Seizure Semiology, Neurotransmitter Receptors and Cellular-stress Responses in Pentylenetetrazole Models of Epilepsy

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Abstract

Ongoing research has elucidated a large variety of genes, proteins and enzyme products that are affected in epilepsy. Despite the pharmacological advances achieved by the development of antiepileptic drugs, numerous patients become pharmacoresistant. Therefore, animal models addressing these complex interactions among compensatory gene-expression cascades and consecutive molecular mechanisms are still a necessity for research-based gene and pharmacotherapy. In this article, we focus on pentylenetetrazole models to study the consequences of tonic–clonic seizures. We address two complex and closely linked aspects: alterations in neurotransmission and oxidative-stress responses. Reviewing just these two aspects highlights the need for a more standardised use of animal models and methods to allow a better integration of data from different lines of research. The latter will be most applicable for the understanding of complex disease-related interactions of gene networks, proteins and enzyme products and timely, research-based development of future therapeutic options.

Keywords

Epilepsy, gene networks, neurotransmission, cyclo-oxygenase, oxidative stress, neuron–glia interaction

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Epilepsy affects about 1% of the human population at some point during their life. The causes of human epilepsies are diverse, ranging from defects during brain development resulting in dysplasias or ectopic cortical neurons to inherited forms involving certain mutations, e.g. ion channel defects or metabolic impairments; however, most causes are still unknown.[1,2] Furthermore, post-traumatic epilepsies are also known;[3] therefore, epilepsies are not a homogeneous pathogenetical entity, but rather are defined as the "occurrence of repeated seizures",[4] which will be grouped into various epileptic syndromes according to the individual semiology of patients.[5]

The causes of the initial mechanisms in the development of epileptic seizures are still elusive and only partial aspects of specific epileptic phenomena may be attributable to mutations at certain gene loci.[6–8] Furthermore, during onset and progression of the disease, multiple changes in the expression patterns of many genes and gene products have been reported.[2,9] This indicates that there are no single and specific gene mutations associated with a certain type of epilepsy, as has been established for Huntington's disease, for example. These multiple molecular responses (at the level of genes, RNA splicing and proteins) provide strong evidence for the induction of pathology-associated responses.

In our view, these changes are best explained as an initiation of compensatory gene-expression cascades (CGECs), i.e. a response to cope with the primary functional alterations caused by the disease (e.g. changes in the expression of neurotransmitter receptors or stress-response genes). These primary CGECs (pCGECs) are followed by additional secondary endogenous responses (e.g. an upregulation of multidrug transporters at the blood–brain barrier or certain neurons[10]) and tertiary responses induced by the short- and long-term effects of specific pharmacological interventions. This tertiary response may also be described as an exogenously or pharmacologically induced phCGEC.

According to these multifactorial and interdependent mechanisms, *in vivo* animal models continue to play a major role in the elucidation and understanding of the ongoing pathomechanisms, as well as the response mechanisms to pharmacological intervention and therapy. Furthermore, due to ethical reasons, animal models are essential for studies addressing the onset mechanisms of epileptic symptoms or questions such as: what are the progressing pathophysiological consequences and therapeutic options after a pathological status is reached from a sample resected tissue of patients with pharmaco-resistant forms of epilepsy?

In order to address these questions, a wide variety of animal models have been developed including genetic models, certain naturally occurring wild-type mutations, 'electrical' models (e.g. electroconvulsive kindling) and several pharmacological models for *in vivo* and/or *in vitro* use such as the kainate, pilocarpin, penicillin, 4-aminopyridine, cholera toxin, bicuculline, picrotoxin and pentylenetetrazole models, etc.[11,12] All of these models address single aspects of epilepsy and have their specific limitations.

