Generation of spike wave discharges in absence epilepsy:
Modulatory effects of serotonin, glutamate and glycine

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Ph.D. thesis

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TABLE OF CONTENTS

ABBREVIATIONS........................................................................................................5

SUMMARY (Hungarian) Összefoglalás.................................................................7

SUMMARY ..............................................................................................................9

INTRODUCTION........................................................................................................11
On epilepsy in general.................................................................................................11
.....and absence epilepsy in particular....................................................................12
Behavioral characteristics.......................................................................................13
Electroencephalographic characteristics..............................................................13
Anatomical origin – the role of thalamo-cortical circuitry......................................14
Spike wave discharges (SWDs): pathophysiology.................................................17
Factors influencing SWD activity..........................................................................17
Mechanisms mediated by neurotransmitter systems in SWDs............................18
GABA–mediated mechanisms in SWDs.................................................................18
Excitatory amino acid-mediated mechanisms in SWDs.......................................20
Monoaminergic mechanisms in SWDs.................................................................21
Serotonergic mechanisms in SWDs......................................................................22
Cholinergic mechanisms in SWDs........................................................................25
Pharmacological control of SWDs.........................................................................26
The role of vigilance in SWDs..............................................................................26
WAG/Rij rat model for absence epilepsy...............................................................27

AIMS OF THE THESIS...........................................................................................30

What is the role of 5-HT_{2C} receptors in seizure generation in WAG/Rij mode?..30
What is the role of 5-HT_{7} receptors in seizure generation in WAG/Rij model?..31
What are the effects of AMPA receptor modulators in WAG/Rij rats on SWD, vigilance and behavior? ..........................................................31

How do the glycine transporter-1 inhibitors influence SWDs and vigilance in WAG/Rij model of absence epilepsy? ..........................................................31

METHODS.................................................................................................33

Animals and surgery..................................................................................33
Electrophysiological recordings.................................................................34
Drugs........................................................................................................35
Statistical analysis.....................................................................................36
Protocols of Experiments (1-4).................................................................36

RESULTS..................................................................................................39

Effects of the 5-HT2C receptor agonist mCPP, the selective 5-HT2C receptor antagonist SB-242084, the SSRI citalopram and their combinations on the number and cumulative duration of SWDs. (Experiment 1)..............................................39

Effect of the selective 5-HT7 antagonist SB-258719 on epileptic activity in the WAG/Rij rat model of absence epilepsy. (Experiment 2).......................................................41

Effect of GYKI 52466 and GYKI 53405 on vigilance and behaviour (Experiment 3.1).42

Effects of two non-competitive AMPA receptor antagonists, GYKI 52466 and GYKI 53405 on number and duration of SWDs. (Experiment 3.2).............................................. 44

Influence of two glycine transporter-1 inhibitors (NFPS and Org 24461) on SWDs and vigilance. (Experiment 4).................................................................48

DISCUSSION..........................................................................................54

CONCLUSION.........................................................................................64
ACKNOWLEDGEMENTS

REFERENCES

FIGURE LEGENDS

BIBLIOGRAPHY

ORIGINAL PUBLICATIONS
Abbreviations:

AED: Antiepileptic drug
AMPA: α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AW: active wakefulness
CBZ: Carbamazepine
CLB: Clobazam
CSF: Cerebrospinal fluid
CxTh: Cortico-thalamic
DOI: 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
ECG: Electrocardiography
EEG: Electroencephalography
EMG: Electromyography
ETS: Ethouximide
FGPE: Feline generalized penicillin epilepsy
GABA: γ-aminobutyrate
GAERS: Genetic Absence Epilepsy Rats of Strasbourg
GEPR: Genetically Epilepsy-prone Rats
GDA: glycyldodecylamide
GlyT: glycine transporters
GHB: γ-hydroxybutyrate
GMA: Generalized seizures on awakening
GTC: Generalized tonic clonic seizures
GYKI 52466: 1-[4-aminophenyl]-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine
GYKI 53405: 7-acetyl-5-(4-aminophenyl)-8-methyl-8,9-dihydro-7H-1,3-dioxolo[4,5-b][2,3]benzodiazepine
i.c.v.: Intracerebroventricularly
i.p.: Intraperitoneally
IGE: Idiopathic Generalized Epilepsy
IPSPs: Inhibitory postsynaptic potentials
JAE: Juvenile Absence Epilepsy
LTG: Lamotrigine
mCPP: meta-chlorophenylpiperazine HCl
MPFC: Medial prefrontal cortex
MK-801: (+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5,10-imine maleate
NAN-190: 1-(2-Methoxyphenyl)-4-[4-2(2-phthalimido)butyl]piperazine
NFPS: N[3-(4’-fluorophenyl)-3-(4’-phenylphenoxy)-propyl]sarcosine
NMDA: N-methyl-D-aspartate
NREM: Non rapid eye movement
Org 24461: R,S- (+/−)N-[(4-trifluoromethyl)phenoxy]-3-phenyl-propylglycine
PHN: Phenytoine
PW: passive wakefulness
REM: Rapid eye movement
RTN: Reticular Thalamic Nucleus
SB-242084: 6-chloro-5-methyl-1-[(2-[2-methylpyrid-3-yloxy]pyrid-5-yl)carbamoyl]indoline dihydrochloride
SB-258719: (R)-3,N-Dimethyl-N-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzenesulfonamide
s.c.: Subcutaneously
SD: Sleep deprivation
SWD: Spike wave discharge
SWS1: Light slow wave sleep
SWS2: Deep slow wave sleep
TCR: Thalamo-cortical
THM: Tüske-hulláminta
VEH: vehicle
VLP: Valproic acid
WAG/Rij: Wistar albino Glaxo strain, bred in Rijswijk
WAY-100635: N-[2-]4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl
cyclohexanecarboxamide maleate
5-HT: Serotonin
8-OH-DPAT: 8-hydroxy-2-(di-n-propylamino)-tetralin hydrobromide
A tüskehullám-mintát befolyásoló neurotranszmitterek vizsgálata absence epilepsziában: A szerotonin, a glutamát és a glicin szerepe a tüskehullám-minta kialakulásában.

