Abstract. Compelling animal and human evidence suggests that serotonin plays an important role in the pathophysiology of epilepsy as it is involved in hyperexcitability, epileptogenesis, seizure generation, depression and psychiatric disorders comorbid with epilepsy. Serotonin involvement in epilepsy is complex; the reasons are twofold i) epilepsy is in reality a spectrum disorder, and ii) serotonin effects vary from one form of epilepsy to another, due also to the different serotonin receptors involved. Here, we will focus on the role of serotonin and its 5-HT$_2$ receptors in absence epilepsy. Our recent pharmacological experimental evidence in GAERS will be reviewed together with our preliminary optogenetic results. 5-HT$_{2C}$ receptor agonists may represent a new approach to interfere with seizure generation and seizure management. Our optogenetic experiments also indicate that by modulating rhythmic cortical activity, optogenetic stimulation of the serotonergic system may provide seizure control without the adverse effects induced by pharmacological activation of 5-HT$_{2C}$ receptors. Thus, targeting the serotonergic system could provide novel insights into the pathophysiological mechanisms of seizure generation and lead to potentially novel treatments.

Keywords: Serotonin receptors, epilepsy, epileptogenesis, antiepileptic drugs, optogenetics, closed-loop control

1 Introduction

1.1 Serotonin systems

The dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) of the midbrain raphe nuclei, send serotonin (5-HT) widespread innervation to all brain areas (Azmitia & Segal, 1978; van der Kooy & Hattori, 1980; Steinbusch, Nieuwenhuys, Verhofstad & der Kooy, 1981; Steinbusch, 1984; Van Bockstaele, Biswas & Pickel, 1993). There is some specificity regarding the two raphe nuclei. The DRN, which is composed of approximately 50% of 5-HT neurons, is mainly responsible for the 5-HT innervation of the mammalian medial prefrontal cortex (mPFC) and neostriatum (Bobillier et al., 1976; Azmitia & Segal, 1978; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992). The MRN contains fewer 5-HT cell bodies, which represent approximately 5% of the neurons in the nucleus. Although the MRN innervates several brain regions, the projections from this nucleus to the basal ganglia are presumed not to release 5-HT (Soubrie, Reisine & Glowinski, 1984; Jacobs & Azmitia, 1992).
DRN are also heterogeneous due to the expression or lack of some differentiation factors such as Lmx1b (Ding et al., 2003) or pet-1 (Kiyasova et al., 2011; Gaspar & Lillesaar, 2012; Smidt & van Hooft, 2013). Six parts of the DRN have been described, based on their anatomy and functional topography (Hale & Lowry, 2011). Dorsal parts of the DRN send projections to the central and basolateral nuclei of the amygdala, the dorsal hypothalamic area and the mPFC (Lowry et al., 2008). The ventral part of the DRN innervates the sensorimotor cortex and the caudate putamen. The lateral part projects mainly to subcortical regions, including the lateral hypothalamus and the superior colliculus. The interfacellular part projects to the HIP and the medial septum. The rostral parts send projections to the caudate putamen and the substantia nigra (SN), whilst the caudal part sends projections to the amygdala, ventral HIP and thalamic nuclei. The DRN also receives several afferents from the lateral habenula, the preoptic area, the lateral dorsal and posterior hypothalamic nuclei, basal telencephalon, bed nucleus of the terminalis stria amygdala, cingulate cortex and PFC. In central and caudal levels, the DRN receives projections from the SN, the reticulate formation, the periaqueductal grey matter, and the parabrachial nucleus (Lowry et al., 2008).

The distribution of 5-HT terminals in the brain has been studied through autoradiography, using the binding of the two 5-HT uptake sites (serotonin transporter, SERT) ligands [3H]-imipramine and [3H]-citalopram (D’Amato, Largent, Snowman & Snyder, 1987; Hrdina, Foy, Hepner & Summers, 1990; Dewar, Reader, Grondin & Descaries, 1991), immunohistochemistry using antibodies directed against SERT (Hrdina et al., 1990) and 5-HT (Steinbusch et al., 1981; Steinbusch, 1984), or by measuring tryptophan hydroxylase (TPH) activity (Saavedra, 1977).