In this article, we will focus on certain aspects of the widely used pentylenetetrazole (PTZ) model. Today, PTZ is no longer of therapeutic use except in rare cases of barbiturate intoxication. Referring to PubMed, more than 5,200 publications are listed using the chemical convulsant PTZ when addressing pharmacological and epileptological questions. PTZ exerts its action by binding to the picrotoxin-recognition site and benzodiazepine-binding site of the post-synaptic gamma-aminobutyric acid A ($GABA_A$) receptor.[13,14] Thus, PTZ reduces the effects of endogenous GABA and other inhibitory transmitters, which renders the system in a hyperexcitable state. In the case of a convulsive dose, PTZ induces generalised tonic–clonic seizure activity within seconds.

The So-called Pentylenetetrazole Model

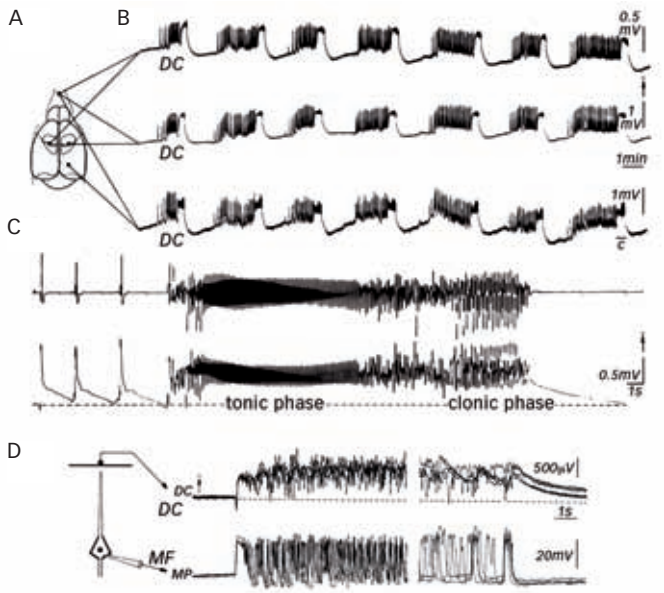
There is no single PTZ model; rather, several varieties exist. This seems to be a major reason for controversy concerning data achieved using one of the different PTZ models. First of all, species-specific differences certainly exist when applying PTZ to mice, rats, dogs and other animals. Furthermore, basic research on epilepsy has shown well-established strain-specific differences, as well as differences between individuals of a single strain or breeding group. In addition to the use of PTZ in various species, PTZ is typically used in three major experimental set-ups: injection of a single convulsive dose, as a kindling model by repeatedly injecting a subconvulsive dose and by repeatedly injecting a low but convulsive dose with longer seizure-free periods in between (usually described as repeated series of seizures).

Confusion often arises when a comparison is made between the results of a single species with a specific type of the aforementioned models, because much of the data are incomparable. This is so because most authors apply different doses of PTZ (range 10–110mg/kg), or in the case of kindling they use different time intervals, such as every 24 or 48 hours. Finally, the total period of treatment varies from two to eight weeks.

These circumstances often cause confusion when referring to observations made by simply referring to the term PTZ model.[15] This article focuses on the common phenomena using the PTZ model. First, in each form of PTZ treatment seizure activity occurs with a short latency, and second, neurodegeneration is absent or induced very late. These are two advantages of this model compared with other models such as the kainate or pilocarpin models, in which neurodegeneration is always associated with the initial seizure response.[16,17] Additionally, the PTZ model has the advantage that one chemical is used to study two different mechanisms associated with epilepsy, i.e. processes primarily related to neurodegeneration that have been observed in tissue resected from patients with epilepsy, and in many aspects are also similar to situations observed in ischaemic cerebral insults and second cellular-response mechanisms induced by seizure activity, which at least at the beginning occur independently of neuronal death or cell death in general.

The pentylenetetrazole model has the advantage that one chemical is used to study two different mechanisms associated with epilepsy.