Az epilepsziás szindrómák közül az idiopátiás generalizált epilepsziák (IGE) nemcsak a rohamforma és az EEG jelenségek, hanem neurofiziológiai és farmakológiai sajátosságaiak alapján is önálló csoportot alkotnak. Jelenlegi ismereteink alapján az elektroencefalográfia felvételen - mindkét agyi féltete felett jelentkező szinkronizált, generalizált tüskehullám-minta (THM) kisülések thalamocorticalis eredetűek. A thalamocorticalis rendszer működésével kapcsolatban számtalan új ismeretre derült fény az utóbbi évtizedben, egyre többet tudunk a thalamikus eredetű ritmikusan alternáló izgalom-gátlás szekvenciák, az alvási orsók és a THM kapcsolatáról. Valószínűsíthető, hogy az IGE kialakulásában meghatározó szerepet játszik a T-típusú Ca\(^{2+}\) csatornák genetikusan determinált eltérése. Ezen ioncsatorna-rendellenesség az NMDA által közvetített fokozott izgalmon és a GABA által mediált gátlás csökkenésén keresztül, a thalamocorticalis rendszer szinkronizált “burst”-ől izgalmai állapotát eredményezi, ami a THM kialakulásához vezet. Más vizsgálatokban kimutatták, hogy a különböző gátló és serkentő neurotranszmitterek, amelyek a thalamocorticalis rendszer működését befolyásolják, a THM előfordulására is hatással bírnak. Vizsgálataink célja a THM-t befolyásoló neurotranszmitterekről ill. Receptorakról eddig megszerzett ismereteink bővítése mellett újabb terápiás lehetőségek keresése volt.

Dolgozatomban két szerotonin-receptor, az 5-HT\(_{2C}\)- és az 5-HT\(_{7}\)-receptor, valamint az AMPA-receptor és a glicin-transzporter-1 szerepét vizsgáltam az absence epilepszia genetikai állatmodelljében szelektív farmakológiai eszközökkel. Kimutattuk, hogy a már ismert GABAerg, glutamáterg és dopaminerg neurotranszmitter-rendszerek mellett a szerotoninerg rendszerek is jelentős hatása van a THM kisülések gyakoriságára. Eredményeinket röviden úgy összegezhetjük, hogy a szerotonin absence epilepsziában kettős hatással bír: az 5-HT\(_{2C}\) receptor agonistával történő aktiválása vagy az extracelluláris szerotoninszint növelése csökkenti a THM előfordulását. Ezzel szemben,
az 5-HT₁₅-receptor agonista vegyületek vagy az extracelluláris szerotoninkoncentráció növelése fokozza a THM-t. A szerotonerg neurotranszmisszió befolyásolásával a THM gyakoriságában létrehozott változások az alvás-ébrenlétére ható változásoktól függetlenek voltak. Ma már jól ismert, hogy az 5-HT₁₅-receptorok mellett a 5-HT₇-receptorok jelentős mennyiségben találhatók a thalamusban is. Az 5-HT₇-receptor szelektív antagonistájával végzett kísérleteink megerősítették hipotézisünket, mely szerint az 5-HT₇-receptorok befolyásolják a THM-t WAG/Rij állatokban: a szelektív 5-HT₇-receptor antagonista szignifikánsan csökkentette a paroxizmusok számát, azok összesített, valamint átlagos időtartamát.

AMPA-receptor antagonistákkal végzett állatkísérletes adatok alapján az AMPA receptorok igéretes célpontnak tűnnek az epilepszia gyógyszeres kezelésében. Eredményeink azonban megkérdőjelezik az AMPA receptorok jelentőségét a THM kialakulásában, és jelzik, hogy a hatás egy része a vigilanciára gyakorolt hatáson keresztül valósul meg az általunk vizsgált modellben. Ehhez hasonlóan, az általunk vizsgált két glicin-transzporter-1 gátló vegyület, csak a legmagasabb dózisában bizonyult átmenetileg hatásosnak, és ez az átmeneti hatás is egyértelműen a vigilanciára gyakorolt hatással függött össze. Eredményeink alapján megállapíthatjuk, hogy az AMPA-receptorok más típusú epilepsziákban játszhatnak szerepet, és ehhez hasonlóan a glicin-transzporter gátlók sem fejtenek ki markáns hatást absence epilepsziában.
SUMMARY

Generation of spike wave discharges in absence epilepsy:
Modulatory effects of serotonin, glutamate and glycine

Idiopathic generalized epilepsies are a developmentally, neurophysiologically and pharmacologically unique group of epilepsy syndromes. Evidence suggests that the basic mechanisms underlying the bilaterally synchronous spike-wave discharge (SWD) burst, a common electrophysiological (EEG) marker that characterizes these seizure types, are related to the thalamo-cortical (TCR) network. In the last decade, the thalamic rhythmogenic mechanisms responsible for sleep spindles and SWDs have been intensively investigated leading to a better understanding of their anatomical-physiological substrate. It has been shown that the aberrant developmental shift in the balance of NMDA-mediated excitation and/or GABAergic-mediated inhibition turns the TCR circuitry in favour of synchronization, “burst firing” mode and consequently to SWD. Further studies investigating the neurotransmitters and their receptors that modulate excitation and inhibition in TCR pathways may help to understand the control mechanisms of the SWD expression.

