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PERSPECTIVE

The Orexin System: A Potential Player in the Pathophysiology of Absence Epilepsy

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Abstract: ***Background*:** Absence epilepsy is characterized by the presence of spike-and-wave discharges (SWDs) at the EEG generated within the cortico-thalamo-cortical circuit. The molecular mechanisms involved in the pathophysiology of absence epilepsy are only partially known. WAG/Rij rats older than 2-3 months develop spontaneous SWDs, and they are sensitive to anti-absence medications. Hence, WAG/Rij rats are extensively used as a model for absence epilepsy with predictive validity.

***Objective*:** The aim of the study was to examine the possibility that the orexin system, which supports the wake status in experimental animals and humans, plays a role in the pathophysiology of absence seizures.

***Methods*:** The perspective grounds its method from recent literature along with measurements of orexin receptor type-1 (OX1) protein levels in the thalamus and somatosensory cortex of WAG/Rij rats and non-epileptic Wistar control rats at two ages (25 days and 6-7 months). OX1 protein levels were measured by immunoblotting.

***Results*:** The analysis of the current literature suggests that the orexin system might be involved in the pathophysiology of absence epilepsy and might be targeted by therapeutic intervention. Experimental data are in line with this hypothesis, showing that OX1 protein levels were reduced in the thalamus and somatosensory cortex of symptomatic WAG/Rij rats (6-7 months of age) with respect to non-epileptic controls, whereas these differences were not seen in pre-symptomatic, 25 days-old WAG/Rij rats.

***Conclusion*:** This perspective might pave the way for future studies on the involvement of the orexinergic system in the pathophysiology of SWDs associated with absence epilepsy and its co-morbidities.

Keywords: Absence epilepsy, SWDs, cortico-thalamo-cortical network, sleep disturbance, orexin system, OX1.

1. INTRODUCTION

1.1. Background: Absence Epilepsy and the WAG/RIJ Rat Model

Absence epilepsy is a form of generalized non-convulsive type of epilepsy characterized by sudden and transient episodes of behavioral arrest. Large amplitude spike-wave discharges (SWDs) are the electroencephalographic hallmark of absence epilepsy in patients and in genetic animal models, *e.g.*, rats of the WAG/Rij (Wistar Albino Glaxo from Rijswijk) strain and GAERS (Genetic Absence Epileptic rats of Strasbourg) [1].

SWDs are generated in a cortico-thalamo-cortical circuit, which includes pyramidal neurons of the somatosensory cortex, thalamo-cortical neurons (TC), and GABAergic neurons of the reticular thalamic (RT) nucleus projecting to the TC neurons in the ventrobasal (VB) thalamus. T-type voltage-sensitive Ca^{2+} channels are critically involved in the pathophysiology of absence seizures [2]. It has been proposed that SWDs develop in the cortex, and next the thalamus gets involved, including the GABAergic RT neurons [3, 4]. The latter cause IPSP in TC neurons, after which the low threshold Ca^{2+} channels in TC cells activate, and they produce the low-threshold Ca^{2+} spike, followed by a burst of action potentials leading to the next cycle of the oscillation [2, 4]. TC neurons fire differentially in two functionally distinct states: they show bursts of action potentials during slow-wave sleep, sleep spindles and SWDs, and they fire tonically during wakefulness and REM sleep.

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2. ABSENCE SEIZURES AND SLEEP