Figure 1: Bioelectric Neocortical Activity During Repetitive Tonic–Clonic Seizures Elicited by Systemic Administration of Pentylenetetrazole in Rat (A–C) and Cat (D) Models



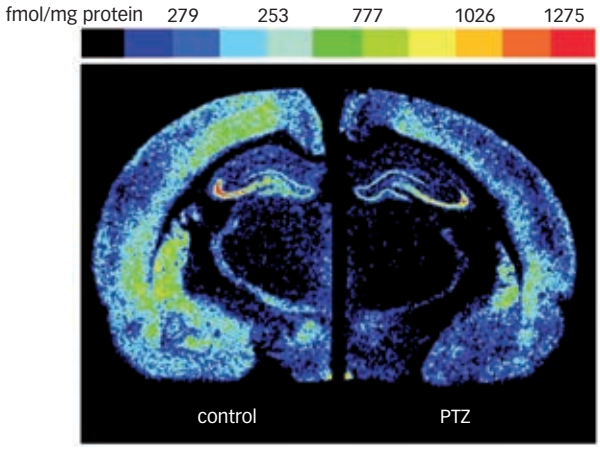
A: A schematic representation of the recording positions in the rat. Three epicortical electrodes were bilaterally positioned over the forelimb and hindlimb motor areas and one right-sided over visual areas were switched against a reference electrode in the frontal nasal bone. B: Simultaneous recordings of the cortical direct current (DC) potential (slow potential fluctuations superimposed by the fast waves of the conventional electroencephalogram [EEG]) from the sites indicated in A. The time-scale has been compressed; the duration of the recording in B circa was 30 minutes. C: Recording of the episode marked in B with example C as conventional EEG and DC potential. The time-scale was extended; duration of the recording in C was circa 30 seconds. D: Simultaneous recordings of the cortical DC potential and of the membrane potential (MP) of a layer V pyramidal cell (motor cortex) during four successive tonic–clonic seizures. Graphical superimposition; interruptions 20–60 seconds. Source: Speckmann E-J, 1986.[18]

Most authors argue that models in which neurodegeneration occurs are justified as they resemble the situation observed in patients with epilepsy.[18–20] However, successful therapeutic intervention in epilepsy may depend on the understanding and rapid treatment of early symptoms, which may precede the situation observed in cerebral tissue dissected from patients with a long history of seizure semiology. Therefore, the PTZ models may offer the opportunity to address this latter aspect, as the PTZ model of 'repeated series of seizures' using PTZ 40mg/kg bodyweight produces a pattern of seizures that matches that observed in patients (see *Figure 1*). As it is beyond the scope of this article to address all aspects of PTZ-induced seizures as previously described and discussed,[4] we would like to focus on effects reported for neurotransmitter receptors and associated stress responses.

Pentylenetetrazole-induced Seizures and Neurotransmission

For PTZ-kindled mice and during acutely PTZ-induced tonic–clonic seizures in mice, clear time-dependent changes in the amount of adenosine (A1) receptors are known, which also depend on the duration of the kindling process.[22–26] In general, A1-binding sites were increased in cortical, hippocampal and cerebellar regions, whereas

Figure 2: Colour-coded Images from Autoradiographs Showing the Overall Changes for Kainate-binding Sites of Control and Pentylenetetrazole-treated Rats



Scale bars code receptor densities in fmol/mg protein. The same scaling was used for pentylenetetrazole (PTZ)-treated and control brains. For further details refer to Cremer et al., 2009.[26]

a persistent decrease occurred in the striatum.[22,23] Within the so-called epileptic circuitry,[12] hippocampal, cerebellar and striatal alterations of A1 binding occurred immediately and persisted, whereas increases in cortical regions developed during kindling. In an elegant comparison, the authors also showed that in a colony of 'tottering mice' (a model closely resembling absence epilepsy), A1 binding is unaltered compared with PTZ models, indicating that different semiology may affect A1 binding.[24]

Furthermore, a clear model-dependent difference was also revealed for the A1 receptor, as mice used in the kainate model exhibited a reduction in hippocampal A1-binding sites due to the early occurring neurodegeneration. This indicates that a significant amount of A1-binding sites are post-synaptically localised,[25] and particularly highlights specific differences between models with initial neurodegeneration and the PTZ model with no or comparatively late neurodegeneration. As neurodegeneration is associated with glial changes (mainly with astrocyte proliferation) leading to gliosis, a hallmark of epilepsy, the above-discussed data indicate that a possible glial A1 expression does not compensate for neuronal A1 reduction in the kainate model. In contrast to the data obtained in mice, repeated series of PTZ-induced seizures in the Wistar rat led to a reduction in G-protein-coupled high-affinity A1-binding sites.[26] The reason for these contradicting results could be related to either species-specific differences or to the use of different ligands in the presence or absence of guanosine 5'[β,y-imido]triphosphate.

