Development of Maximal Dentate Activation in the Hippocampus of Anesthetized Rats

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SUMMARY

Aims: Substantial evidence indicates that 5-HT$_{2C}$ receptors are involved in the control of neuronal network excitability and in seizure pathophysiology. Here, we have addressed the relatively unexplored relationship between temporal lobe epilepsy (TLE), the most frequent type of intractable epilepsy, and 5-HT$_{2C}$Rs. **Methods:** In this study, we investigated this issue using a model of partial complex (limbic) seizures in urethane-anesthetized rat, based on the phenomenon of maximal dentate activation (MDA) using 5-HT$_{2C}$ compounds, electrophysiology, immunohistochemistry, and western blotting techniques. **Results:** The 5-HT$_{2C}$ agonists mCPP (1 mg/kg, i.p) and lorcaserin (3 mg/kg, i.p), but not RO60-0175 (1–3 mg/kg i.p.), were antiepileptogenic reducing the MDA response duration. The selective 5-HT$_{2C}$ antagonist SB242084 (2 mg/kg, i.p) unveiled antiepileptogenic effects of RO60-0175 (3 mg/kg, i.p) but did not alter those induced by mCPP and lorcaserin. Compared with control rats, electrically stimulated rats showed an increase in glutamic acid decarboxylase levels and a heterogeneous decrease in 5-HT$_{2C}$R immunoreactivity in different hippocampal areas. **Conclusions:** In our animal model of TLE, mCPP and lorcaserin were anticonvulsant; likely acting on receptor subtypes other than 5-HT$_{2C}$. Epileptogenesis induced early adaptive changes and reorganization in the 5-HT$_{2C}$R and GABA systems.

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The first three authors contributed equally to this work.

Introduction

The serotonin (5-hydroxytryptamine; 5-HT) 2C receptor (5-HT$_{2C}$R) subtype is one of the most studied members of the serotonin receptor family that holds up to 14 subtypes [1–4]. This is not surprising, considering that it is widely expressed within the central nervous system (CNS) and is thought to play a major role in 5-HT regulation of a plethora of behaviors. Despite the importance of the 5-HT$_{2C}$R, our understanding of its complex signal transduction properties remains incomplete. This is due to its distinctive regulatory properties, such as constitutive activity and RNA-editing *in vivo* and especially the scarcity of subtype-selective drugs [5,6]. Nevertheless, 5-HT$_{2C}$R has been shown by experimental and clinical observation to represent a possible therapeutic target for the development of drugs for a range of CNS disorders such as schizophrenia, depression, drug

abuse, eating disorders, and Parkinson's disease to name but a few [1,5–7]. As activation of 5-HT_{2C}Rs suppresses neural network hyperexcitability in different brain areas [7–9], it might play a similar role in the hippocampus. This hypothesis is corroborated by the high 5-HT_{2C}R mRNA and protein hippocampal expression [10,11] with immunoreactivity for the 5-HT_{2C}R, wildly located in the polymorphic cell layer of the dentate gyrus (DG), in the pyramidal cell layer of hippocampus proper (CA1, CA2, and CA3 fields), in the mossy fibers of CA3 and in the subiculum [12,13]. Moreover, 5-HT_{2C} knock out (KO) mice show a selective impairment of DG plasticity *in vitro*, spatial learning impairment and emergence neophobia [14]. Consistently, 5-HT_{2C}R activation decreases theta oscillations [15] implying that 5-HT_{2C}R antagonists might have therapeutic significance in psychiatric or neurological disorders associated with impaired cognitive functions and epilepsy. 5-HT_{2C}R KO mice are extremely susceptible to audiogenic seizures [16], and prone to spontaneous death from seizures [17]. Furthermore, an up-regulation of 5-HT_{2C}Rs with an increase in hippocampal gene expression and inositol triphosphate content and associated depressive mood behavioral changes have recently been shown in pilocarpine-induced temporal lobe epilepsy (TLE) in rats [18].

Despite these compelling data, research on the role of 5-HT_{2C}Rs in TLE, the most frequent type of intractable epilepsy, has been relatively scarce and lead to conflicting results [8,19].

In this study, we used a model of partial complex (limbic) seizures based on the phenomenon of maximal dentate activation (MDA) recorded in the DG, induced by repetitive electrical stimulation of the perforant path (PP) in anesthetized rats [20,21]. To answer the question of a possible involvement of 5-HT_{2C}R in TLE, we evaluated the anticonvulsant properties of some old and newer 5-HT_{2C}R agonists, that is, mCPP, RO60-0175, and lorcaserin and the selective 5-HT_{2C}R antagonist SB 242084 using the MDA animal model. Moreover, standard immunohistochemistry, and western blotting were used with the aim to elucidate whether any adaptive changes in the 5-HT_{2C} and GABA systems occurred in early-stage hippocampal epileptogenesis by evaluating the expression of 5-HT_{2C}R and GAD67 in the hippocampus of rats that underwent the MDA protocol compared with the control group.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles Rivers, IT) weighing between 250 and 300 g were used. Procedures involving animals and their care were in accordance with Council Directive 86/609/EEC and with the Animals Scientific Procedures Act 1986, and local regulations regarding in animals in research. Surgery and field potentials recordings were performed as previously described [20,22]. Briefly, the population spike (PS) was evoked by stimulating the medial perforant path (PP) (AP:-8.3 L:4.8 V:3.4; [23]) and recorded with a bipolar stimulating electrode (bifilar stainless steel wire, CFW, CA, USA) in the hilus of the DG of the hippocampus (AP: -4.8 L: 2.2 V: 3.6) (Figure 1A). Electrical activity was recorded by a NeuroLog amplifier (Digitimer Ltd, high pass: 0.2 Hz, low pass: 5000 Hz, gain: 200). A digitally controlled constant current stimulator (Digitimer Ltd, model DS3) was used to apply square-wave pulses of 0.3 ms duration, 1 per min. PS amplitude was calculated as shown in Figure 1B. Stimulus intensity was set to evoke 45–50% of the maximum amplitude of the PS. Responses were digitized by a CED 1401 plus analog-digital converter (Cambridge Electronic Design Ltd., Cambridge, UK), stored on a computer and averaged offline using Signal 1.9 software. Sampling rate was set to 10 kHz. Location of the electrodes was verified histologically (Figure 1A).

Maximal Dentate Activation

The induction of the MDA was started when normal DG excitability was revealed, 30 min or more following surgery. This was assessed by paired pulse stimulation with two different interpulse intervals (i.e., 25 and 150 msec), capable of inducing fast inhibition and excitation, respectively [20,22]. MDA was characterized electrophysiologically according to published criteria [20,24]. Stimulus trains of 10 second (pulses of 0.3 ms duration, at 20 Hz) were delivered through the PP electrode at an initial intensity of 100 μA. If MDA was not elicited, the stimulus intensity was increased in 50 μA steps and redelivered every 2.5 min until MDA was induced. Threshold was reached at 350 $\pm$ 100 μA, and stimulus intensity was further increased by 100 μA. For each stimulus, the duration of MDA, time to onset and after discharge (AD) were measured as shown in Figure 1C.

Repeated trains inducing seizure were delivered every 10 min for 4 h (total of 24 stimulus trains). As shown in Figure 1D, the latency to MDA onset was measured from stimulus onset to the point of PS appearance with half of the maximal amplitude [20].

After the AD began to lengthen, either drug or vehicle was administered after six stimulus train. In the vehicle group, the duration of MDA increases and the time to onset gradually decreases [20,21]. To make comparisons across animals, the measured durations of MDA and time to onset were "normalized" by subtracting their duration in response to the first stimulus from the duration in response to each subsequent stimulus train. Thus, for individual stimulus trains after the first, a change in duration (or time to onset) was calculated. In this way, data from separate animals were averaged and comparisons across groups of animals were made [20,21].

Immunohistochemistry, Western Blot analysis, and Statistical analysis

See supporting information.