In the thesis we examined the role of two 5-HT receptors, 5-HT$_{2C}$ and 5-HT$_{7}$, AMPA receptors and glycine-transporter-1 in the generation of SWDs in the accepted rat model of human absence epilepsy. The results provide further evidence that in addition to the known GABAergic, glutamatergic and dopaminergic control, serotonergic mechanisms also play an important role in the triggering and maintenance of epileptic activity, demonstrating that serotonin play a dual effect in absence epilepsy: activation of 5-HT$_{2C}$ receptors by receptor agonists or by increase in endogenous 5-HT concentration inhibit SWD, although this inhibitory effect is not significant at basal 5-HT tone. In contrast, activation of 5-HT$_{1A}$ receptors by receptor agonists or increase in endogenous 5-HT concentration promotes SWD. Changes caused in the SWD activity were independent of the effects on vigilance and sleep. Since the selective 5-HT$_{7}$ receptor antagonist reduced the cumulative duration of SWDs, as well as the number and average duration of paroxysms compared to vehicle in the WAG/Rij rat model of absence epilepsy, our
results strengthened the hypothesis that 5-HT\textsubscript{7} receptors, which density is relatively high within the thalamus, has influence on SWD in WAG/Rij rats. Studies with selective AMPA receptor antagonists in animal seizure models have indicated that AMPA receptors are potentially promising anticonvulsant drug targets. However, our results may raise doubts about the strong involvement of AMPA receptors in generating SWD, and may indicate that part of the effect of AMPA receptor antagonists in regulation of epileptic activity occur through vigilance effects in this model. Similarly, the examined glycin-transporter-1 inhibitors caused a transient antiepileptic activity only at the highest dose, what was clearly a result of their effects on vigilance. Our results suggest that AMPA modulators may have a therapeutic action in other types of epilepsies and glycin-transporter-1 inhibitors have no significant effects in absence seizures.

Bibliography*


*Only the author’s original publications related to the subject of the Ph.D. thesis are listed here.*
Introduction

On epilepsy in general...

The epilepsies represent a heterogeneous group of disorders with diverse aetiologies, electrographical and behavioural seizure patterns, and pharmacological sensitivities. Although epileptic syndromes and their causes are diverse, the cellular mechanisms of seizure generation appear to fall into only two categories: rhythmic or tonic "runaway" excitation or the synchronized and rhythmic interplay between excitatory and inhibitory neurons and membrane conductance.

Various studies report the prevalence of epilepsy as 5 to 8 in 1000. The risk of epilepsy from birth through age 20 years is approximately 1% and reaches 3% at age 75 (3). The concept of epileptic syndromes was first considered in 1985 (77). In 2001, the International League Against Epilepsy (ILAE) proposed a revised classification of epilepsies and epileptic syndromes to replace the 1989 revision of the Classification of Epilepsies and Epileptic Syndromes of the ILAE (23, 51, 78). The International Classification of Epilepsies begins by dividing epilepsies according to the overall seizure type: partial (focal, localization-related, local) and generalized. Epilepsies are next divided according to the aetiology: idiopathic, symptomatic or familial. There are also three special categories: reflex epilepsies, epileptic encephalopathies and progressive myoclonic epilepsies. Finally, there is a list of conditions that may result in seizures but that are not likely to result in epilepsy, chronic unprovoked seizures. An epileptic syndrome is characterized by exact clinical and EEG manifestations. The same type of seizures may occur in different syndromes, but different types of seizures can also belong to the same syndrome. This concept of epileptic syndromes helps in selecting the appropriate investigations, deciding on optimal antiepileptic treatment, predicting the outcome, has value regarding the pathogenesis of epilepsy and finally this concept of epileptic syndromes is useful in research and comparative studies.
...and absence epilepsy in particular

Idiopathic generalized epilepsies (IGE) (Table 1.) are age-related, have well defined EEG characteristics, affected subjects have no associated intellectual or neurological deficits. The genetic bases of the idiopathic generalized epilepsies are increasingly established. The syndrome of juvenile myoclonic epilepsy was found to be linked to chromosome 6p and 15q14 (155). An allelic association of juvenile absence epilepsies with kainate-selective GluR5 receptor on chromosome 21q22.1 has been reported. Linkage to another locus, 8q24, has recently been confirmed in a large family with absence epilepsy and generalized tonic-clonic seizures (155).

III. Idiopathic generalized epilepsies
   A. **Childhood absence epilepsy** (ABS, TCS)
   B. **Juvenile absence epilepsy** (ABS, TCS)
   C. **Juvenile myoclonic epilepsy** (MYO, TCS, ABS)
   D. Epilepsy with tonic-clonic seizures on awakening (TCS)
   E. Epilepsy with random tonic-clonic seizures (TCS)
   F. Epilepsy with myoclonic astatic seizures (MYO, ATO)

Table 1. Generalized Epilepsies according to Classification and Terminology of the ILAE 2001.
ABS: absence seizure; ATO: atonic seizure; MYO: myoclonic seizure; TCS: tonic-clonic seizure

I. Typical absence seizures
   A. Simple-impairment of consciousness only
   B. Complex
      1. With mild clonic components
      2. With changes in tone
      3. With automatisms
      4. With autonomic components

II. Atypical absence seizures

III. Absence status epilepticus

Table 2. Classification of absence seizures according to Classification and Terminology of the ILAE 2001.
**Behavioural characteristics**