Additionally, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA)-receptor binding increases in a transient time-dependent manner[27] following PTZ-induced seizure episodes, whereas no significant changes were reported for the rat 24 hours after the last tonic–clonic seizures.[26] However, focusing on the N-methyl-D-aspartic acid (NMDA) receptor in mice, a long-lasting upregulation of NMDA-binding sites occurs in mice, which has also been seen in rats.[26,28]

Kainate receptors have long been associated with epilepsy, but despite the obvious use of the so-called kainate model, their direct relation to epileptic seizures is still a matter for discussion due to their ubiquitous distribution and multiple functions. In fully PTZ-kindled rats and in the PTZ model of a repeated series of seizures, kainate-binding sites are significantly reduced (see *Figure 2*).[26,30] Therefore, the overall differences reported for the glutamate receptors among mice and rats could be related to the post-seizure times at which the measurements were performed. Despite these model-specific differences, it should be emphasised that a combined inhibition of AMPA and NMDA receptors prevents PTZ-induced tonic–clonic seizures.[29] In contrast to the more ubiquitous downregulation of kainate receptors, dopamine receptors (D1 and D2) become reduced in a more region-specific manner, mostly affecting the amygdala.[31] Effects have also been described or postulated for the other monoaminergic receptors, mainly as compensatory responses to PTZ kindling.[32,33]

Despite the fact that PTZ has been shown to be an antagonist at $GABA_A$ receptors by means of a rat model of repeated series of seizures, no changes in $GABA_A$ binding occurred. However, a significant increase in benzodiazepine (BZ)-binding sites was observed. Since GABA and BZ-binding sites are localised at different positions within the functional $GABA_A$-receptor complex, the subunit composition of the $GABA_A$ receptors may change in such a way that BZ can potentiate GABAergic inhibition, as discussed in further detail by Cremer et al.[26]

Taken together, there are multiple PTZ-induced changes in neurotransmission at the receptor level, indicating that these alterations occur in order to compensate for seizure-induced adverse neurological effects. As the major changes remain restricted to cerebral regions belonging to the so-called epileptic circuitry,[12] the observed alterations are most likely seizure-associated and not attributable to more general pharmacological effects of PTZ. Therefore, the changes reported in terms of receptors and/or transmitter synthesis, release and recycling are indicative of the induction of multiple neurotransmission-related CGECs.

Oxidative and Nitrosative Stress

For various PTZ models, oxygen- and pH-related changes were reported early in the history of seizure semiology.[34] As in most other models of experimental epilepsy, clear signs of oxidative stress occur shortly after PTZ application, such as alterations of the thiol redox state and lipid and protein oxidation.[35–37] After PTZ-induced seizures and kindling, the formation of the reactive hydroxyl radical and an increase in NO metabolites has been reported,[38,39] which consequently results in seizure-induced, region-specific protein nitration.[40] Seizure- and NO-related protein nitration are part of an initially protective response cascade. This hypothesis is supported by the fact that convulsive doses of PTZ are lethal to mice lacking

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neuronal nitric oxide synthase (nNOS) expression, and subconvulsive doses of PTZ affect nNOS knockout mice more severely than wild-type mice. It is further supported by the fact that NO-modulated seizure augmentation versus seizure suppression in wild-type mice seems to be dependent on the concentration of NO.[41]

The Topography of Stress Responses

As explained above, it is now well-established that seizure-related hyperactivity is associated with oxidative stress. From a neurophysiological point of view, certainly one of the most convincing aspects for an association of neuronal excitation and oxidative responses is related to the arachidonic acid pathway and prostaglandin synthesis via cyclo-oxygenases (COX-1, COX-2); in particular, the inducible form COX-2 is constitutively expressed in a region-specific manner in many cerebral neurons in which COX-2 expression and activity is regulated by synaptic activity. In addition,