Drug Administration Protocols

The drugs were all administered i.p., and the effect on the MDA parameters was recorded in a dose volume of 1 ml/kg. Ro60-0175 (1, 3, and 10 mg/kg), mCPP (1 mg/kg), and lorcaserin (3 mg/kg) were dissolved in saline, SB242084 in 20%

DMSO and saline. Lorcaserin was a gift from Arena pharmaceutical; the other compounds were purchased from Sigma-Aldrich, Gillingham, UK.

Results

Effect of RO60-0175, mCPP, Lorcaserin, and SB242084 on the Maximal Dentate Activation Parameters

The effects of the different 5-HT_{2c}/ ligands were compared with those of their vehicles (saline and 20% DMSO) on MDA duration and the time to onset of MDA (Figure 1C and 2A). The results from the vehicle-treated animals were determined on nine animals (n = 5 for saline and 4 for 20% DMSO) and averaged together because they were not statistically significant (not shown) and indicated from now on as control group. Time of onset gradually decreased over the first eight stimuli (−1.9 ± 4) seconds and then stabilized for the remainder of the experiment (Figures 1C and 2C). Conversely, MDA increased steadily, reaching a peak at the 24th stimulus (+24.6 ± 2.4 seconds), although a value fluctuation was observed over the last stimuli. The lengthening of the MDA duration was significantly and differently affected by the 5-HT_{2C} compounds used in this study (Figures 2–4). Similarly, a pattern of effects was seen for the AD (data not shown).

Effect of RO60-0175 on MDA Parameters and Role of 5-HT_{2C} Receptors

The effect of RO60-0175 (1, 3 and 10 mg/kg, i.p) on the magnitude of MDA elongation is reported in Figure 2A. Despite a trend toward a decrease after 1 or 3 mg/kg, RO60-0175 did not significantly alter the duration of MDA (one-way ANOVA, $F_{3,22}$ = 0.711; P = 0.556) (Figure 2A).

Figure 2B reports the effect of the 5-HT_{2C} antagonist SB242084 (2 mg/kg i.p.) on MDA responses obtained in the presence or the absence of RO60-0175 (3 mg/kg i.p.).

Statistical analysis revealed a significant reduction of the MDA elongation by cotreatment with SB242084 (2 mg/kg, i.p.) and RO60-0175 (3 mg/kg) (one-way ANOVA, $F_{3,23}$ = 3.418; P = 0.0342). However, SB242084 was without effect by itself (SB vs. control, Fisher PLSD test, P = 0.5585; n = 5) (Figure 2B). The inhibitory effect produced by this combination was observed as early as frame 10 (Figure 2B; n = 7).

Despite a trend toward inhibition, RO60-0175 (1, 3, 10 mg/kg n = 6) did not alter the onset of the MDA (one-way ANOVA, $F_{3,22}$ = 0.054, P = 0.6552) (Figure 2C). Pre-treatment with the

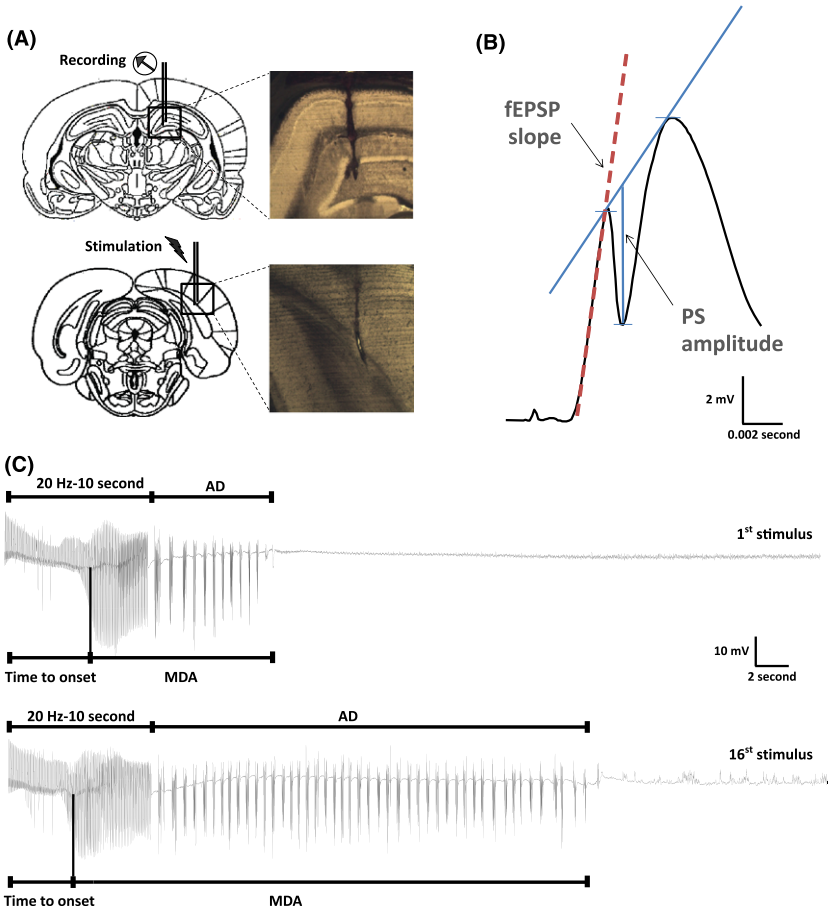

Figure 1 (**A**) Stereotaxic coronal plates in which the recording (above) and the stimulating (below) electrodes were placed. The two photomicrographs of 100 μm-thick coronal sections on the right panels represent the anatomical location of the electrodes in the dentate gyrus (DG) and angular bundle (perforant pathway, PP), respectively. (**B**) Representative trace of evoked field potential recorded in DG in response to stimulation of PP fibers. The field excitatory postsynaptic potential (fEPSP) slope is represented by the maximum slope of the line that best fitted to seven steepest points of the rising phase of the local field potential (dashed line). The population spike (PS) amplitude is measured as the distance of the vertical line from the tangent line drawn connecting the first peak with the second peak of the positive deflection and the valley of the evoked field potential (arrow). (**C**) Measurement of the parameter of Maximal dentate activation (MDA) in control animals. The Time to onset is defined by the time that occurs from the beginning of the stimulus train to the midpoint of the maximum amplitude of the PSs. The duration of the MDA is measured from the time to onset and the end of the epileptic afterdischarges (AD). AD is represented by spontaneous bursts of PSs that appear at the end of the stimulus train.