In childhood absence epilepsy (Table 2.), the frequency of absence seizures is high, with seizures occurring up to several hundred times per day. The hallmark of the typical absence seizure is the suppression of mental function, usually to the point of complete abolition of awareness, responsiveness, and memory. The seizure start abruptly, without an aura, and typically last from a few seconds to half a minute, although, at times, they last more than 1 minute. In a simple typical absence seizure, the child stares with a motionless, distant appearance. At the end of the seizure the child usually returns to the gesture, sentence, or other activity that the seizure interrupted. The condition appears to be inherited in an autosomal dominant pattern with incomplete penetrance. In juvenile absence epilepsy, the seizure frequency is much lower. In juvenile myoclonic epilepsy, myoclonic and generalized tonic-clonic seizures are also seen, and for children with the syndrome to have many absence seizures is unusual. Childhood absence epilepsy usually begins between age of 3 years and puberty; juvenile absence epilepsy and myoclonic epilepsy begin during or after puberty. Although generalized tonic-clonic convulsions can occur in both syndromes, the incidence is higher in children with juvenile absence seizures than with the childhood form (23, 68).

**Electroencephalographic characteristics**

A hallmark of absence seizure is the sudden onset of either generalized symmetric spike-wave or multiple spike-and-slow-wave complexes. In typical absence seizures, the spike-and-slow wave complexes usually occur at a frequency of 3 Hz (range 2.5 to 3.5 Hz) (Figure 1.)
Over the last decade there have been significant advances in the understanding of the neurophysiological, anatomical and pharmacological mechanisms that underlie generalized epilepsies at cellular, neuronal network and whole brain levels (133, 136, 142, 143). Both human and animal data strongly suggest that generalized epilepsy, SWDs arise from aberrant thalamo-cortical rhythms. To understand the cellular basis of the thalamo-cortical rhythms generating the SWDs, it is necessary first to briefly review the functional aspects of this circuit, of the unique oscillatory thalamo-cortical rhythms generated by this circuitry, the cellular mechanisms underlying these neural oscillations and the involved neurotransmitters.

**Anatomical origin - the role of thalamo-cortical circuitry - functional aspects**

The thalamus constitutes the major relay information for sensory information to the cortex, except for olfaction. Thalamic neurons send an ordered atonal projection principally to layers III/IV and V/VI of the neocortex, synapsing on both pyramidal neurons and interneurons. In turn, thalamic neurons receive a feedback projection from layer VI neurons in the same cortical area to which they project. In addition, both the thalamo-cortical (TCR) and cortico-thalamic (CxTh) projections send axon collaterals to the reticular thalamic nucleus (RTN), which is an entirely GABA-ergic nucleus at ventral
posterolateral side of the thalamus. RTN provides a strong inhibitory innervation to the thalamus (5, 39, 141) (Fig.2.). The world of the thalamus is not to be regarded merely as a set of nuclei that relay afferent impulses en route to the cerebral cortex. Rather, it should be viewed as an unifying entity that operates as the ultimate gate master and can, in fact, conjure from the intrinsic properties of neurons the resting and active states of the brain (61, 63, 139, 140).

Figure 2. The basic thalamo-cortical circuit. (RTN-Reticular Thalamic Nucleus, TCR-Thalamo-cortical Relay Cell, CxTh-Cortico-thalamic cell). Schematic diagram of thalamo-cortical circuitry showing the relationship between neurons in the RTN, TCR and CxTh neurons in layer VI of the cerebral cortex. The figure illustrates the reciprocal excitation between TCR and cortical neurons (I), recurrent inhibition between RTN neurons (II), reciprocal inhibition between TCR and RTN neurons (III), and parallel excitation between CxTh neurons and RTN and TCR (IV). (Modification of a figure published by O. Carter Snead III. M.D., 1995)

Thalamic neurons are endowed with an ensemble of voltage-dependent ionic conductances, which are activated and inactivated by changes in membrane potential overlapping with the normal range of potentials occurring during the resting state of these cells. These conductances play a significant role in generating normal cellular behaviour in these neurons. The role of these conductances is particularly notable in the generation of the state-dependent changes in behaviour evident in thalamic neurons. In the waking state, thalamic neurons faithfully transduce information and relay it from the periphery to the cortex. Falling asleep, thalamic neurons hyperpolarize, and a portion of the
conductances which are inactivated at normal “awake” membrane potentials “de-
activate”, and alter the behaviour of thalamic neurons, and of the system as a whole (40,
88, 139, 142, 144). The system becomes generally unresponsive to external input. It tends
to oscillate spontaneously, generating slow sleep rhythms such as spindle waves. The
thalamic neurons tend to shift their action potential firing mode from single-spike tonic
firing mode, characterized by desynchronized EEG activity, corresponding to waking state
to a phasic, oscillatory, rhythmic burst firing mode, characterized by synchronized EEG
activity, corresponding to sleep state. The uniqueness of this situation relates to the
circuitry of the GABA-ergic and glutamatergic neurons in the thalamus and cortex, rather
than the intrinsic ability of the neurons themselves to oscillate (136). The cellular event
that underlies the ability of RTN neurons to shift between tonic and burst firing mode is
the low-threshold calcium current. This event is triggered via GABA-B-mediated late
inhibitory postsynaptic potentials (IPSPs). These low threshold Ca²⁺ potentials represent a
key membrane property involved in burst firing excitation and are associated with the
oscillatory activity observed in the thalamo-cortical cells during synchronized sleep.