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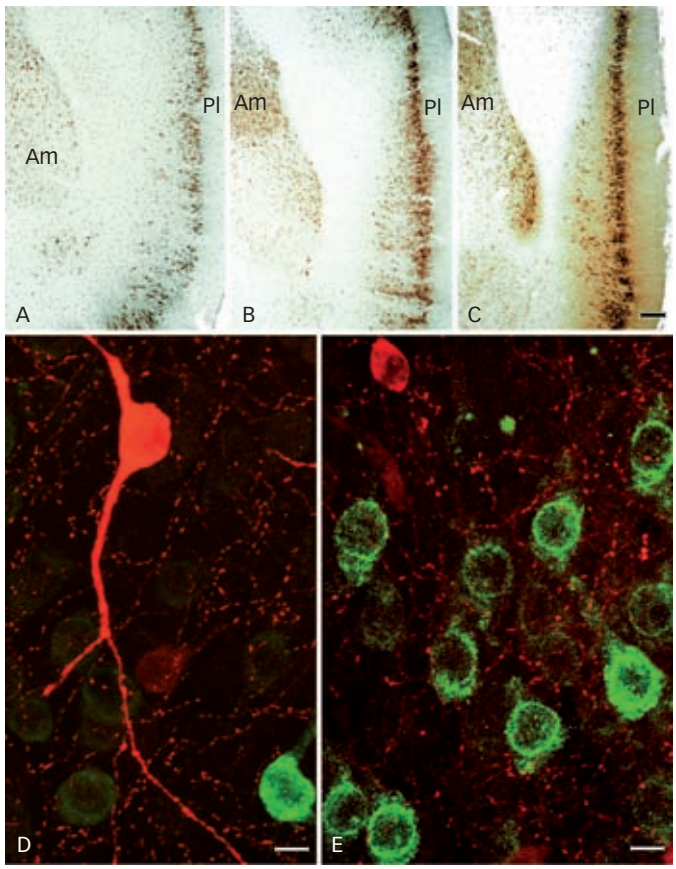
it should be noted that under normal physiological conditions cerebral regions well-known for their high constitutive COX-2 expression are those that belong to the epileptic circuitry.[12] Furthermore, NMDA-receptor-induced c-fos expression is prostaglandin-dependent and the calcium-dependent activation of cyclooxygenases results in superoxide production (for a review see Yermakova and O'Banion[42]).

For PTZ models, it is known that COX inhibitors (anti-inflammatory drugs and non-steroidal anti-inflammatory drugs [NSAIDs]) attenuate seizure activity.[43,44] Although not as strong as in rat models for cerebral ischaemia,[45,46] COX-2 expression is induced in human patients with epilepsy[47] and in the model of repeated PTZ-induced seizures (see *Figure 3*). COX-2-expressing neurons are shown to be closely associated with the processes of neurons expressing nNOS, thus revealing a direct topographical link between COX-2-related superoxide and NOS-I-related NO production. As the normal constitutive neuronal expression of COX-2 and the seizure-induced induction of COX-2 take place in a region-specific manner, COX-2-related production of oxygen radicals may represent the basis for region-specific pathological changes attributed to seizure-induced oxidative stress.

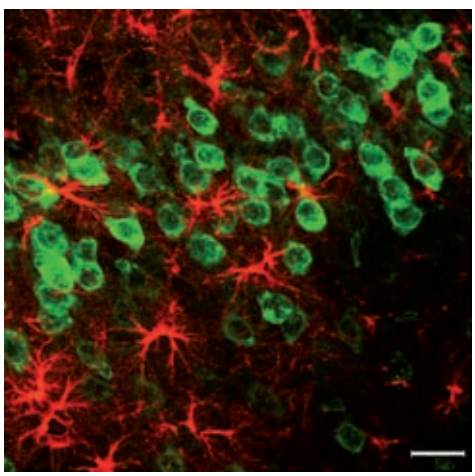
Some of the most affected cortical regions are the piriform and entorhinal cortices, which exhibit not only high COX-2 expression (see *Figure 3*), but also one of the highest packing densities of NOS-I- expressing cortical neurons.[48,49] This association of neurons responding with the production of radicals such as superoxide and nitric oxide, which results in the formation of peroxynitrite,[46] may be one reason for the fact that seizure-induced protein nitration and glutamine synthetase inhibition are much more easily detectable in piriform and entorhinal cortices than in other regions.[40] The latter may also explain why certain other changes affecting