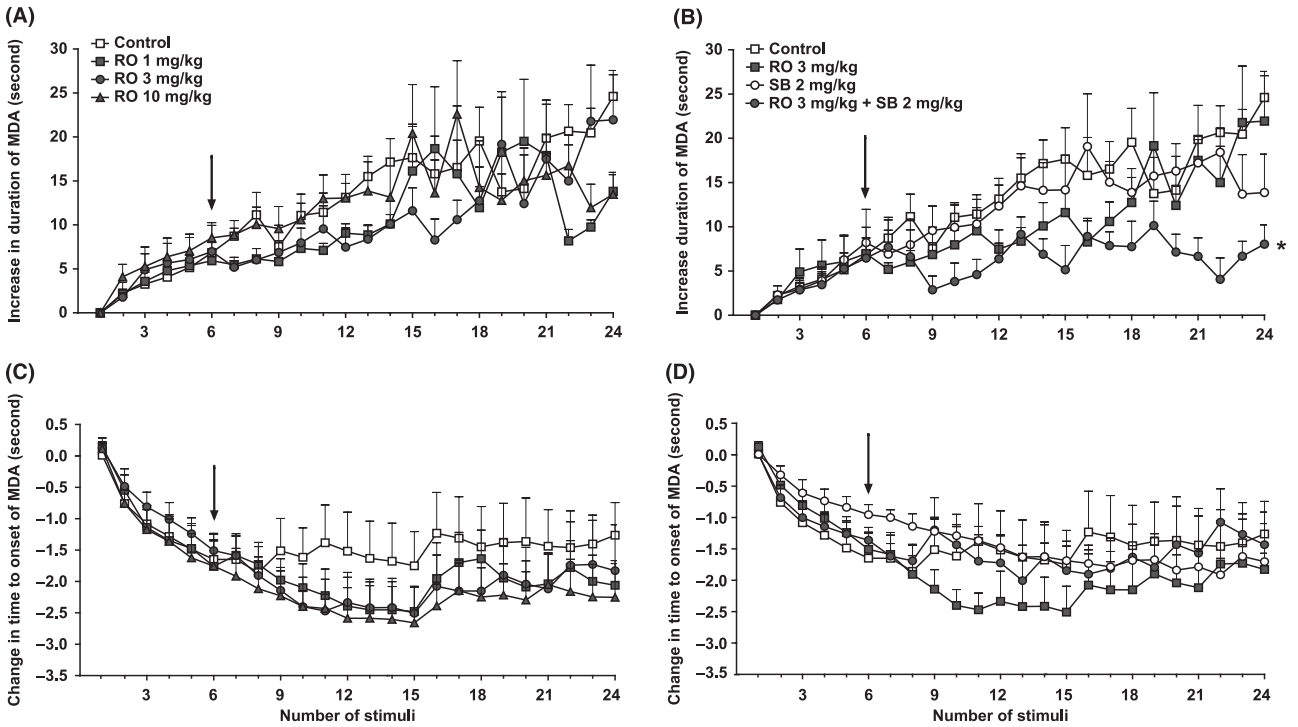

Figure 2 Effect of RO60-0175 (RO) on the parameters of the maximal dentate activation (MDA). The duration and time to onset of MDA were measured for each stimulus train. These values were then normalized, averaged and plotted (±SEM) against stimulus number. Drugs were administered i.p. at the arrows. The open square line indicates the mean values from the vehicle control animals (n = 9). The effect of RO at 1 mg/kg (n = 5, filled squares), 3 mg/kg (n = 7, filled circles) and 10 mg/kg (n = 6, filled triangles) on the increase in duration of MDA **(A)** and on the change in the time to onset of MDA **(C)**. The effect of SB242084 2 mg/kg alone (n = 5, empty circles) or combined with RO 3 mg/kg (n = 7, filled circles), on the increase in duration of MDA **(B)** and on the change in the time to onset of MDA **(D)**. One-way ANOVA for repeated measures followed by Fisher's PLSD post hoc test; $*P < 0.05$ versus vehicle group.

selective 5-HT_{2C} antagonist SB242084 (2 mg/kg, i.p, n = 7), without effect by itself (SB vs. control, Fisher's PLSD test, $P = 0.9635$), did not reveal any interaction with 3 mg/kg RO60-0175 on the time to onset of MDA (Figure 2D). Conversely, RO60-0175 (1, 3, 10 mg/kg, n = 6 for each dose), SB242084 (2 mg/kg, n = 7) and cotreatment with 3 mg/kg RO60-0175 and SB242084 did not effected the onset of the MDA (Figure 2C,D).

Effect of mCPP on MDA Parameters and Role of 5-HT_{2C} Receptors

The average effect of mCPP (1 mg/kg i.p.; n = 5) treatment on MDA elongation is shown on Figure 3A. ANOVA analysis revealed a dramatic decrease in duration of the MDA induced by mCPP (one-way ANOVA $F_{3,20} = 5.316$, $P = 0.0074$), nearly blocking it 40 min (stimulus 10) after its administration (mCPP vs. control, 2.7 ± 0.8 vs. 11.1 ± 1.4, Fisher's PLSD test, $P = 0.0017$), an effect which lasted for the entire duration of the experiment with variable entity. SB pre-treatment tended to potentiate mCPP (1 mg/kg) effects (mCPP + SB vs. mCPP, Fisher's PLSD test, $P = 0.5441$) (Figure 3B). The effects of mCPP (1 mg/kg i.p.; n = 5) or its cotreatment with SB242084 (n = 5) did not significantly affect the onset of the MDA (one-way ANOVA, $F_{3,20} = 0.052$, $P = 0.9837$).

Effect of Lorcaserin on MDA Parameters and Role of 5-HT_{2C} Receptors

As shown in Figure 4A, administration of lorcaserin (3 mg/kg, i.p; n = 5) produced a significant sustained decrease of the MDA elongation (one-way ANOVA, $F_{3,20} = 4.328$, $P = 0.0166$). Post hoc analysis revealed that the peak effect was attained at stimulus 10 (LOR vs. control, 7.5 ± 4.0 vs. 11.1 ± 1.4; Fisher's PLSD test, $P = 0.0389$) and sustained for 90 min after. SB pre-treatment tended to potentiate lorcaserin (3 mg/kg) effects (LOR + SB vs. LOR, Fisher's PLSD test, $P = 0.5752$) (Figure 4A). The effects of lorcaserin (3 mg/kg i.p.; n = 5) or its cotreatment with SB242084 (LOR + SB, n = 5) did not significantly affect the onset of the MDA (one-way ANOVA, $F_{3,20} = 0.202$, $P = 0.8936$) (Figure 4B).

Distribution of 5-HT_{2C} Receptor Immunoreactivity in the Dentate Gyrus, Hippocampus Proper, and Entorhinal Cortex

Control Rats

General Staining Features

The 5-HT_{2C} immunoreaction product was usually limited to a dark cell body but was not present in the proximal dendrites. The neu-

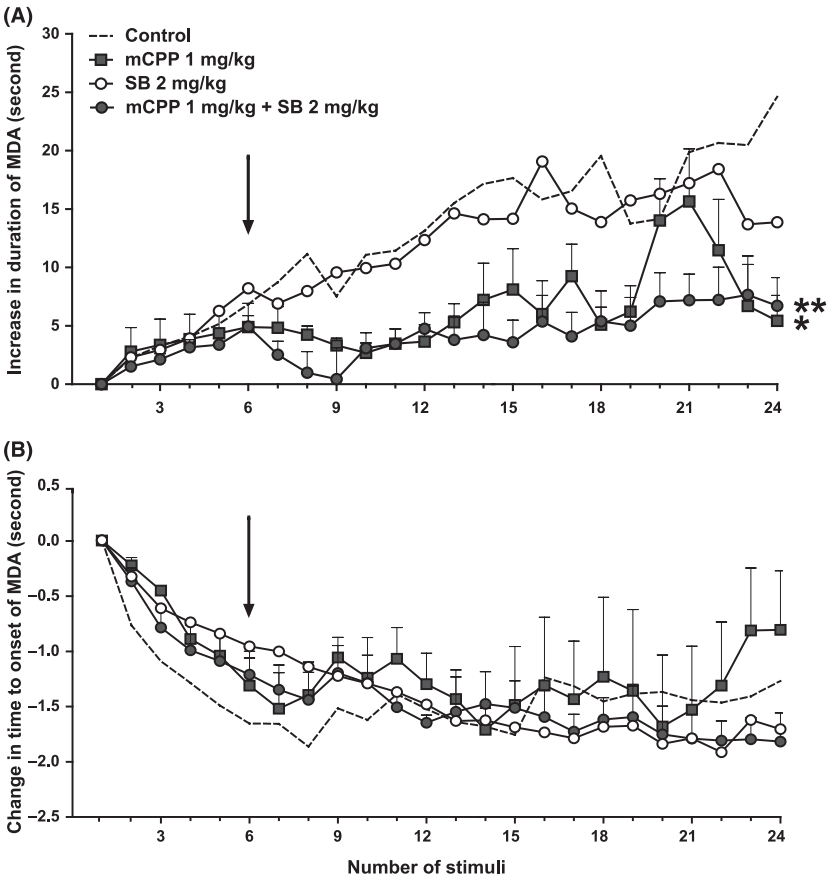

Figure 3 Effect of mCPP and SB242084 (SB) on the parameters of the maximal dentate activation (MDA). The duration and time to onset of MDA were measured for each stimulus train. These values were then normalized, averaged and plotted (±SEM) against stimulus number. Drugs were administered i.p. at the arrows. The dashed line indicates the mean values from the vehicle control animals (n = 9). The effect of mCPP at 1 mg/kg (n = 5, filled squares), SB 2 mg/kg (n = 5, opened circles) and the combined administration of mCPP and SB (n = 5, filled circles) on the change in duration of MDA **(A)** and on the change in the time to onset of MDA (**B**). One-way ANOVA for repeated measures followed by Fisher's PLSD post hoc test; *$P < 0.05$ versus vehicle group; **$P < 0.01$ versus vehicle group.

ropil staining consisted only of diffuse staining without any visible dendrite. The diffuse neuropilar labeling could not be associated with any specific neuronal elements.