In summary there are three main elements responsible for switching from the tonic
to oscillatory modes. 1) The intrinsic membrane properties of the thalamic neurons: the
non inactivating Na⁺ conductance, the low-threshold somatic Ca²⁺ spike, the high-
threshold dendritic Ca²⁺ conductance, and a series of voltage- and Ca²⁺-dependent K⁺
currents. 2) The synaptic networks that galvanize these neuronal elements into active
groups, the depolarizing oscillations in GABA-ergic reticular thalamic neurons that
generate cyclic hyperpolarization-rebound sequences in thalamo-cortical neurons. The
disappearance of spindling in thalamic nuclei deprived of connections from the reticular
nucleus and the preservation of spindle oscillations in the reticular nucleus deafferented
from its input sources indicate that this thalamic nucleus is the pacemaker of spindle
activity. 3) The ascending systems from the brain stem that modulate, in a gentle or
startling manner, the intrinsic state of these gatemasters. These ascending systems can
gently lull one to sleep or brutally discharge one from sleep in the presence of danger.
Stimulation of cholinergic pathways originating in rostral brain stem reticular neurons,
leads to suppression of synchronized oscillations in both the relay and reticular thalamic
cells (140, 141).
**Spike wave discharges (SWDs): pathophysiology**

Absence epilepsy is characterized by the spontaneous occurrence of bilateral synchronous spike-wave discharges that involve the entire cortical mantle (36, 122, 136, 159). Thalamus, as a pacemaker structure for the rhythmic cortical oscillations very likely represents the primary neuronal dysfunction underlying the generation of spike-wave discharges (6, 36, 102, 122, 143). Within the thalamus, sleep spindles are generated as a recurrent interaction between thalamocortical and thalamic reticular cells (140). It has been suggested based on the resemblance in the EEG and the similar circadian pattern that spike-wave discharges are modified sleep spindles (102, 140, 159). Sleep spindles and other rhythmic oscillations (e.g. delta waves) are produced by thalamic neurons by their ability to spontaneously generate rhythmic bursts of action potentials due to the interaction of the \( \text{Ca}^{2+} \) current \( I_T \) and the inward “pacemaker” current \( I_h \). The amplitude, or voltage sensitivity of \( I_h \) adjusts the rate at which the thalamic cells oscillate and this sensitivity is adjusted by the release of modulatory neurotransmitters (103).

**Factors influencing SWD activity**

Seizures may occur frequently or infrequently, only at night or after awakening, in a cyclic pattern suggesting vigilance dependence, or without any apparent predictability. Factors (Fig. 3.) that precipitate seizure occurrence are at present poorly understood, but clinical observations and experimental data indicate that specific mechanisms, environmental and physiological factors modulate the probability of seizure occurrence in IGE. It is not known, however, how these perturbations are translated into increased epileptic susceptibility at the cellular level. Changes that alter neuronal excitability or the potential for synchronous interaction among neurons are undoubtedly important but remain to be specified in detail.
Mechanisms mediated by neurotransmitter systems in SWDs:

External factors that play a significant role in thalamo-cortical circuitry and SWD generation are glutamatergic, gabaergic, cholinergic, dopaminergic, noradrenergic and serotonergic mechanisms (6, 44, 136, 143) (Fig. 3).

GABA-mediated mechanisms in SWDs

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the CNS. It is synthesized from glutamic acid by the enzyme glutamic acid decarboxylase and is degraded by GABA-transaminase and is also taken up by a reuptake process. The GABA receptor complex consists of pentamer (5 peptides) arranged around the chloride ionophore channels. When GABA contacts the receptor, there is an opening of the chloride channel and Cl⁻ moves along its electrochemical gradient to enter the cell. This results in a more positive membrane potential (hyperpolarization), inhibiting action potential generation. Two main varieties of subreceptors have been identified: GABA_A
and GABA$_B$. The former is a ligand Cl$^-$-gated ion channel, the latter is associated with K$^+$, Ca$^{2+}$ channels and regulated by G proteins. There are at least 5 different subunit families ($\alpha, \beta, \gamma, \delta, \sigma$), giving rise to a very large number of potential GABA$_A$ receptor types. Specific binding sites, distinct from the GABA binding site, have been identified on the GABA receptor complex for benzodiazepines, barbiturates, picrotoxin and certain steroids. GABA$_B$ receptors located postsynaptically on pyramidal cell dendrites may inhibit NMDA function by virtue of the GABA-induced hyperpolarization, thus decreasing cell excitability. GABA$_B$ receptor on inhibitory interneuron somata or on inhibitory interneuron terminals may reduce the amount of GABA released from the interneuron, leading to increased cell excitability.

![Figure 4](image-url)

Enhancement of GABA-ergic inhibition in the brain potentiates clinical and all experimental forms of generalized absence (136, 146). In genetic animal models of absence epilepsy GABA$_A$ receptor agonist muscimol caused dose-dependent increase in SWDs, GABA$_A$ receptor antagonist bicuculline inhibited SWDs (97, 158). Acute i.p.
administration of indirect GABA<sub>A</sub> receptor agonist diazepam dose-dependently decreased SWD activity in WAG/Rij rats (117). Over the last few years a significant amount of research in genetic animal models has focused on the role of the inhibitory GABA<sub>B</sub> receptors. In various rodent genetic models expressing absence, systemic administration of GABA<sub>B</sub> antagonists had a strong anticonvulsant effect, whereas localized perfusion of GABA<sub>B</sub> agonists into the thalamus has a marked proconvulsant effect in these animals. Initial studies provided evidence that the GABA<sub>B</sub> receptor was capable of generating the low threshold calcium spike required for initiation of the burst firing, leading researchers to hypothesize that the GABA<sub>B</sub> receptors played a significant role in these seizures. Subsequent research took advantage of the new generation of GABA<sub>B</sub> antagonists that became available in the early 1990s and demonstrated that in a number of models the seizures could be eliminated by the administration of one of these compounds (39, 40). GABA<sub>B</sub> agonist baclofen exacerbated SWDs in the GHB models of absence epilepsy (136). GABA<sub>B</sub> antagonist GCP 35348 was shown either to attenuate or block SWDs in the GAERS (97). Animal model data suggest the role of GABA<sub>B</sub>-mediated IPSP in regulating thalamo-cortical oscillatory behavior via low threshold calcium currents.