Figure 3: Changes in COX-2- and NOS-I-positive Neurons in Control and Pentylenetetrazole-treated Rats



Images from frontal sections through brains of adult Wistar rats showing the constitutive presence of cyclo-oxygenase-2 (COX-2) (brown or green) in control (A, D, saline-treated) and after repeated pentylenetetrazole (PTZ) injections for two weeks, as described in reference 40, 24 hours after the last seizure episode in an individual with low seizure score (B) and in one with highest seizure score (C, E). Neurons expressing COX-2 (green, D, E) and neurons expressing neuronal nitric-oxide-synthase (NOS-I) (red, D, E) are tightly connected via the numerous long NOS-I-containing neuronal processes. In control animals, only a few single strongly COX-2-positive neurons were present, whereas after seizure episodes most neurons showed strong COX-2 expression. In rats with high seizure scores, neuronal processes containing NOS-I also appeared to be affected (E). As NO and oxygen radicals are highly reactive diffusible gases,[46] this tight association is most important for their interaction when produced in an excessive, uncontrolled manner. Am = amygdala; Pl = piriform cortex. Bars: 0.2mm A–C; 10µm D–E.

Figure 4: Association of HSP-27-positive Astrocytes and COX-2-positive Neurons in Piriform Cortex in a Pentylenetetrazole-treated Rat



40 Bar: 30µm. Image showing the co-distribution of seizure-induced heat-shock protein (HSP)-27 expression (red) in astrocytes and the neuronal induction of cyclo-oxygenase-2 (green) in piriform cortex of a Wistar rat 24 hours after the last pentylenetetrazole-induced seizure episode in a sequence of repeatedly induced seizures for two weeks.

neuro-transmitter metabolism seen in other rodent models of epilepsy seem to occur in a most pronounced manner in these cortices.[50] However, the question of why a neuronal induction of the heat shock protein 70 (HSP-70) family does not occur in PTZ models, whereas it is a normal finding in other rodent models of epilepsy,[51] still has to be explained, especially as HSP-70-overexpressing mice seem to be partly protected against PTZ- induced seizure activity.[52] As oxidative and nitrosative stress are not restricted to neurons that are functionally connected with glial cells and blood vessels, it may not be surprising that concomitant glial and endothelial changes do occur. The latter are seen by strong region-specific focal glial HSP-27 expression (see *Figure 4*). HSP-27 is a well-established marker for oxidative stress.[21] As glial cells are well equipped to cope with pathological alterations and ongoing processes such as during scar formation, it is understandable that glial cells respond in a more delayed manner.[21] When focusing on currently used experimental models of epilepsy, glial responses have to be viewed as a secondary consequence of neuronal seizure activity[26] rather than an initial cause of seizures, as is sometimes suggested.[53,54] Nevertheless, these secondary glial responses may subsequently contribute to a continuation or progression of epileptic seizures, for example during successful kindling.

Conclusion

In this article we have concentrated on two aspects of the PTZ model: neurotransmitter–receptor alterations and oxidative stress. Consideration of these two topics alone is sufficient to reveal seizure-induced changes in the expression patterns of several genes, interactions among their gene products (proteins, e.g. receptor subunits and enzymes) and interactions among the enzyme products (e.g. prostaglandins) or even their by-products and reaction products (e.g. superoxide, NO and peroxynitrite). To gain insight into the complex *in vivo* interactions, animal experiments are essential. The complexity of these changes points to limitations of experimental gene therapy,[55] because enhancing the expression of genes that contribute to the production of increased amounts of inhibitory neurotransmitters, for example, may reduce the severity of the acute symptoms but still be far from curing the disease and its progression. ■