GAD67 immunohistochemistry distributions are described in the supporting information.

Dentate Gyrus

In the DG, most of the 5-HT_{2C}R-immunoreactive (IR) somata were located in the granule cell and polymorphic cell layers (Figure 5A1,B1; Table 1). Virtually, all granule cells were positive for the 5-HT_{2C}R (Figure 5A1,B1). A low density of 5-HT_{2C}R-IR neurons was located also in the molecular layer (Table 2). A strong diffuse neuropilar staining could be observed especially in the polymorphic cell layers (Figure 5A1,B1; Table 2).

Hippocampus Proper

5-HT_{2C}R immunoreactivity was quite similar in the different fields of the hippocampus proper (Figure 6A1,B1; Tables 1 and 2). 5-HT_{2C}R-IR cell bodies were primarily located in the pyramidal cell layer and, presumably, belong to pyramidal neurons (Figure 6A1, B1; Table 1). A low density of immunopositive cells could be observed also in strata oriens, radiatum and lacunosum moleculare (Figure 6A1,B1; Table 1). The diffuse neuropilar staining was evident throughout the layers of hippocampus proper (Figure 6A1,B1; Table 2). However, the stratum lucidum of the CA3 field (where the mossy fibers are present) showed a low level of diffuse neuropilar immunoreactivity.

Entorhinal Cortex

A high density of 5-HT_{2C}R-IR somata could be observed in the entorhinal cortex (Figure 6C1; Table 1). The great majority of 5-HT_{2C}R-IR cell bodies, which probably belonged to pyramidal or modified pyramidal cells, were located in layers II, III, V, and VI (Figure 6C1). The diffuse neuropilar staining was high in every layer (Figure 6C1; Table 2).

MDA-Stimulated Rats

The 5-HT_{2C}R immunostaining was more prominent in control than in MDA-stimulated rats (Figure 5A1–A2, B1–B2; Figure 6A1–A2, B1–B2, C1–C2; Tables 1 and 2). Moreover, the MDA-stimulated left (MDAL) hemisphere, ipsilateral to the PP stimulation, presented more effects in all the areas examined, compared with MDA-stimulated right (MDLR) hemisphere and nonstimulated control brains (Tables 1 and 2). In fact, a diffuse reduction of the number of immunoreactive somata could be observed in MDA-stimulated rats (Figure 5A1–A2, B1–B2; Figure 6A1–A2, B1–B2, C1–C2; Table 1). This aspect was statistically significant in the granular cell layer, polymorphic cell layer, pyra-

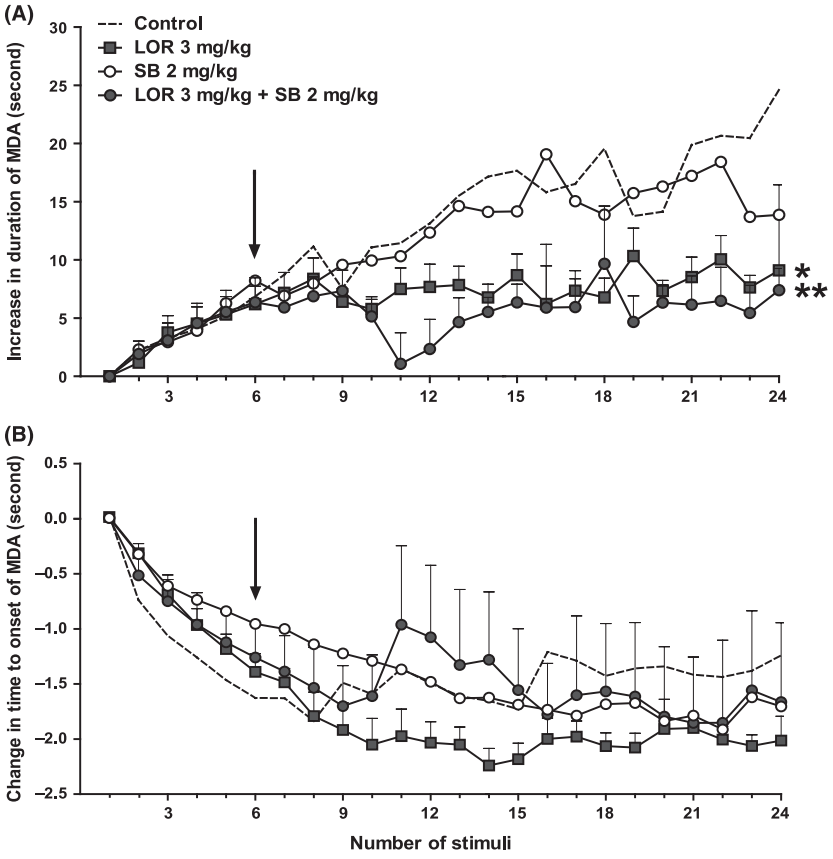

Figure 4 Effect of lorcaserin (LOR) and SB242084 (SB) on the parameters of the maximal dentate activation (MDA). The duration and time to onset of MDA were measured for each stimulus train. These values were then normalized, averaged and plotted (±SEM) against stimulus number. Drugs were administered i.p. at the arrows. The dashed line indicates the mean values from the vehicle control animals (n = 9). The effect of LOR at 3 mg/kg (n = 5, filled squares), SB 2 mg/kg (n = 5, opened circles) and the combined administration of LOR and SB (n = 5, filled circles) on the change in duration of MDA (**A**) and on the change in the time to onset of MDA (**B**). One-way ANOVA for repeated measures followed by Fisher's PLSD post hoc test; $*P < 0.05$ versus vehicle group; $**P < 0.01$ versus vehicle group.

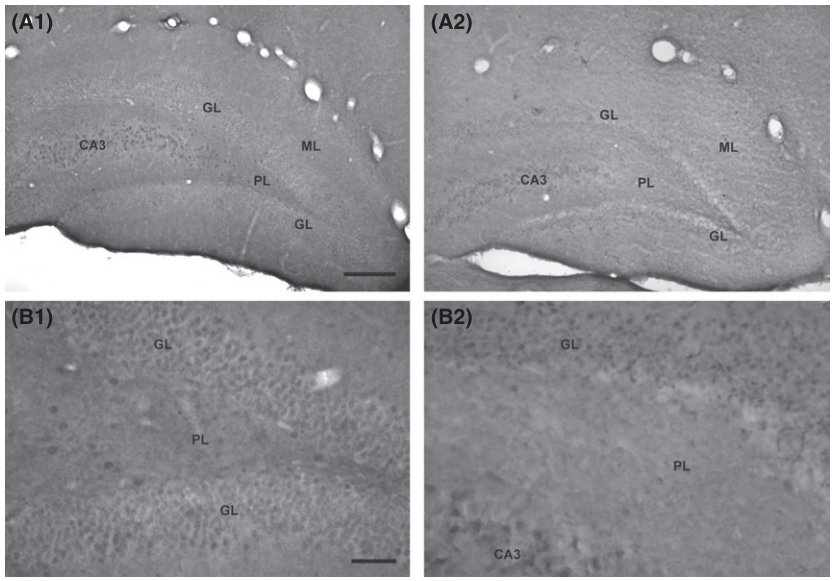

Figure 5 Distribution of 5-HT_{2C} receptor (5-HT_{2C}R) immunoreactivity in the dentate gyrus (DG) of control (**A1, B1**) and MDA-stimulated rats (**A2, B2**). **(A1–A2)** Granule cells are more numerous in control than in MDA-stimulated rats. Also the intensity of diffuse neuropilar immunostaining is higher in the control than in MDA-stimulated rats. **(B1–B2)** Polymorphic cell layer. The number of immunostained neurons, as well as the intensity of diffuse neuropilar staining, are more evident in the control than MDA-stimulated rats. Scale bar = 200 μm in **A1** (applies to **A1**, **A2**); 50 μm in **B1** (applies to **B1**, **B2**). GL, granule cell layer; ML, molecular layer; PL, polymorphic cell layer.

midal cell layer, and entorhinal cortex (Table 1). In addition, the intensity of neuronal immunostaining was less evident in MDA-stimulated rats than in control rats. In every region analyzed, the intensity of diffuse neuropilar immunostaining was highest in control rats than in MDA-stimulated ones (Figure 5A1–A2, B1–B2; Figure 6A1–A2, B1–B2, C1–C2; Table 2).