The organization of 5-HT contacts on other neural elements lacks specificity. Axon terminals of 5-HT neurons are in contact with a variety of structures, including axon terminals, dendritic spines and shafts, but rarely neuronal somata. This organization of 5-HT varicosities and synapses in the brain lends support to the hypothesis that diffusion processes or volume transmission are the main features of 5-HT transmission (Descaries, Seguela & Wakins, 1991; Umbriaco, Garcia, Beaulieu & Descaries, 1995; Descaries & Mechawar, 2000).

### 1.2 Serotonin and Epilepsy

Compelling animal and human studies have shown 5-HT involvement in many psychiatric and neurological diseases, including epilepsy. It is well known that 5-HT controls, directly or indirectly, neuron excitability by modulating various ion channels, controlling the release of other neurotransmitters and the activation of intracellular pathways via the activation of its plethora (fourteen) receptor subtypes (Barnes & Sharp, 1999; D’Adamo et al., 2013). Therefore, 5-HT is logically involved in the cascade of events that can change a normal neuronal network into a hyperexcitable one (Bagdy, Kecskemeti, Riba & Jakus, 2007; Jakus & Bagdy, 2011; Ghanbari, El Mansari & Blier, 2012). 5-HT is likely to play a role in the initiation, propagation and maintenance of seizure activity, apart from the epileptogenesis. Here, we will focus on the evidence of a 5-HT2R control of epilepsy. 5-HT2A, along with 5-HT2B and 5-HT2C, belong to the 5-HT2 subfamily that consists of three Gq/G11-coupled receptor. 5-HT2A/2C-Rs in general mediate excitatory effects of 5-HT on CNS neurons (Di Giovanni, Di Matteo, Pierucci, Benigno & Esposito, 2006; Millan, Marin, Bockaert & la Cour, 2008).

Classically, epilepsy syndromes are classified into two distinct types, focal and generalized, according to the brain circuitry that sustain the oscillations that lead to seizures, site of seizure onset, electroencephalographic and behavioural characteristics (Berg et al., 2010). Generalized and focal epilepsy also differ in the nature of the pathological and neurochemical imbalance between glutamate and GABA function. Indeed, drugs that increase extracellular GABA levels and/or GABA transmission are first choice in focal/generalized convulsive epilepsy, whereas they exacerbate generalized nonconvulsive seizures. As matter of fact, a structural GABA analogue, gabapentin, which increases GABA synthesis, exacerbates generalized nonconvulsive absence seizures (ASs) and is not indicated in non-convulsive seizures (Manning, Richards & Bowery, 2003). Consistently, we have shown that an increase of tonic GABA inhibition is a conditio sine qua non for the generation of absence seizure in rat and mouse models of the this form of epilepsy (Cope et al., 2009; Errington, Gibson, Cruenlli & Cope, 2011, 2014).

### 1.3 Absence Seizures

A typical AS consists of a sudden and relatively short period of a lack of consciousness, which is invariably accompanied by a stereotypical EEG activity of synchronous and generalized spike and wave discharges (SWDs). ASs are present in various idiopathic generalized epilepsies (IGEs), while they are the only phenotype in childhood absence epilepsy (CAE). In CAE, the average age in which ASs start is 3 to 8 years and they are neither induced or generated by either visual or other sensory stimuli. The majority (~60%) of children suffering from CAE show spontaneous remission often around adolescence, although, in approximately a third of cases, absences continue later in life. This benign outcome of CAE concords with a lack of metabolic and neuropathological signs in this epilepsy. Nevertheless, in up to 90% of CAE sufferers for whom ASs persist during adulthood, there is the occurrence of generalized tonic clonic seizures (GTCSs) (Cruenlli & Leresche, 2002).
The annual incidence rate of CAE is 2–8 per 100,000 children under 15 years of age, and its prevalence is 2–10% among children with any type of epilepsy. CAE is genetically determined, with a 16–45% positive family history. Although penetrance is incomplete, a concordance of 70–85% and 33% has been reported in monozygotic twins and first-degree relatives, respectively. Thus, CAE is commonly described as a familial disease with a complex genotype, and evidence exists that it may represent a channelopathy. Indeed, the emerging picture from the vast majority of genetic studies of AS cohorts preferentially points to abnormalities in genes encoding either calcium channels and/or GABA receptors, though it needs to be stressed that in many of these studies, ASs were not the only epileptic phenotype (Crunelli & Leresche, 2002).