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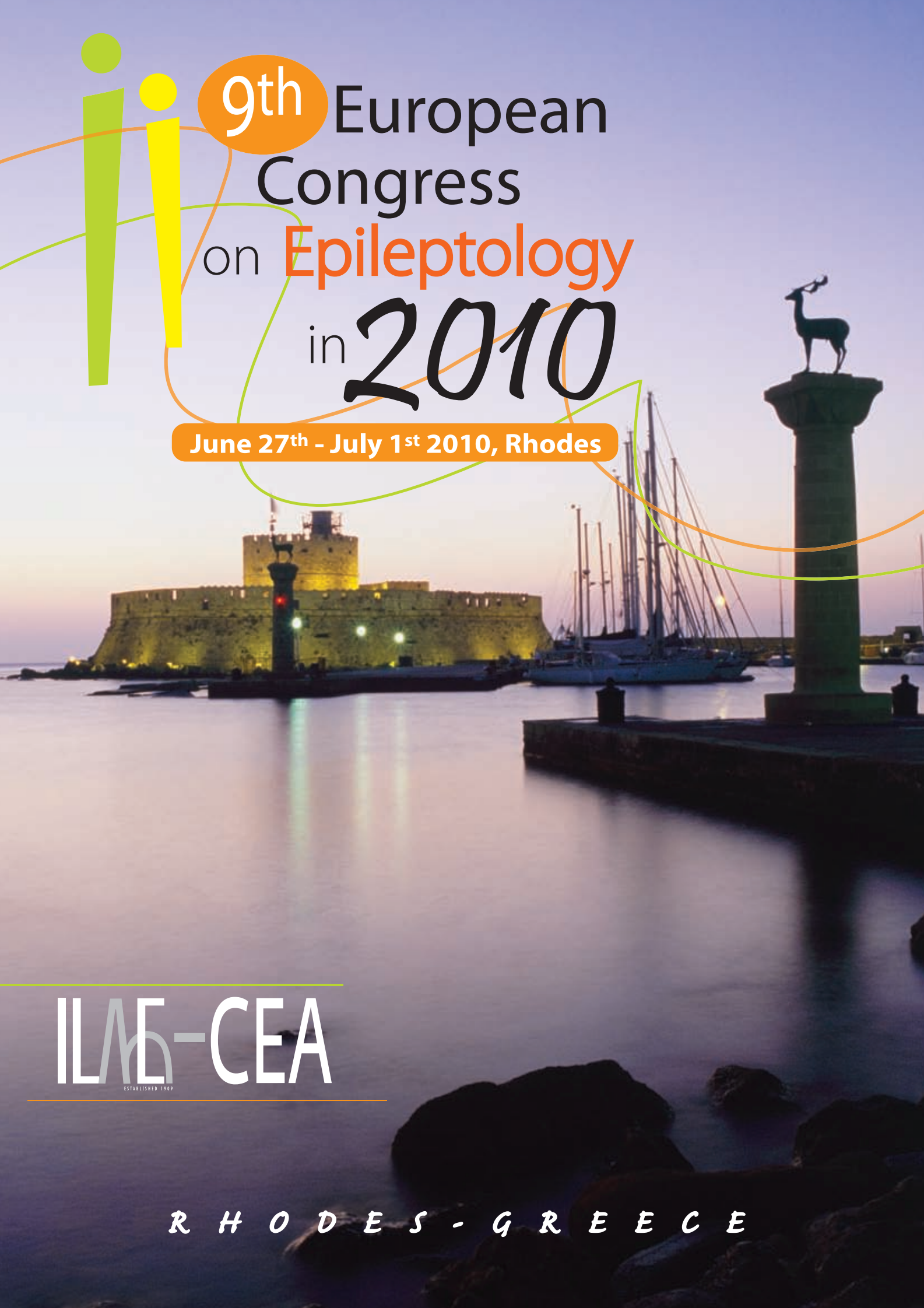
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