There is a bidirectional link between sleep and epilepsy; sleep has a clear influence on seizure threshold, and both focal and generalized seizures have a strong impact on sleep architecture. The most common disturbances reported in patients with epilepsy are sleep fragmentation, reduction of various sleep stages, difficulties in sleep initiation, and increased daytime sleepiness [5]. The relationship between sleep and absence epilepsy is also well recognized [6]. Absence seizures preferably occur during somnolence or mental and physical inactivity, at the transition between wakefulness and sleep and during unstable periods of light non-REM sleep, and are virtually absent during deep slow wave and REM sleep in people with absence seizures and in the genetic models [6, 7]. WAG/Rij rats also show abnormalities in the sleep architecture with a lower incidence of REM episodes, more arousals and greater duration of the intermediate stage of sleep (the transition from non-REM to REM sleep), while the circadian distribution of sleep and wake and depth of sleep seems normal [7-9]. Pharmacotherapy of absence epilepsy may also influence sleep parameters since a 4-month treatment with the anti-absence drug ethosuximide decreased REM sleep duration and REM sleep/total sleep time in the same model [10]. Importantly, both SWDs and sleep spindles are generated in the same cortico-thalamo-cortical networks [11] and both increase with age in WAG/Rij rats [1, 9]. Hence, it is reasonable to hypothesize that systems involved in the generation and/or control of absence seizures may also have a role in sleep-wake regulation. This hypothesis has potential translational value because wake-promoting agents are already marketed for the treatment of excessive daytime sleeping associated with narcolepsy or obstructive sleep apnea [12, 13].

3. THE OREXIN SYSTEM

Orexin-producing neurons represent one of the major wake-promoting systems in the brain, and their degeneration is associated with narcolepsy, a disorder characterized by sudden onset of REM episodes, excessive daytime sleeping, and cataplexy [14]. These neurons are localized in the lateral hypothalamus and project to brain centers involved in wake/sleep regulation, such as monoaminergic nuclei in the brain stem [15-18]. Orexins A and B activate two G-protein coupled receptors, named Orexin-1 and Orexin-2 (OX1 and OX2, respectively) which share 94% and 95% identity in humans and rats [16, 19, 20]. Orexin A displays a 5-100-fold greater affinity than orexin B for OX1 receptors, whereas orexins A and B show similar affinities for OX2 receptors [21]. Orexin receptors couple to multiple G proteins in heterologous expression systems, although $G_{q/11}$-mediated stimulation of inositol phospholipid hydrolysis with the ensuing increase in intracellular Ca^{2+} is considered a leading transduction mechanism activated by orexin receptors [22]. Activation of OX1 receptors by orexin A can also enhance intracellular Ca^{2+} by activating transient receptor potential channel 3 (TrpC3) in the cell membrane [23]. The transcript of OX1 receptor is abundant in the thalamus and is also found in the neocortex [20, 24, 25]. Orexins may excite thalamic neurons either directly (*i.e.*, by local activation of orexin receptors) or indirectly (by activating wake-promoting pathways projecting to the thalamus) [26-29]. There are analogies between type-1 narcolepsy (*i.e.*, narcolepsy associated with a detectable loss of orexin levels and cataplexy) and epilepsy. For example, cataplexy can be misdiagnosed as epileptic drop attacks and atonic seizures [30], and praxis-induced epileptic seizures can be misdiagnosed as cataplexy [31]. Association between type-1 narcolepsy and epileptic syndrome has been reported [32, 33], but to our knowledge, a link between changes in the orexin system and absence epilepsy has never been demonstrated in animal models or humans.

4. REDUCED OX1 RECEPTOR EXPRESSION IN THE THALAMUS AND SOMATOSENSORY CORTEX OF SYMPTOMATIC WAG/RIJ RATS.

WAG/Rij rats develop spontaneous SWDs after 2-3 months of age and present a genetic absence epilepsy model with excellent face, predictive and construct validity [1, 34]. We used protein extracts collected from the thalamus and somatosensory cortex of male presymptomatic (25 days old) or symptomatic (6-7 months old) male WAG/Rij rats and age-matched non-epileptic male Wistar rats to measure OX1 receptors by immunoblotting. Tissue was collected from rats bred in IRCCS Neuromed [Authorization no. 855/2020-PR]. Immunoblots were analysed as described before [35, 36]. OX1 receptors were detected by using rabbit polyclonal antibody (anti-OX1, AB3092, Chemicon, Millipore Corporation, Billerica, MA, 1:1000) and mouse monoclonal antibody for β-actin (1:50000, Sigma, St. Louis, MO).