As far as the pathophysiological mechanisms of ASs are concerned, invasive experimental work (Williams, 1953) and more recent non-invasive imaging studies in humans (Holmes, Brown & Tucker, 2004; Hamandi et al., 2006; Bai et al., 2010) have indicated that these seizures are generated by paroxysmal electrical activity of cortical and thalamic networks. In particular, studies in mouse and rat genetic absence models have shown that SWDs initiate in somatosensory cortex, from where they rapidly spread to other cortical areas and to the thalamus. The presence of a cortical “initiation site” for SWDs of ASs has now been conclusively demonstrated in CAE and other patients with ASs, challenging the classical view of a SWD as a fully generalized EEG paroxysm, at least at its onset.

The main activity of layer V/VI cortical neurons during SWDs are rhythmic depolarizations that occur in phase with the EEG spike, and whose waveform is drastically different from the classical paroxysmal depolarizing shifts of convulsive epilepsies. Possible candidates for cortical abnormalities underlying the expression of this firing pattern may include an increased NMDA-mediated excitation in deep layers, a decreased GABAergic inhibition in layer II/III and/or abnormalities in HCN channels. In NRT neurons in vivo, the enhanced and more synchronous cortical volley of SWDs, together with the convergence of the corticothalamic input, results in bursts of EPSPs, that at times generate a T-type Ca$$^{2+}$$ channel dependent high frequency burst of action potentials in correspondence to each spike of the SWD. Alterations in GABA-A $\gamma 2$ subunits, T-type Ca$$^{2+}$$ channels, gap-junction coupling and/or excitatory and inhibitory synaptic strengths have been suggested to occur in the NRT of genetic absence models. The strong and prolonged inhibitory output of the NRT, coupled to the deficient GABA transporter-1, lead TC neurons to the presence of rhythmic sequences of 4–6 GABA$\text{A}$ IPSPs and a marked increase in tonic GABA$\text{A}$ inhibition. Thus, the firing rate of these thalamic neurons during ASs decreases and only occasional action potentials are observed in synchrony with the spike component of SWDs. Notwithstanding, there is always a synchronized output from thalamus to cortex during ASs (Crunelli & Leresche, 2002).