**Excitatory amino acid-mediated mechanisms in SWDs**

An integral part of the modulation of thalamo-cortical rhythmicity is effected through glutamate-mediated, recurrent excitation between thalamo-cortical and cortico-thalamic pathways, both of which project excitatory axon collaterals to the RTN (Fig. 1.). Glutamate activates at least four different classes of receptors, named for their prototypic pharmacological agonist: 1. NMDA – (N-methyl-D-aspartate), 2. AMPA – mainly responsible for excitatory glutamatergic transmission, 3. Kainate – excited by the glutamate analogue kainic acid, 4. Metabotropic – coupled to G proteins. AMPA and NMDA receptors have specific agonists and antagonists thus allowing specific pharmacological manipulation (37). Recent experiments demonstrated that pharmacological manipulation of NMDA-mediated excitation results in a profound effect on SWD duration in a number of experimental models of generalized absence seizures. In the GHB model of absence seizures and in the GAERS (4, 97), administration of either
NMDA agonists or antagonists resulted in attenuation of SWD duration. In the WAG/Rij genetic model of absence epilepsy, the NMDA receptor antagonist MK-801 reduced SWD activity (54, 119). NMDA receptors are more abundant in the cerebral cortex of rats with absence-like seizures than in the cortex of control rats (126). Local application of NMDA antagonist into the reticular nucleus of the thalamus failed to disrupt the generation of SWDs (13, 136). Authors of other studies drew a similar conclusion (85, 108, 94). These findings point to the role of the NMDA-mediated activation of SWDs at cortical level (13, 136). Considering the ubiquity the of AMPA receptors in sensory thalamo-cortical cells, the involvement of sensory relay nuclei in this type of epilepsy, a stronger effect of the AMPA receptor antagonists would be suggested. Although, Peeters et al. (118) demonstrated that high doses of intracerebroventricularly administered AMPA increase the amount of SWD in this model, AMPA receptors had not been long considered seriously as a potential targets for antiepileptic drugs.

The AMPA receptor is a member of the ion channel family of the glutamate receptors (GluR). It is a hetero-oligomer formed from GluR1, GluR2, GluR3, GluR4 subunits (89). Two classes of antagonists that selectively block AMPA and not other excitatory amino acid receptors have been extensively studied: (a) quinoxalinedione (and structurally related nonquinoxalinedione) competitive antagonists that bind to the agonist (AMPA) recognition site on AMPA receptors and (b) 2,3-benzodiazepine non-competitive antagonists that bind to a distinct allosteric regulatory site (131). Both types of antagonist are effective in vivo in the maximal electroshock test (166) and against reflex (audiogenic) seizures (142), various chemoconvulsant seizures (166) and kindled seizures (49).

**Monoaminergic mechanisms in SWDs**

There is some evidence that noradrenergic neurotransmission may participate in the control of generalized absence seizure activity. Drugs that decrease α-noradrenergic neurotransmission such as the α1–antagonist prazosin or the α2–agonist, clonidine, exacerbate experimental absence seizures while pharmacological manipulation that increases α-noradrenergic neurotransmission (α1–agonists, α2–antagonists) reduces SWD
duration in experimental models of absence epilepsy. Drugs that influence β-noradrenergic neurotransmission seem to be ineffective in experimental absence seizures (107). Dopaminergic pathways may also be operative in the control of generalized absence seizures. Mixed dopaminergic D1/D2 agonists such as L-dopa, apomorphine, and amphetamine, result in a dose-dependent reduction of SWD duration in experimental models, while mixed dopaminergic D1/D2 antagonists (haloperidol, flupentixol) exacerbate experimental absence seizures (163). The mechanism by which the dopaminergic pathway interacts with thalamo-cortical circuitry is not clear (136).

**Serotonergic mechanisms in SWDs**

While serotonin has some role in the peripheral neuron systems, e.g. in the gut, cardiovascular system, the majority of its action is in the central nervous system (7, 9, 10, 11). Serotonin (5-hydroxytriptamine, 5-HT) neurons in the raphe nuclei give rise to collateralized projections that provide potent modulatory input into most networks throughout the central nervous system (7, 132). 5-HT has been implicated in the aetiology of many diseases, but is particularly important in several neurologic and mental illness, such as depression, anxiety, schizophrenia, migraine, panic disorder, obsessive compulsive disorder. 5-HT has a significant role in further brain functions like arousal and sleep, modulates release of stress-related neuropeptides and hormones, and regulates appetite (8, 7, 110).

There is growing evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures and involved in the enhanced seizure susceptibility observed in some genetically epilepsy prone rodents (24, 41, 54, 58, 74, 86, 127, 132, 134). While, Marescaux C. et al. failed to show major involvement of serotoninergic neurons in the pathogenesis of generalized absence seizures in genetic absence epilepsy model rats from Strasbourg (GAERS) (97). Studies investigating the role of different serotonin agents, and subtypes in various rat epilepsy models showed different, sometimes contradictory results or failed to show involvement of the serotoninergic system in SWD activity modulation. Generally, agents that elevate extracellular serotonin (5-HT) levels, such as 5-hydroxytryptophan and 5-HT reuptake
blockers, inhibit both limbic and generalized seizures (43, 91, 96, 124, 165). In hippocampal pyramidal cells high K(+)‐induced seizure‐like activity was inhibited by fluoxetine (41). Conversely, depletion of brain 5-HT lowers the threshold to audiogenically, chemically and electrically evoked convulsion (24, 43, 138).