OX1 receptor protein levels were significantly lower in the thalamus of symptomatic 6-7 months WAG/Rij rats as compared to both age-matched non-epileptic Wistar rats, and were compared with presymptomatic WAG/Rij rats. This decrease was not seen in rats of the Wistar strain. There were no differences found between 25 days old WAG/Rij and Wistar pups in the thalamus. The cortex showed, besides significant age (increase) and strain (Wistar > WAG/Rij) effects, lower protein levels in adult WAG/Rij rats compared to age-matched Wistar rats, and again, this difference was not present in pups (Fig. **1**). Thus, at least in the WAG/Rij model, age-dependent development of SWDs was associated with a unique down-regulation of OX1 protein level in the thalamus, while the age-dependent increase in the cortex was diminished in symptomatic WAG/Rij rats. In all, it seems that the major stations of the neuronal circuit underlying the generation of SWDs and sensory processing are characterized by a reduction of OX1 receptor protein levels.

5. DISCUSSION AND PERSPECTIVES

Orexins depolarize thalamic neurons in the dorsal lateral geniculate nucleus [37] and paraventricular nucleus [38], and elicit anxiety-like behavior when microinjected in the paraventricular nucleus [39]. T-type voltage-sensitive Ca^{2+} channels are low threshold ion channels that are rapidly inactivated by membrane depolarization [40]. We speculate that,