Effects of MDA on 5-HT_{2C} and GAD67 Expression Levels in the Hippocampus Measured by Western Blotting

The expression levels of 5-HT_{2C}R and GAD67 were investigated in protein lysates of hippocampus derived from control and

Table 1 The density of 5-HT_{2C} receptor-immunoreactive neurons in control and right and left hippocampal formation/entorhinal cortex of MDA-stimulated rats

Area	Layer	Control (n = 3)	MDAL (n = 3)	MDAR (n = 3)
Dentate Gyrus	Polymorphic cell layer*	355 ± 36.5	105.9 ± 18.2*	103.1 ± 18.3*
	Granule cell layer*	8537 ± 151	1853 ± 121*	1332 ± 98.9*,#
	Molecular layer	72.5 ± 13.6	48.3 ± 14.1	44.1 ± 14.8
Hippocampus proper				
CA1	Stratum oriens	127 ± 18.9	46.4 ± 6.3	44.2 ± 5.9
	Pyramidal cell layer	5661 ± 123	3812 ± 115.9*	3581 ± 114*
	Stratum radiatum	15.1 ± 5.1	13.1 ± 4.5	10.0 ± 4.1
	Stratum lacunosum-moleculare	70.1 ± 15.9	57.1 ± 16.4	55.1 ± 16.1
CA3	Stratum oriens	89.4 ± 15.8	61.7 ± 14.3	57.3 ± 12.8
	Pyramidal cell layer	2366 ± 98.6	1447 ± 89.1*	1404 ± 87.5*
	Stratum radiatum	33.2 ± 9.6	46.4 ± 13.3	44.1 ± 12.1
	Stratum lacunosum-moleculare	65.7 ± 13.9	62.5 ± 13.4	58.1 ± 11.1
Entorhinal cortex		561 ± 41.7	297 ± 27.3*	289 ± 23.1*

The density of 5-HT_{2C} receptor-immunoreactive neurons is expressed as the mean/mm^2; n = number of rats in each group. *$P < 0.05$ MDAL and MDAR versus control. #$P < 0.05$ MDAL versus MDAR. MDAR: right hippocampus of MDA-stimulated rats. MDAL: left hippocampus of MDA-stimulated rats.

Table 2 Density of 5-HT_{2C}R dendrites-immunoreactive and intensity of the diffuse neuropilar staining in control and MDA rats

		Control		MDA	
Area	Layer	Dendrites	Diffuse staining	Dendrites	Diffuse staining
Dentate Gyrus	Polymorphic cell layer	–	+++	–	+(L)/++(R)
	Granule cell layer	–	–	–	–
	Molecular layer	–	++	–	+
Hippocampus proper					
CA1	Stratum oriens	–	++/+++	–	+
	Pyramidal cell layer	–	–	–	–
	Stratum radiatum	–	++/+++	–	+
	Stratum lacunosum-moleculare	–	++/+++	–	+
CA3	Stratum oriens	–	++/+++	–	+
	Pyramidal cell layer	–	–	–	–
	Stratum radiatum	–	++/+++	–	+
	Stratum lacunosum-moleculare	–	++/+++	–	+
Entorhinal cortex		–	++/+++	–	+

The density of dendrites and the intensity of the diffuse neuropilar staining is expressed as + + + high, + + medium, + low, – absent. L: left; R: right.

MDA-stimulated rats. 5-HT_{2C} and GAD67 expression levels were normalized for beta actin expression levels. Western blotting analysis for 5-HT_{2C} did not show any significant differences within the groups (Figure 7A,B; $P > 0.05$), while the levels of GAD67 were significantly increased ($P < 0.05$, Figures 7C and 3D) in the MDAR hippocampus compared to MDAL and control rat hippocampi. GAD67 immunohistochemistry analysis are described in the supporting information.

Discussion

In the present study, we report an important impact of the rat MDA model of hippocampal seizures on the distribution of 5-HT_{2C}R in the hippocampal regions without changes of its total expression. Surprisingly, although mCPP and lorcaserin behaved as antiepileptogenic agents, their effects were not blocked by selective 5-HT_{2C} SB242084 antagonist. Therefore, it seems that 5-HT_{2C}Rs do not control the electrophysiological features of the TLE model used here. Our data suggest that 5-HT_{2C}R could be linked to TLE via an anatomo-functional reorganization of GABAergic transmission in the hippocampus during epileptogenis rather than influence directly paroxysmal discharges of the DG granular cells.

Our results are in line with data stressing the important role of 5-HT transmission in regulating hippocampal excitability and seizure activity [19] but dampen the idea that 5-HT_{2C}R play a role in this activity. Thus, to our knowledge, the present electrophysiological study is the first *in vivo* investigation of the effects of acute treatment of a number of agonists with different chemical structures, different affinity and selectivity over the different 5-HT_2 subtypes [2] namely mCPP [25,26], lorcaserin [27] and RO60-0175 [28] and the 5-HT_{2C} antagonist SB242084 [29] on the DG