1.4 Role of 5-HT in Generalized Epilepsy: Pharmacological Evidence

Bonnycastle, Giarman and Pasanen (1957) proposed the implication of 5-HT in epilepsy for the first time in the late fifties. Successively, a large body of evidence has confirmed a direct relationship between 5-HT impairment and epilepsy, both in generalized and focal epilepsy (see Svob Strac et al., 2016). For instance, an increase in 5-HT CNS concentration, by using 5-HT transporter (SERT) blockers or increasing its metabolism by introducing more tryptophan to the diet, has been associated with an antiepileptic activity, while a decrease in 5-HT brain concentration leads to a diminished threshold for various types of convulsive seizures (see Bagdy et al., 2007). Moreover, an increase of the firing rate of DRN neurons contextually to ASs (Lörincz, Olah, Baraeskay, Szilagyi & Juhasz, 2007), a decrease in 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) level in the thalamus and a significant negative correlation between the severity of epilepsy (as time spent in ASs) and the thalamic levels of 5-HT (Midzyanovskaya et al., 2006) have been observed in WAG/Rij (Wistar Albino Glaxo/Rijswijk) rat model of absence epilepsy. Many anti-epileptic drugs (AEDs) also act by elevating brain extracellular 5-HT, and many SSRIs show an antiepileptic effect (Bagdy et al., 2007). Moreover, a common serotonin dysfunction may underlie both epilepsy and comorbid depression, seen in epileptic patients (Kanner et al., 2012; Guiardi & Di Giovanni, 2015; Svob Strac et al., 2016). There is a clear association between the ASs and behavioural states, with clinical seizures occurring preferentially in states of ‘decreased or fluctuating vigilance’, and very few during deep stages of non-REM sleep. This behavioral state-dependence implies that, as it is the case for other oscillations generated by the thalamocortical loop, the expression of SWDs is controlled by various neuromodulatory systems (Steriade & McCarley, 2005; Saper, Fuller, Pedersen, Lu & Scammell, 2010). These include, among others, monoaminergic systems in the brainstem (Lörincz & Adamantidis, 2017), cholinergic systems in the basal forebrain and brainstem (Steriade & McCarley, 2005), and serotoninergic system originating in the raphe nuclei. The activity of neurons in these neuromodulatory neurons is highly brain state-dependent (Lörincz & Adamantidis, 2017) and their selective stimulation or inhibition leads to brain state changes (Carter et al., 2010; Xu et al., 2015; Eban-Rothschild, Rothschild, Giardino, Jones &
de Lecea, 2016). Given the behavioural-state dependence of ASs and the prominent effects of neuromodulators on brain states, external changes in the neuromodulator systems could in principle affect the expression of ASs. This novel therapeutic approach would be beneficial as monotherapy with gold-standard anti-absence drugs is only effective in 50% of CAE patients (Glauzer et al., 2010). Indeed, it has been shown that drugs that affect the tone of the 5-HT system can modulate, and in some cases abolish ASs when injected systemically.

Nevertheless, none of the common AEDs target the 5-HT system, at least as main mechanism. Selective 5-HT ligands may indeed induce dangerous off-target effects. Therefore, the challenge for pharmaceutical research is to develop new 5-HT compounds with better side effects profile, potentially efficacious for both epilepsy and its comorbid depression (see our recent review Svb Strac et al., 2016).

Of the plethora of 5-HTRs, the 5-HT$_2C$R seems to be the most promising since it has been shown to be involved in generalized convulsive epilepsy, seizure generation and network excitability (Isaac, 2005; Jakus & Bagdy, 2011). Moreover, 5-HT$_2C$ knock down (KO) mice show spontaneous, occasionally lethal, tonic-clonic seizures (Tecott et al., 1995). Furthermore, in 5-HT$_2C$ KO mice the threshold for electrical (kindling, electroshock), audiogenic and chemical-induced (i.e., by pentylenetetrazol; PTZ) seizures was decreased (Applegate & Tecott, 1998; Heisler, Chm & Tecott, 1998). Consistently, treatments with 5-HT$_2CR$ agonists increased the threshold for PTZ and electroshock-induced seizures in mice (Upton, Stean, Middlemiss, Blackburn & Kennett, 1998). On the other hand, 5-HT$_2C$Rs do not seem to affect focal epilepsy. For instance, metachlorophenylpiperazine, lorcaserin, but not RO60-0175, 5-HT$_2C$R agonists with different pharmacological profiles (Fletcher & Higgins, 2011; Higgins et al., 2013), were able to stop the hippocampal maximal dentate gyrus activation (MDA) in a rat lateral lobe epilepsy (TLE) model (Orban et al., 2014). mCPP and lorcaserin anti-epileptic effects were not blocked by SB 242084 pre-treatment, a selective 5-HT$_2C$R antagonist, but rather potentiated (Orban et al., 2014). Therefore, our findings suggest that 5-HT$_2C$Rs are pro-epileptic and the mCPP and lorcaserin activate other 5-HTRs, most likely 5-HT$_1A$Rs (Orban et al., 2013). The fact that RO60-0175 was not effective in blocking the MDA elongation further supports the evidence that this compound is far from being a specific 5-HT$_2C$R tool (Damjanoska et al., 2003; Navailles, Lagriere, Le Moine & De Deurwaerder, 2013; Orban et al., 2014).