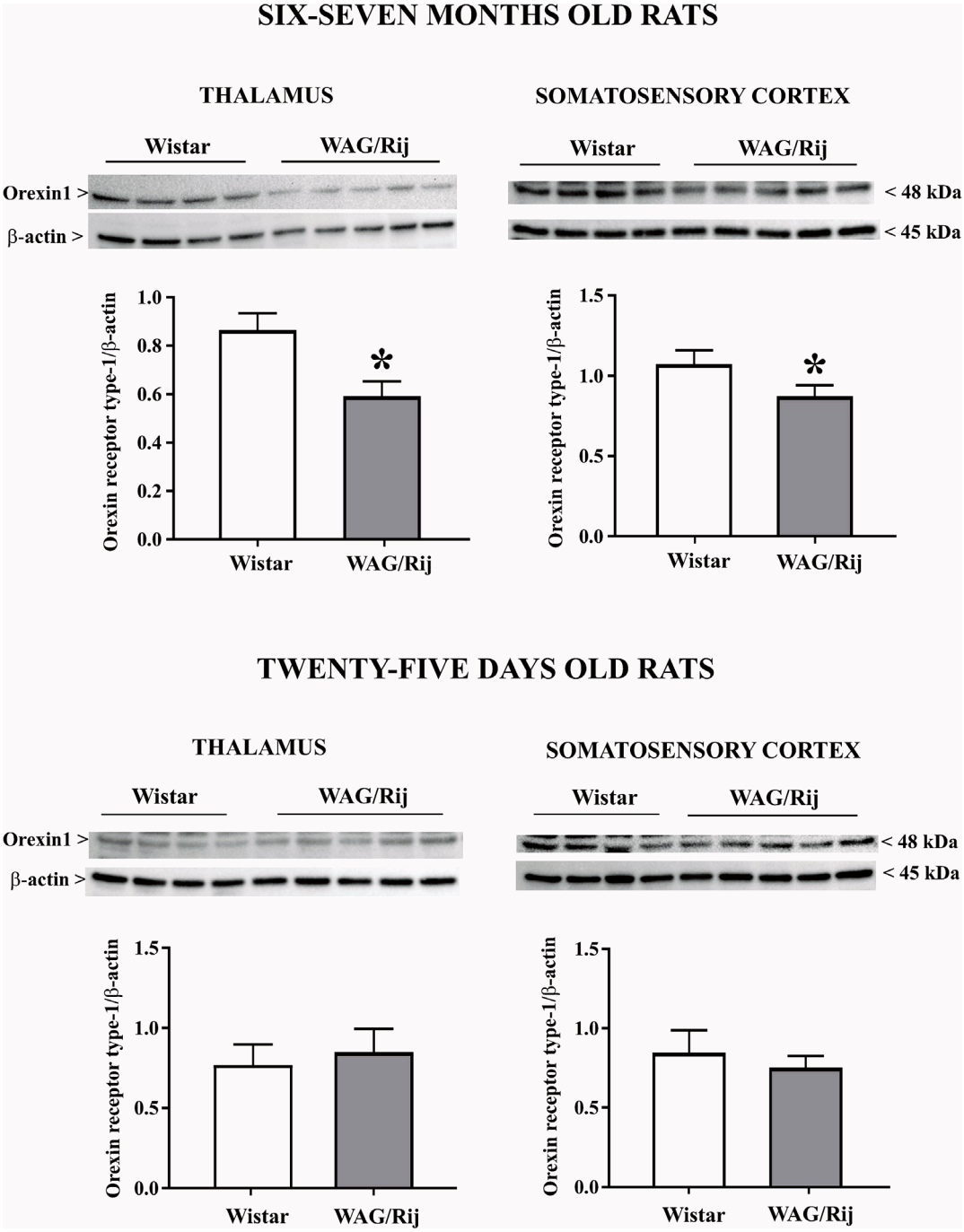

Fig. (1). Immunoblot analysis of OX1 receptors in the thalamus and somatosensory cortex of Wistar and WAG/Rij rats. Representative immunoblot of OX1 receptors levels in thalamus and cortex; (**A**) symptomatic WAG/Rij rats (6-7 months old) and (**B**) pre-symptomatic WAG/Rij rats (25 days old) as compared to age-matched control Wistar rats. Values are means ± S.E.M. with 4–5 animals in each group. Data points are from individual experiments. Post-hoc t-tests showed that symptomatic WAG/Rij rats differ from non-epileptic age matched controls (*: Thalamus: t = 6.27, df=7, and 95% two-tailed confidence interval for difference of means: 0.170 to 0.377; two-tailed P-value = 0.000418; Somatosensory cortex: t = 3.86, df=7, and 95% two-tailed confidence interval for difference of means: 0,0767 to 0,320; two-tailed P-value = 0,00624). There were no statistical differences detected between age-matched Wistar and pre-symptomatic WAG/Rij rats, neither in thalamus, nor in cortex (Thalamus: t = -0.956, df=7, and 95% two-tailed confidence interval for difference of means: -0,312 to 0,132; two-tailed P-value = 0,371; Somatosensory cortex: t = 1.207, df=7, and 95% two-tailed confidence interval for difference of means: -0,086 to 0,265. two-tailed P-value = 0,267). Next, and not illustrated, there was an age-dependent increase in the cortex (F=15.28, df=1,14, P-value = 0,002), but not in thalamus (F= 2.40 df = 1,14, P-value = 0,144). *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