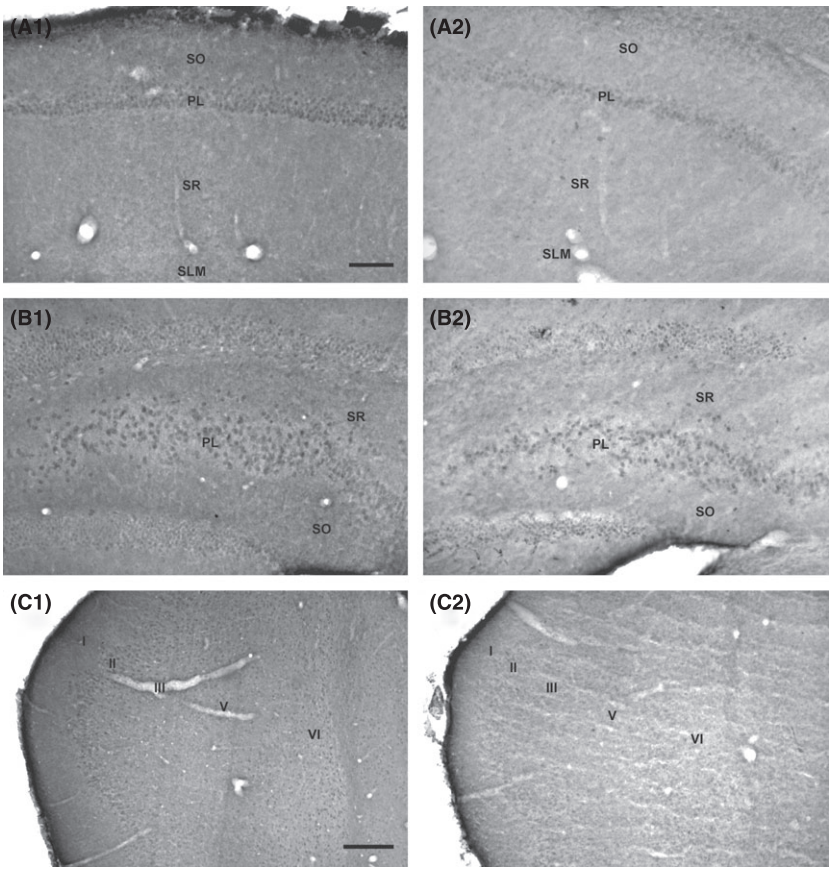

Figure 6 Distribution of 5-HT_{2C} receptor (5-HT_{2C}R) immunoreactivity in the hippocampus proper and entorhinal cortex of control (**A1, B1, C1**) and MDA-stimulated rats (**A2, B2, C2**). (**A1**–**A2**) CA1 and (**B1**–**B2**) CA3 fields. In the pyramidal cell layer, the density of immunoreactive pyramidal neurons is lower in MDA-stimulated rats than in control rats. Also the density of the 5-HT_{2C}R-IR diffuse neuropilar staining is low in MDA-stimulated rats than in control rats. (**C1**–**C2**) Entorhinal cortex. Note that in the MDA-stimulated rats the number of immunopositive somata, as well as the diffuse neuropilar immunostaining, are lower than in control rats. Scale bar = 100 μm in **A1** (applies to **A1**–**B2**); 200 μm in **C1** (applies to **C1**, **C2**). Abbreviations: PL, pyramidal cell layer; SLM, stratum lacunosum-moleculare; SO, stratum oriens; SR, stratum radiatum.

granular cell hyperexcitability. It is interesting to note that RO60-0175 was devoid of any significant antiepileptic effects over a wide range of doses (1–10 mg/kg). This regimen has been previously shown to be efficient on various electrophysiological, biochemical, and behavioral experiments [30–35] strongly suggesting that 5-HT_{2C}R stimulation is not involved in the control of MDA elongation. The presence of the 5-HT_{2C} antagonist SB242084 unmasked a decrease in MDA response highlighting the antiepileptic properties of RO60-0175. Interestingly, mCPP and lorcaserin significantly reduced the MDA elongation over the 4-h period of recording. Their effects, not blocked by the pretreatment with SB242084, tended instead to be potentiated by the antagonist.

The mere involvement of 5-HT_{2C}R in the electrophysiological feature of MDA has been also confirmed on the time to onset of MDA. The latency to onset of MDA can be used as a gauge of seizure threshold (anticonvulsant) and the duration of MDA as a measure of processes that terminate seizure activity in the limbic system and its decrease has been considered to be antiepileptogenic [36]. The anticonvulsant and the antiepileptogenic processes likely involve different mechanisms [37] and in accordance none of the agonists significantly affected the onset of the MDA, while mCPP and lorcaserin strongly decreased the MDA-associated after discharges elongation.

Overall, these data suggest that MDA does not respond to phasic stimulation of 5-HT_{2C}R. Moreover, our data show also that MDA response is under a poor tonic influence exerted by 5-HT_{2C}R. Indeed, the selective 5-HT_{2C}R antagonist SB242084 (2 mg/kg, i.p.) did not induce any proconvulsant effects seen such as elongation of the MDA and AD in rats or reduction of the MDA latency. Thus, in contrast to the situation reported in 5-HT_{2C} KO mice [17], the lack of a 5-HT_{2C}R tonic control on epilepsy is in agreement with data from generalized epilepsy models [19]. It has been previously reported that SB242084 or the other selective antagonist SB243213 were unable to reduce seizure threshold in adult rodents [8]. Although a constitutive activity of 5-HT_{2C}R could have hardly been unmasked with SB242084 [38], previous data have reported that the prototypical inverse agonist SB206553 did not alter on its own seizure threshold [39]. Thus, it appears that endogenous 5-HT_{2C}R tone exerts a poor influence on the general activity.

The nonselective and distinct effects triggered by the agonists used in this study deserve comments. It is possible that the different effects of the agonists are related to their different affinity to 5-HT_{2A}R [2]. Another possible candidate for the antiepileptic effects is the 5-HT_{1A}R, for which among all the 5-HTRs the most compelling evidence exists for a causative role in TLE [40]. Consistently, we recently showed, using the same experimental approach, that 8-OH-DPAT decreased the MDA elongation in a similar way to mCPP and lorcaserin [20]. Moreover, mCPP [26], lorcaserin [41] and RO60-0175 [28] bind 5-HT_{1A}Rs. The loss of selectivity of mCPP and RO60-0175 has already been reported in the literature at these regimens. Indeed, 1 mg/kg mCPP has been shown to enhance c-fos expression in the striatum or alter locomotor activity in part via mechanisms involving 5-HTR other than

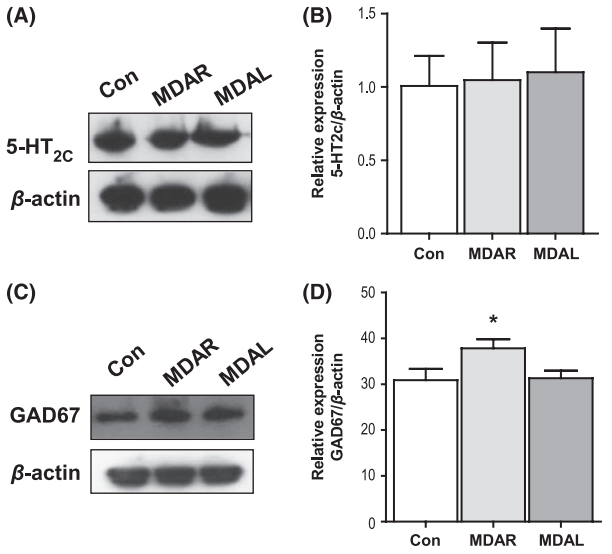

Figure 7 5-HT_{2C} and GAD67 expression in the hippocampus of control and MDA-stimulated rats. (**A**) Representative western blot bands for 5-$HT_{2C}R_s$ in control (Con), right hippocampus of MDA-stimulated rats (MDAR) and left hippocampus of MDA-stimulated rats (MDAL). β-actin was used as an internal control. (**B**) Relative expression of 5-HT_{2C}. Optical density of 5-HT_{2C} protein expression is shown as the expression level of 5-HT_{2C} divided by the expression level of β-actin, as the mean $\pm$ SD. (**C**) Representative western blot bands for GAD67 in Con, MDAR and MDAL. β-actin was used as internal control. (**D**) Relative expression of GAD67. Optical density of GAD67 protein expression is shown as the expression level of GAD67 divided by the expression level of β-actin, as the mean $\pm$ SD. One-way ANOVA for repeated measures followed by Bonferroni post hoc test; $^*P < 0.05$ versus vehicle group.

5-HT_{2C}R [42–44]. Similarly, 5 mg/kg RO60-0175 stimulates adrenocorticotrophic hormone, oxytocin, prolactin secretion, and c-Fos expression in basal ganglia by mechanisms independent of the activation 5-HT_{2C}Rs or 5-HT_{2A}Rs [45]. Also, the effect of 3 mg/kg RO60-0175 on c-Fos expression in basal ganglia is not totally reversed by the 5-HT_{2C} antagonist SB243213 [44].

While we are unable to indicate a clear pro- or antiepileptogenic influence of 5-HT_{2C}R, it has been reported that agomelatine, a 5-HT_{2C}R antagonist and potent MT1 and MT2 melatonin receptor agonist, showed anticonvulsant activity in limbic pilocarpine-induced seizure models [46]. Moreover, 5-HT_{2C} agonists and antagonists potentiated [47] or inhibited cocaine-induced convulsions [48], respectively. Thus, the lack of involvement of 5-HT_{2C}R in our model does not exclude their participation in other TLE models or in humans. Moreover, our findings must be interpreted with caution because they have been obtained under urethane anesthesia, which is known to alter neuronal, including DG granular cell [49], excitability. Nevertheless, for consistency with available data, we selected urethane as the most suitable anesthetic for our experiments as all the studies investigating the effect of drugs on MDA have been carried out under this type of anesthesia [20,21,24,50–52].