On the other hand, the involvement of 5-HT$_2C$Rs in non-convulsive generalized seizures is more compelling compared to focal epilepsy. Hitherto, the results have been hampered by the lack of selectivity of the 5-HT$_2C$R available drugs (Bagdy et al., 2007; Gui & Di Giovanni, 2015).

mCPP decreased the duration of SWDs via the activation of 5-HT$_2C$Rs in WAG/Rij rats since its effect was blocked by SB 242084 (Jakus et al., 2003). On the other hand, 5-HT$_2C$Rs seem not to play a role in basal modulation of ASs (Jakus et al., 2003; Jakus & Bagdy, 2011). Similarly, DOI, a 5-HT$_2A$/$2C$ mixed agonist and the two 5-HT reuptake inhibitors fluoxetine and clomipramine, reduced the time spent in seizure in groggy model of ASs (Tokuda et al., 2007). DOI-elicted decrease in ASs was blocked by ritanserin, a non-selective 5-HT$_2$ antagonist, which had no effect on its own (Ohno et al., 2010), further confirming that phasic 5-HT activation of 5-HT$_2$Rs does not modulate the occurrence of SWDs. Moreover, mCPP had no effect in modulating absence seizures, while DOI reduced the total time spent in seizure in the AY-9944 model of atypical AS (Bercovici, Cortez, Wong & Snead, 2006).

We have recently investigated the effects of pharmacological manipulation of 5-HT$_2$Rs in the expression of absence seizures of in GAERS (Genetic Absence Epilepsy Rat from Strasbourg), another polygenic model of ASs using selective 5-HT$_2C$ drugs (Venzi et al., 2016). Since early results obtained with unselective 5-HTR ligands (Marescaux, Vergnes & Depaulis, 1992b, 1992a) (see Danoher, Deransart, Depaulis, Vergnes & Marescaux, 1998) did not show any significant 5-HT modulation of AS in GAERS, we used lorcaserin and CP809, 101, the most selective 5-HT$_2C$ agonist available (Siuciak et al., 2007) and the selective 5-HT$_2C$ antagonist SB 242084. 5-HT$_2C$Rs activation reduced total time spent in seizures in GAERS. Moreover, we observed an overexpression of 5-HT$_2C$Rs in the ventrobasal (VB) thalamus in GAERS compared to non-epileptic control (NEC) rats (unpublished observations). Therefore, a dysfunction of 5-HT$_2C$Rs might be involved in the pathogenesis of ASs and selective agonists at these receptors may be potential targets for new anti-absence drugs.

5-HT$_2C$Rs are widely expressed in the CNS, including key areas involved in the pathogenesis of the ASs, such as the cortex and thalamus (Cruneell & Leresche, 2002) or areas known to modulate SWDs i.e., striatum, nucleus accumbens and substantia nigra pars reticulata (Depaulis, David & Champier, 2016). Therefore, since we administered the drugs intraperitoneally, it is difficult to rule out which brain area is involved in the anti-absence effect of 5-HT$_2C$R agonists.

5-HT$_2C$Rs are expressed in thalamocortical (TC) neurons in the dorsal lateral geniculate nucleus (dLGN) (Coulon et al., 2010). GABAergic interneurons of the dLGN contain 5-HT$_2C$R mRNA, and their activation induces an increase of phasic GABA$_A$R inhibition in
The intracellular pathways that couple the 5-HT$_2$Rs to the Ca$^{2+}$-influx mechanism depend on the PLC system and the transient receptor potential (TRP) protein TRPC4 (Munsch et al., 2003). We instead showed that mCPP (ineffective in mice; Munsch et al., 2003) decreased phasic inhibition as well as tonic GABA$_A$R current in dLGN neurons in rats, an effect blocked by pretreatment with SB 242084 (Crunelli & Di Giovanni, 2015). 5-HT$_2CR$ GABAergic modulation is not limited to the dLGN but is also present at the level of the somatosensory VB thalamus, where RO 60-0175 decreased both tonic and phasic inhibition GABA$_A$ (unpublished observations). The control of tonic inhibition seems to be phasic in nature, since SB 242084 did not have any effects on its own, but blocked RO 60-0175 effect in wistar rats. RO 60-0175 produces a normalization of the increased GABA$_A$ tonic current in GAERS, thought to be a necessary mechanism for the development of ASs (Cope et al., 2009).