5-HT effects are mediated by multiple receptor subtypes with distinct regional distribution patterns, pre- and postsynaptic localization, receptor structure and transduction system. A number of serotonin receptor subtypes have been characterized pharmacologically using selective radioligands (14, 75, 120, 121). Thus far, the brain is known to contain more than 14 types of 5-HT receptors which differ in structure, function and anatomical distribution (71, 75). Based on current, most widely accepted classification, 5-HT receptors are divided into 7 types (families). Definitive characterization of 5-HT receptors in relation of drug action, structural and transductional term of approach are indicated in Table 3.

<table>
<thead>
<tr>
<th>5-HT Receptor (family) type and subtypes</th>
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<tbody>
<tr>
<td>5HT_{1A}</td>
</tr>
<tr>
<td>5HT_{1B(Dβ)}</td>
</tr>
<tr>
<td>5HT_{1E}</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Superfamily: Transductional characteristics</th>
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<tbody>
<tr>
<td>G protein linkage</td>
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<tr>
<th>Intracellular Response</th>
</tr>
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<tbody>
<tr>
<td>Adenylyl cyclase ↓</td>
</tr>
<tr>
<td>IP₃/DG↑</td>
</tr>
</tbody>
</table>
Table 3. Classification of 5-HT receptors (in brackets the previous name of the receptor, prior to the classification)

Serotonin receptor types have a distinct and heterogeneous distribution in the brain. Highest densities of the 5-HT$_{1A}$ receptor were found in the hippocampus, neocortex and raphe nuclei (112, 138). Somatodendritic 5-HT$_{1A}$ autoreceptors in the raphe nuclei cause reduction in 5-HT synthesis and release, while postsynaptic 5-HT$_{1A}$ receptors in the hippocampus, neocortex or other brain structures cause neuronal hyperpolarization (71, 75). Several knock out mouse models suggest a relation between 5-HT, hippocampal dysfunction, and epilepsy. 5-HT$_{1A}$ knock out mice display lower seizure thresholds and higher lethality in response to kainic acid administration (114). Administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT causes marked, dose-dependent increases in the number and mean cumulative duration of spike-wave discharges in WAG/Rij rats (54, 58). However, the antagonism of 5-HT$_{1A}$ receptors may increase or augment seizure severity in rats with stage 1 kindled seizures (164), and activation of 5-HT$_{1A}$ receptors can retard the development of amygdaloid kindling (161).

Activation of 5-HT$_{2}$ receptors causes, in general opposite effects as 5-HT$_{1A}$ receptors. For example, 5-HT$_{2}$ receptors play a facilitatory role in the developmental seizure process in amygdaloid kindling (161). Extracellular and intracellular recordings revealed that 5-HT-induced inhibition of burst firing in nucleus reticularis thalami (NRT) is mediated through 5-HT$_{2}$ receptors with possible involvement of 5-HT$_{1C}$ (currently 5-HT$_{2C}$) receptors (100). Other experiments using pharmacological probes have also implicated 5-HT$_{2}$ receptors in the development of amygdala-kindled limbic seizures (161) and the expression of electrically induced generalized seizures (128) in rodents. The possible involvement of 5-HT$_{2C}$ receptors has been suggested by the finding that mutant mice lacking this receptor subtype are extremely susceptible to audiogenic seizures and are prone to spontaneous death from seizures, suggesting that serotonergic neurotransmission mediated by 5-HT$_{2C}$ receptors suppresses neuronal network hyperexcitability and seizure activity (22, 151). In addition, studies investigating the role of 5-HT$_{2C}$ and 5-HT$_{2B}$ receptors in the generation of pentylentetrazol and electroshock-evoked seizures in rodents revealed that the observed anticonvulsant effects of mCPP are likely to be mediated by activation of 5-HT$_{2C}$ receptors in these models (156). In contrast,
the results of a study of Watanabe coworkers (164) obtained in rats with stage 1 kindled hippocampal seizures suggest that the antagonism of 5-HT$_{2B,C}$ do not lower or raise seizure threshold. 5-HT$_7$ receptor expression is relatively high within the thalamus(14, 157), which brain region most probably represents the primary neuronal dysfunction underlying the generation of spike-wave discharges (36, 102, 122, 140). Furthermore, the role of both 5-HT$_{1A}$ and 5-HT$_7$ receptors has been suggested in serotonin-induced responses in thalamic neurons (26, 27, 109, 122).

In conclusion, the results of systemic drug administrations suggest that 5-HT receptors might play a role in the modulation of rat TCR oscillations and SWDs. However when drugs are administered systemically, their peripheral effects cannot be excluded. It should also be mentioned that i.c.v. infused serotoninergic drugs may exert their effects on SWD activity by affecting the behavioural or motor activity, and by influence on the vigilance state. Investigations with different doses of more selective ligands, with longer EEG recordings and investigating effects on more systems (e.g. effects on SWD and vigilance in parallel, or parallel involvement of other neurotransmission systems, serotonergic and dopaminergic, or glutamatergic) should be performed to be able to interpret the results, and the role of serotoninergic mechanisms in SWD activity modulation in generalized epilepsy. Moreover, these experiments performed on animals may vary according to the model of epilepsy and/or species of animal.

Previously, Filakovszky and co-workers (54, 55) examined the role of the 5-HT$_{1A}$ receptor parallel with glutamatergic influence of spike wave discharges in WAG/Rij rats. They also found in their study, that the 5-HT$_{1A}$ agonist and the NMDA antagonist-induced effects on spike-wave discharges were independent of the changes caused in sleep-wake patterns.