Although our electrical stimulation protocol is only 4 hours-long, we have detected 5-HT_{2C}Rs and GAD67 expression changes (see also supporting information). Indeed, an increase in GAD67 and a decrease in 5-HT_{2C}Rs immunoreactivity were observed in the hippocampus of rats that underwent the electrical stimulation paradigm when compared to nonstimulated rats.

In control subjects, 5-HT_{2C}R-IR was located in the cell bodies of the granules of the DG, and pyramidal neurons of the CA3 and CA1 fields of the hippocampus proper and also in the soma of GABA interneurons in line with previous evidence [10,11,53]. Regarding GAD67, there was no difference in the distribution of the immunoreactivity among the control and stimulated rat, but in PP-stimulated rats, GAD67-IR around the CA3 pyramidal neurons and in the polymorphic layer appears much more intense (see supporting information). WB analysis showed an increase of GAD67 protein expression in the MDAR hippocampus.

This is an important demonstration that adaptive changes, namely up-regulation of the GABA system, can occur at an early stage of epileptogenesis. In the animals which underwent the MDA stimulation protocol, 5-HT_{2C}R-IR was present at much lower levels, although the WB did not reveal any change in total protein in the whole hippocampus. These changes may be compensatory, with the aim of preventing excessive firing of the hippocampal principal cells and development of spontaneous recurrent seizures. In contrast, a previous study reported hippocampal 5-HT_{2C}R up-regulation 2 weeks after the administration of pilocarpine in rats [18]. These differences can be explained by the different TLE models used, although it might be possible that the chronic 5-HT_{2C}R hyperfunction is preceded by an early 5-HT_{2C}R down-regulation. Further work is required to establish this possibility.

Our results suggest subtle changes and possible reorganization of the hippocampal neural network. These findings are particularly interesting, especially in consideration of the fact adaptive changes are typically visible only after a latent period (1–4 weeks) from the epileptic insult [37,54]. Our data confirm that the generation of seizures in the hippocampal-parahippocampal circuits produces structural alterations and/or adaptations [52,55,56] and show for the first time that these can be seen earlier (4 h) at the level of neurotransmission.

It is very difficult to predict the exact brain area in which these 5-HT_{2C} compounds elicit their antiepileptic effect. The systemic drug application route used here does not allow us to rule out the involvement of serotonergic mechanisms in extra-hippocampal areas known to be under a 5-$HT_{2A/2C}$ control, such as mesencephalic dopaminergic areas [57]. In addition, 5-HT_2Rs are both expressed in principal and GABAergic interneurons in the hippocampus ([53] and present observations) as well as in other nuclei, increasing the level of complexity of a general activation. For instance, 5-HT can act on receptors of the 5-HT_2 subtype family to depolarize and excite GABAergic interneurons of the CA1 region [58]. Detailed studies employing local drug application and antagonists for different 5-HT subtypes are required to characterize the precise targets of the putative 5-HT_{2C} ligands in mediating their antiepileptic influences on hippocampal hyperexcitability.

In conclusion, the MDA model of TLE used here allowed us to propose that TLE may profoundly alter the expression of 5-HT_{2C}R and increase GABA neurotransmission. The reorganization of 5-HT_{2C}R after MDA might be an important factor modifying the

impact of other 5-HTR in the control of tissue excitability as previously suggested [18,42]. Indeed, we show for the first time a strong anticonvulsant effects and/or antiepileptogenic activity induced by lorcaserin and mCPP that are not mediated by 5-HT_{2C}Rs at least in this model of TLE. Further studies are warranted to further explore lorcaserin antiepileptic activity in various animal models of TLE, especially in consideration of its availability on the market for the treatment of obesity. Revealing the exact mechanism of 5-HT_2R ligands may help to discover new potential drug targets for this form of epilepsy.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Di Giovanni G, Esposito E, Di Matteo V, eds. *5-HT2C Receptors in the Pathophysiology of CNS Disease*. The Receptors. New York: Springer/Humana Press, 2011; 1–557.
2. Higgins GA, Sellers EM, Fletcher PJ. From obesity to substance abuse: therapeutic opportunities for 5-HT2C receptor agonists. *Trends Pharmacol Sci* 2013;**34**:560–570.
3. De Deurwaerdere P, Lagiere M, Bosc M, Navailles S. Multiple controls exerted by 5-HT2C receptors upon basal ganglia function: from physiology to pathophysiology. *Exp Brain Res* 2013;**230**:477–511.
4. Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* 2002;**71**:533–554.
5. Di Giovanni G, Di Matteo V, Pierucci M, Benigno A, Esposito E. Central serotonin2C receptor: from physiology to pathology. *Curr Top Med Chem* 2006;**6**:1909–1925.
6. Navailles S, Lagiere M, Guthrie M, De Deurwaerdere P. Serotonin2c receptor constitutive activity: in vivo direct and indirect evidence and functional significance. *Cent Nerv Syst Agents Med Chem* 2013;**13**:98–107.
7. Crunelli V, Di Giovanni G. Monoamine modulation of tonic GABAA inhibition. *Rev Neurosci* 2014;**00**:1–12.
8. Jakus R, Bagdy G. The Role of 5-HT2C Receptor in Epilepsy. In: Di Giovanni G, Esposito E, Di Matteo V, editors. *5-HT2C Receptors in the Pathophysiology of CNS Disease*. New York: Humana Press, 2011; 429–444.
9. Isaac M. Serotonergic 5-HT2C receptors as a potential therapeutic target for the design antiepileptic drugs. *Curr Top Med Chem* 2005;**5**:59–67.
10. Pompeiano M, Palacios JM, Mengod G. Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. *Brain Res Mol Brain Res* 1994;**23**:163–178.
11. Abramowski D, Rigo M, Duc D, Hoyer D, Staufenbiel M. Localization of the 5-hydroxytryptamine2C receptor protein in human and rat brain using specific antisera. *Neuropharmacology* 1995;**34**:1635–1645.
12. Li QH, Nakadate K, Tanaka-Nakadate S, Nakatsuka D, Cui YL, Watanabe Y. 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* 2004;**469**:128–140.
13. Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KCF. Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. *Neuropharmacology* 2000;**39**:123–132.
14. Tecott LH, Logue SF, Wehner JM, Kauer JA. Perturbed dentate gyrus function in serotonin 5-HT2C receptor mutant mice. *Proc Natl Acad Sci USA* 1998;**95**:15026–15031.
15. Sorman E, Wang D, Hajos M, Kocsis B. Control of hippocampal theta rhythm by serotonin: role of 5-HT2c receptors. *Neuropharmacology* 2011;**61**:489–494.
16. Brennan TJ, Seeley WW, Kilgard M, Schreiner CE, Tecott LH. Sound-induced seizures in serotonin 5-HT2c receptor mutant mice. *Nat Genet* 1997;**16**:387–390.
17. Tecott LH, Sun LM, Akana SF, et al. Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. *Nature* 1995;**374**:542–546.
18. Krishnakumar A, Nandhu MS, Paulose CS. Upregulation of 5-HT2C receptors in hippocampus of pilocarpine-induced epileptic rats: antagonism by Bacopa monnieri. *Epilepsy Behav* 2009;**16**:225–230.
19. Bagdy G, Kecskemeti V, Riba P, Jakus R. Serotonin and epilepsy. *J Neurochem* 2007;**100**:857–873.
20. Orban G, Pierucci M, Benigno A, et al. High dose of 8-OH-DPAT decreases maximal dentate gyrus activation and facilitates granular cell plasticity in vivo. *Exp Brain Res* 2013;**230**:441–451.
21. Stringer JL, Lothman EW. Maximal dentate activation: a tool to screen compounds for activity against limbic seizures. *Epilepsy Res* 1990;**5**:169–176.
22. Di Giovanni G, García I, Colangeli R, et al. N-(furan-2-ylmethyl)-N-methylprop-2-yn-1-amine (F2MPA): a Potential Cognitive Enhancer with MAO Inhibitor Properties. *CNS Neurosci Ther* 2014; In Press.
23. Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. New York: Academic Press, 2007.
24. Stringer JL, Williamson JM, Lothman EW. Induction of paroxysmal discharges in the dentate gyrus: frequency dependence and relationship to afterdischarge production. *J Neurophysiol* 1989;**62**:126–135.
25. Hoyer D. Functional correlates of serotonin 5-HT1 recognition sites. *J Recept Res* 1988;**8**:59–81.
26. Hamik A, Peroutka SJ. 1-(m-chlorophenyl)piperazine (mCPP) interactions with neurotransmitter receptors in the human brain. *Biol Psychiatry* 1989;**25**: 569–575.
27. Chan EW, He Y, Chui CS, Wong AY, Lau WC, Wong IC. Efficacy and safety of lorcaserin in obese adults: a meta-analysis of 1-year randomized controlled trials (RCTs) and narrative review on short-term RCTs. *Obes Rev* 2013;**14**:383–392.
28. Martin JR, Bos M, Jenck F, et al. 5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential. *J Pharmacol Exp Ther* 1998;**286**:913–924.
29. Di Matteo V, Di Giovanni G, Esposito E. SB 242084: a Selective 5-HT2C Receptor Antagonist. *CNS Drug Rev* 2000;**6**:195–205.
30. Beyeler A, Kadiri N, Navailles S, et al. Stimulation of serotonin2C receptors elicits abnormal oral movements by acting on pathways other than the sensorimotor one in the rat basal ganglia. *Neuroscience* 2010;**169**:158–170.
31. Di Matteo V, Pierucci M, Esposito E. Selective stimulation of serotonin2C receptors blocks the enhancement of striatal and accumbal dopamine release induced by nicotine administration. *J Neurochem* 2004;**89**:418–429.
32. Invernizzi RW, Pierucci M, Calcagno E, et al. Selective activation of 5-HT(2C) receptors stimulates GABA-ergic function in the rat substantia nigra pars reticulata: a combined in vivo electrophysiological and neurochemical study. *Neuroscience* 2007;**144**:1523–1535.
33. Navailles S, Lagiere M, Le Moine C, De Deurwaerdere P. Role of 5-HT2C receptors in the enhancement of c-Fos expression induced by a 5-HT2B/2C inverse agonist and 5-HT 2 agonists in the rat basal ganglia. *Exp Brain Res* 2013;**230**:525–535.
34. Di Giovanni G, Di Matteo V, Pierucci M, Esposito E. Serotonin-dopamine interaction: electrophysiological evidence. *Prog Brain Res*, 2008; **172**:45–71.
35. Di Matteo V, Di Giovanni G, Pierucci M, Esposito E. Serotonin control of central dopaminergic function: focus on in vivo microdialysis studies. *Prog Brain Res* 2008;**172**:7–44.
36. White HS. Animal models of epileptogenesis. *Neurology* 2002;**59**(9 Suppl 5):S7–S14.
37. Loscher W. Strategies for antiepileptogenesis: Antiepileptic drugs versus novel approaches evaluated in post-status epilepticus models of temporal lobe epilepsy, 2012.
38. De Deurwaerdere P, Navailles S, Berg KA, Clarke WP, Spampinato U. Constitutive activity of the serotonin2C receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *J Neurosci* 2004;**24**:3235–3241.
39. Upton N, Stean T, Middlemiss D, Blackburn T, Kennett G. Studies on the role of 5-HT2C and 5-HT2B receptors in regulating generalised seizure threshold in rodents. *Eur J Pharmacol* 1998;**359**:33–40.
40. Martinez A, Finegersh A, Cannon DM, et al. The 5-HT1A receptor and 5-HT transporter in temporal lobe epilepsy. *Neurology* 2013;**80**:1465–1471.
41. Thomsen WJ, Grottick AJ, Menzaghi F, et al. Lorcaserin, a novel selective human 5-hydroxytryptamine2C agonist: in vitro and in vivo pharmacological characterization. *J Pharmacol Exp Ther* 2008;**325**:577–587.
42. Dalton GL, Lee MD, Kennett GA, Dourish CT, Clifton PG. mCPP-induced hyperactivity in 5-HT2C receptor mutant mice is mediated by activation of multiple 5-HT receptor subtypes. *Neuropharmacology* 2004;**46**:663–671.
43. Higgins GA, Fletcher PJ. Serotonin and drug reward: focus on 5-HT2C receptors. *Eur J Pharmacol* 2003;**480**:151–162.
44. Navailles S, Lagiere M, Roumegous A, et al. Serotonin2C ligands exhibiting full negative and positive intrinsic activity elicit purposeless oral movements in rats: distinct effects of agonists and inverse agonists in a rat model of