5-HT in the thalamus induces depolarization of TC neurons and change in their firing pattern from burst to single spike activity (McCormick, 1992). 5-HT induces membrane depolarization by inhibition of a leak K$^+$ conductance (Meuth et al., 2006) and hyperpolarization-activated non-selective cation current (Ih) (Pape & McCormick, 1989; Chapin & Andrade, 2001). 5-HT$_2CR$ agonist CP809,101 and 5-HT produce similar depolarization effects activating Gq protein-coupled receptors (Coulon et al., 2010). Ketanserin, a 5-HT$_1A/2CR$ antagonist, was capable of blocking 5-HT-induced switch in the NRT neuronal pattern activity. Therefore, 5-HT modulation of sleep-waking activity might depend on GABAergic neurons of the NRT (McCormick & Pape, 1990; McCormick & Wang, 1991).

5-HT promotes waking and suppress REM sleep but on the other hand 5-HT$_2CR$ KO mice have an increase of waking and a reduction in NREM sleep. Different results come from pharmacological experiments where selective 5-HT$_2CR$ antagonists and nonselective 5-HT$_2A/2CR$ antagonists increase SWS (slow wave sleep) and reducing REM sleep respectively (Popa et al., 2005). On the other hand, nonselective 5-HT$_2A/2CR$ agonists and selective 5-HT$_2CR$ agonists increased waking and reduced SWS and REM sleep.

During ictal activity recorded in animal models of absence epilepsy, TC neurons are generally silent (Pinault et al., 1998; Pulack et al., 2007) due to an increased corticothalamic excitatory inputs into NRT neurons compared to TC neurons. We hypothesize that during ASs, 5-HT$_2CR$ agonists have antiabsence effects by decreasing GABA release form NRT neurons into VB TC neurons leading to a reduced GABA$_A$ phasic and tonic current. Nevertheless, that it is not impossible that 5-HT$_2CR$s have both anti- and pro-epileptic effects, depending which brain area receptor population is activated. For example in the cortex, 5-HT$_2CR$s are both highly expressed on inhibitory interneurons (S. Liu, Bubar, Lanfranco, Hillman & Cunningham, 2007) and pyramidal cortical neurons and 5-HT$_2CR$ activation can induce increase of thalamic glutamate release (Puig, Celada, Diaz-Mataix & Artigas, 2003).

Another potential way by which 5-HT$_2CR$s modulate ASs may be through other neurotransmitters such as dopamine, and noradrenaline known to modulate the arousal state and affecting thalamic and cortical pathological oscillations seen in absence epilepsy (Di Giovanni, Di Matteo & Esposito, 2008, 2010; Di Giovanni, 2013).

### 1.5 Therapeutic Potential of 5-HT2C Drugs in Epilepsy

5-HT modulates normal and pathological brain excitability via the plethora of 5-HT$_2$s. The complexity of this control it might be due to the opposing effects of different receptors and the different 5-HT modulation of the different brain areas involved in the various types of epilepsy. 5-HT$_2CR$s modulate generalized convulsive tonic-clonic and non-convulsive epilepsy. On the other hand, 5-HT$_2CR$s do not seem to be involved in focal epilepsy.

Our findings with 5-HT$_2CR$s agonists are promising and suggest a therapeutic potential of these drugs for the treatment of human generalized convulsive and non-convulsive epilepsy. Since lorcaserin has received FDA approval for treatment of obesity it will be easy to conduct a well-controlled studies to demonstrate its efficacy in epilepsy.

One of the negative side effects of 5-HT$_2CR$s agonists may be their potential anxiogenic effects. As a result, new AEDs based on 5-HT$_2CR$s agonism should be lacking of this and other 5-HT$_2CR$s aversive off-target effects. Ideally optogenetic treatment of ASs would be devoided of all the typical synthetic 5-HT ligands side effects. Our going work using optogenetics to stop ASs will also clarify the role of 5-HT in this type of epilepsy. Increasing our understanding of the role of 5-HT might reveal novel mechanisms of potential translational significance.