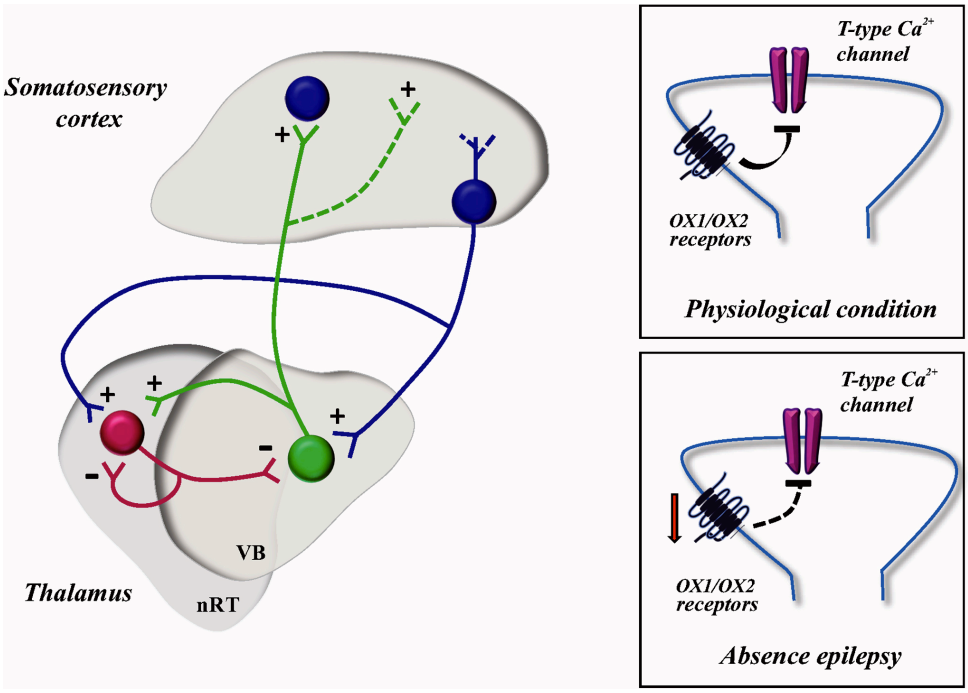

Fig. (2). Cortico-thalamo-cortical network underlying absence seizures. VB = Ventrobasal thalamic nuclei; nRT = reticular thalamic nucleus. Fig. (**2**) is partially adapted from Celli *et al.*, *Curr Neuropharmacol.*, **2017**, *15*(6), 918-925. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

under physiological conditions, orexin-induced depolarization may facilitate T-channel inactivation in VB or in RT neurons, thereby restraining the development of SWDs. The inhibitory control by the GABA-ergic RT cells might be less efficient in WAG/Rij rats because of the downregulation of OX1 receptors in the thalamus, and this might play a permissive role in the generation of absence-like seizures (Fig. **2**). This, however, remains to be demonstrated.

The following question should be addressed: (i) are changes in OX1 receptors causally related to SWDs? Thus, studies involving other models of absence epilepsy are necessary; (ii) how do OX2 receptors behave? (iii) are OX1 or OX2 receptor agonists able to reduce the incidence or duration of SWDs in WAG/Rij rats? (iv) can genetic depletion or pharmacological blockade of orexin receptors worsen the phenotype in the WAG/Rij model? (v) do SWDs occur more easily in models of narcolepsy? (vi) is the OX1 receptor downregulation restricted to cortex and thalamus; and (vii) are changes in orexin levels detectable in the blood or cerebrospinal fluid of patients affected by absence epilepsy? Answers to these questions may shed light on the pathophysiology of absence epilepsy and perhaps find a place for absence seizures in the complexity of an "orexin system spectrum disorder", in which sleep and eating abnormalities are major hallmarks.

Of note, OX2 receptor agonists (*e.g.*, compound TAK-925) are under clinical development for the treatment of narcolepsy [41]. Another OX2 receptor agonist, compound YNT-185, showed efficacy in mouse models of narcolepsy, and also behaved as a wake-promoting agent in control mice [42], suggesting a potential use of OX2 receptor agonists in disorders associated with excessive daytime sleep. In contrast, orexin receptor antagonists are under development for the treatment of insomnia [43, 44], and a dual OX1/OX2 receptors antagonist, lemborexant, has been approved with this indication in Japan [45]. OX1 receptor antagonists have also been developed for the treatment of obesity, and they are shown to restrain feeding behavior and reduce weight gain in models of obesity, metabolic syndrome, and binge eating in rodents [46-49]. Thus, a number of validated pharmacological tools are available for an in-depth study of the orexin system in WAG/Rij rats and other absence models.

Last but not least, alterations in the orexin system might also be involved in the pathophysiology of psychiatric comorbidities associated with absence epilepsy and with the modulation of pain. A recent report showed that WAG/Rij rats have a much lower pain threshold compared to Wistar controls in four different pain assays [50]. Next, this strain has depressive-like properties and is considered as having comorbidity for depression [51]. Interestingly, orexin receptors are also considered as a novel target for the treatment of depression, and orexin receptor antagonists are currently under clinical development for the treatment of major depressive disorders [52]. Similarly, preclinical research shows that a deficient orexin system could imply poor antinociceptive action in the brain and spinal cord in different types of rodent pain assays, and that orexin agonists exert analgesic action in different pain tests [53]. Pain transmission is *via* the dor-

sal thalamus, a part of the brain circuit involved in the occurrence of the SWDs.

CONCLUSION

In all, WAG/Rij rats are a valuable model for studying the link between orexin receptors, absence epilepsy, hyperalgesia and psychiatric comorbidities. Of note, WAG/Rij rats show abnormalities in the central melatonergic system [54], which represents a further link between sleep, absence seizures, and psychiatric comorbidities.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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