Parkinson's disease. *Int J Neuropsychopharmacol* 2013;**16**:593–606.
45. Damjanoska KJ, Muma NA, Zhang Y, et al. Neuroendocrine evidence that (S)-2-(chloro-5-fluoro-indol- l-yl)-1-methylethylamine fumarate (Ro 60-0175) is not a selective 5-hydroxytryptamine (2C) receptor agonist. *J Pharmacol Exp Ther* 2003; **304**:1209–1216.
46. Aguiar CC, Almeida AB, Araujo PV, et al. Anticonvulsant effects of agomelatine in mice. *Epilepsy Behav* 2012;**24**:324–328.
47. O'Dell LE, Kreifeldt MJ, George FR, Ritz MC. Serotonin (2C) receptors appear to mediate genetic sensitivity to cocaine-induced convulsions. *Psychopharmacology* 1999;**146**:313–319.
48. O'Dell LE, Kreifeldt MJ, George FR, Ritz MC. The role of serotonin(2) receptors in mediating cocaine-induced convulsions. *Pharmacol Biochem Behav* 2000;**65**:677–681.
49. Shirasaka Y, Wasterlain CG. The effect of urethane anesthesia on evoked potentials in dentate gyrus. *Eur J Pharmacol* 1995;**282**:11–17.
50. Stringer JL, Lothman EW. Use of maximal dentate activation to study the effect of drugs on kindling and kindled responses. *Epilepsy Res* 1990;**6**:180–186.
51. Bough KJ, Schwartzkroin PA, Rho JM. Calorie restriction and ketogenic diet diminish neuronal excitability in rat dentate gyrus in vivo. *Epilepsia* 2003;**44**:752–760.
52. Stringer JL, Agarwal KS, Dure LS. Is cell death necessary for hippocampal mossy fiber sprouting? *Epilepsy Res* 1997;**27**:67–76.
53. Mengod G. Serotonin 5-HT2C Receptors: Chemical Neuronatomy in the Mammalian Brain. In: Di Giovanni G, Esposito E, Di Matteo V, editors. *5-HT2C Receptors in the Pathophysiology of CNS Disease*. New York: Humana Press, 2011; 17–27.
54. Sloviter RS, Bumanglag AV. Defining "epileptogenesis" and identifying "antiepileptogenic targets" in animal models of acquired temporal lobe epilepsy is not as simple as it might seem. *Neuropharmacology* 2013;**69**:3–15.
55. Sloviter RS, Damiano BP. Sustained electrical stimulation of the perforant path duplicates kainate-induced electrophysiological effects and hippocampal damage in rats. *Neurosci Lett* 1981;**24**:279–284.
56. Stringer JL. Repeated seizures increase GFAP and vimentin in the hippocampus. *Brain Res* 1996;**717**:147–153.
57. Di Giovanni G, De Deurwaerdere P, Di Mascio M, Di Matteo V, Esposito E, Spampinato U. Selective blockade of serotonin-2C/2B receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. *Neuroscience* 1999;**91**:587–597.
58. Shen RY, Andrade R. 5-Hydroxytryptamine2 receptor facilitates GABAergic neurotransmission in rat hippocampus. *J Pharmacol Exp Ther* 1998;**285**: 805–812.

Supporting Information

The following supplementary material is available for this article:

Data S1. Material and Methods and GAD67 immunohistochemistry distributions.