### 1.6 Role of 5-HT in Generalized Epilepsy: Optogenetic Evidence

#### Optogenetic Studies of the Serotonergic System

The activity of raphe nuclei neurons is tightly correlated with changes in brain state changes (Urbain, Creamer & Debonnel, 2006). While correlating neuronal activity with specific behavioural events is essential for elucidating the neuronal mechanisms underlying a variety of brain functions, establishing causal relationships require

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tools to directly interact with neuronal populations to boost or silence their activity and monitor the effects on various physiological and/or behavioural functions. The classical tools to reach these aims were to either electrically stimulate various nuclei or to systemically or locally interact with groups of neurons using pharmacological tools. Electrical stimulation acts by directly triggering action potentials in neurons or axons in the proximity of the stimulating electrode. Because most nuclei consist of populations of neurochemically heterogeneous neurons (see Introduction) and fibers of passage, this technique lacks specificity in terms of selectively influencing the electrical activity of various neurochemically heterogeneous elements of the network albeit and its great temporal specificity. On the other hand, as we showed in the first part of this review pharmacological tools, can be relatively specific, but lack both temporal specificity and when agonists are being used these can reach receptors outside the area of action of axon terminals from which they are released. Recent progress in genetic engineering offers new opportunities to directly control specific neuronal populations. Two light-sensitive proteins, namely Channelrhodopsin-2 (ChR2) and Halorhodopsin (Halo) can now be used to optically activate or silence specific neuronal subtypes (Zhang, Wang, Boyden & Deisseroth, 2006, 2008). The use of these proteins presents compelling advantages: 1) their expression can be targeted to specific cell types and/or subcellular compartments; 2) ChR2 can drive action potential firing with millisecond precision in response to light pulses. Animals expressing ChR2 and/or Halo have already been produced (Gradinaru, Mogri, Thompson, Henderson & Deisseroth, 2009), and optical interfaces for delivering the light to specific brain regions in behaving rodents have been designed (Aravanis et al., 2007). It has been recently shown that when used in combination with electrophysiological recordings, optogenetic techniques can be used to distinguish between action potentials of different classes of neocortical neurons by selectively triggering light evoked action potentials in only one type of cells, a method termed photostimulation-assisted identification of neuronal populations (Lima, Hromadka, Znaenenskiy & Zador, 2009). These recently developed optogenetic tools have several advantages over classical techniques: can be targeted to specific neuronal populations, have millisecond temporal precision and can be used to both stimulate and inhibit neuronal activity.

Selectively stimulating raphe nuclei 5-HT neurons using optogenetic tools has been shown to profoundly affect the spontaneous activity of a large proportion of neurons in the primary olfactory cortex (Lottem, Lőrincz & Mainen, 2016). Specifically, the baseline activity of most neurons in the olfactory cortex is reduced in a rapid and transient manner, most neurons being affected by 5-HT photostimulation in less than 100 ms. Optogenetic stimulation of raphe nuclei neurons has been shown to suppress hippocampal ripple activity and inhibition of these neurons increased ripple activity (Wang et al., 2015). Thus, changes in the 5-HT concentration throughout the forebrain can also powerfully influence rhythmic cortical activity.

Optogenetic targeting of various neuromodulatory systems in vivo could lead to altered seizure dynamics, the effects being mediated by local and global neuromodulatory actions in the brain causing changes in network dynamics. Given the prominent effect of serotonergic stimulation on cortical activity, the effect on SWDs in animal models would be expected to be dramatic. This could be beneficial for two reasons. First, by changing 5-HT neuromodulatory tone, both globally (using somatic 5-HT neuron photostimulation in the raphe nuclei) and locally (photostimulating ChR2 expressing 5-HT fibers in various cortical and thalamic regions) one could gain new insights in the cellular and network mechanisms involved in the generation of SWDs. This is crucial information for developing more targeted medications. Second, since the effect of 5-HT is rapid (< 100 ms; Lottem et al., 2016), this method could in theory be used for closed-loop seizure detection and ablation. Indeed, although the dominant frequency of SWDs in various animal models is 2-3 times higher than in humans, SWDs could be stopped after only one cycle (the interval between individual spikes of an SWD is > 100 ms) if proper spike detection is used. We are using a closed loop system (Berenyi, Belluscio, Mao & Buzsaki, 2012) in which the first spike of an SWD is used to trigger a series of light flashes delivered from a laser connected to an optic fiber situated in close proximity of ChR2 expressing serotonergic neurons. This way, this simple closed loop system quickly interacts with the generating networks and blocks the seizure before its full blown manifestation. ChR2 can readily be expressed in DRN 5-HT neurons (Dugue et al., 2014; Lottem et al., 2016) and photostimulation, using very low light intensity, reliably evokes action potentials in serotonergic neurons with millisecond precision (Dugue et al., 2014) and our preliminary data (Fig. 1). 5-HT thus has the potential to alter ensemble activity in cortical and/or thalamic networks. Our preliminary data confirm the ability to powerfully affect neocortical neuronal activity as reflected in the suppression of individual neuron firing (Fig. 2).

The activity of unidentified DRN neurons (Lőrincz et al., 2007) and identified DRN 5-HT neurons (Zhan et al., 2016) has been shown to be affected during seizures of various types, including SWDs. It has long been thought that, as a neuromodulator, 5-HT can have a sustained influence on its targets without temporal mod-
Targeting the Serotonin (5-HT) System to Control Seizures

Figure 1: EYFP-ChR2 expression in the DRN of a Tph2-ChR2(H134R)-EYFP mouse (Zhao et al., 2011). (A) Low magnification epifluorescent image of a coronal brainstem section. The boxed area is shown in B. Aq: aqueduct.

Figure 2: DRN 5-HT photostimulation results in a prominent suppression of neocortical baseline activity. (A) (Top) Example recording of the electrical activity of a neocortical neuron recorded in the motor cortex of an anesthetized mouse. 5-HT photostimulation (indicated by the horizontal blue bar) results in a rapid and prominent suppression of action potential firing. The inset shows the superimposed spikes, red trace is the averaged spike waveform. The blue bar marks the 5-HT photostimulation (train of 10 ms pulses at 10 Hz). (Bottom) Firing rate of the recorded neuron. (B) Peri stimulus time histogram of the neuron illustrated in (A). The photostimulation is illustrated by the blue bar. (C) Scatter plot comparing firing rates under control and photostimulated conditions for 9 recorded neurons. A linear regression fit is superimposed.

Interestingly, recently it has been shown, that the firing of some DRN neurons is also modulated at a faster timescale and can be phase-locked to specific behavioural events. Specifically, a subset of identified serotonergic neurons show phasic activation to reward predicting cues (Cohen, Amoroso & Uchida, 2015) and encode reward (Z. Liu et al., 2014), suggesting the importance of serotonergic system in guiding behaviour.

Given the transient activation of DRN 5-HT neurons linked to specific behavioural effects and the ability of the 5-HT system to rapidly influence cortical electrical activity, the serotonergic system appears as an attractive neuromodulatory candidate to control pathological synchronous cortical electrical activity on a rapid timescale.
2 Conclusion

The findings reviewed here highlight an important role for 5-HT and its receptors, especially the 5-HT\textsubscript{2C}Rs, in both pathologic neuronal excitability in epilepsy and comorbid affective disorders. The available literature suggests that antagonism at 5-HT\textsubscript{2C}Rs might have beneficial effects on TLE patients, while their activation shows a clear anti-absence effect. These paradoxical anticonvulsant efficacy of 5-HT\textsubscript{2C}R antagonists and agonists can be reconciled, taking into consideration that i) the two types of epilepsy have a different network substrate, ii) both agonism and antagonism induce 5-HT\textsubscript{2C}-R desensitization or downregulation (Graybiel, 2004), and/or iii) the existence of different populations of 5-HT\textsubscript{2C}Rs with different signal transduction mechanisms. Moreover, the anti- versus pro-epileptic effects of the 5-HT\textsubscript{2C}R activation might depend on the dose of the ligands used, with a pro-convulsive effects being present when the receptors are excessively activated.

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