**Cholinergic mechanisms in SWDs**

Cholinergic projections from the nucleus basalis to the cerebral cortex exert profound influence on the activity of the thalamo-cortical system in terms of arousal (99). Because both clinical and experimental generalized seizures are closely related to level of arousal, being suppressed during activity and deep SWS sleep, cholinergic mechanisms
have been thought to play a role in the pathogenesis of SWD. Anticholinesterases, muscarinic cholinergic agonists appear to have a biphasic effect on SWDs in absence models. The effects of cholinergic agonists and antagonists may be related to their action on arousal rather than to a direct effect on thalamo-cortical mechanisms (136).

**Pharmacological control of SWDs**

Generalized absence epilepsy has an unique pharmacological profile with respect to control anti-epileptic drugs (AEDs). The specific generalized absence drugs ethosuximide and trimethadione, which have little or no efficacy in other forms of epilepsy, both block T-type calcium current as one cellular mechanism of action (39). Broader-spectrum anticonvulsants with absence efficacy have variable cellular mechanisms, including augmentation of GABA-ergic inhibition (benzodiazepines, valproate) and T-type calcium current block (ethosuximide, valproate). The selective efficacy of benzodiazepines in controlling absence is apparently due to the selective augmentation of GABA-ergic neurotransmission in the cerebral cortex by these drugs, with much reduced efficacy in thalamus. This may be due to regional variation in the structure and function of GABA_A receptors in the brain. Because augmenting inhibition in the thalamus is clearly proconvulsant in absence, the selective effects of benzodiazepines in the cortex are consistent with their clinical utility in this seizure disorder (39).

**The role of vigilance in SWDs**

It is now well established that several neurotransmitters including, e.g., acetylcholin, noradrenaline, serotonin are involved in the cortical activation and in the modulation of sleep-wake cycle and thus, may modulate SWD through the regulation of vigilance.

It is well known that a close relationship exists between sleep-wake states and absence epilepsy in both human and genetic animal models, including GAERS and WAG/Rij rats. Observations in humans and genetic epilepsy rat models of absence epilepsy showed, almost exclusive occurrence of SWD during passive wakefulness (PW) and light sleep (SWS1) (62, 66, 67, 70). Spike wave discharge activity is known to be strongly
influenced by the altering of wakefulness and sleep in absence epilepsy (64, 66, 67). The relationship between spindles and SWD are supported by clinical observations and experimental data: in all absence rodent models SWD appear spontaneously in vigilance states at which spindles occur, and never during REM sleep or active wakefulness, where spindles are absent. SWDs never develop in genetic rat absence models with lesions in their thalamic reticular nucleus, which is considered the primary pacemaker structure of the spindle rhythm. Spontaneous paroxysms are inhibited by sudden increase of vigilance, as arousing stimuli or calling by name, and also by experimental stimulation of reticular arousal system (65, 70). Coenen and co-workers (36) with agreement of the human data found in WAG/Rij rats that a high level of arousal either spontaneously present during wakefulness or induced by REM sleep deprivation, by a learning task or by photic stimulation, all lead to a reduction of SWDs.

**WAG/Rij rat model for Absence Epilepsy**

In 1986 a rat model for absence epilepsy, the Wistar albino Glaxo strain, bred in Rijswijk, The Netherlands was described by Van Luijtelaar and Coenen (36, 158), in addition to previous genetic absence epilepsy rat models GAERS described by Marescaux (97). The name of the strain is generally abbreviated as WAG/Rij. This strain is an inbred strain of rats in which brother-sister breeding has taken place for more than 100 generations, implying that the rats are homozygous. WAG/Rij rats show SWD in the cortical EEG with frequency of 7-11 Hz, a duration of 1-45 sec and an amplitude of 200-1000 μV, bilaterally symmetrical and generalized over the cortex.
The number and mean duration of SWD increase with age, whereas sex differences are minimal. In addition to electrophysiological signs, behavioural changes were detected prior to and during spike wave discharges, such as immobile behaviour, vibrissal, eye twitching or accelerated breathing (35). The states of vigilance in which SWD predominantly occur, were also established in WAG/Rij rats (36, 158). The highest prevalence of SWD was found in drowsiness and superficial slow wave sleep (SWS1) (158). Deep slow wave sleep (SWS2) showed intermediate effect, while during REM sleep and active wakefulness SWD rarely occurred. In pharmacological studies anti-absence drugs (VLP, ETS) suppressed SWD while anti-convulsive drugs (CBZ, PHN) aggravated SWD (116).

Further pharmacological studies concerned the GABA and glutamate systems. It is thought that seizures are generated when the excitatory glutamatergic system is in imbalance with the inhibitory GABA-ergic system. The glutamate antagonist, MK-801 decreases the number of SWD in a dose-dependent way (119). GABA agonist muscimol enhances the number of SWD, while GABA antagonist biccuculine reduces the number of SWD in the WAG/Rij model (117).

The contribution of GABA-ergic mechanisms in thalamic relay nuclei to SWDs during spontaneous seizures in the WAG/Rij strain of rats, an established genetic model of absence epilepsy, in combination with single-unit recordings and micro-iontophoretic techniques in the ventrobasal thalamic complex in vivo were studied by Staak and Pape (137). Spontaneous SWDs occurring on the electroencephalogram at 5-9 Hz were associated with burst firing in thalamo-cortical neurons, which was phase-locked with the spike component. Micro-iontophoretic application of the GABA$_A$ receptor antagonist bicuculline significantly increased the magnitude of SWD-related firing in all tested cells. Application of the GABA$_B$ receptor antagonist CGP 55845A exerted a statistically insignificant modulatory effect on neuronal activity during spontaneous SWDs but significantly attenuated the bicuculline-evoked aggravation of SWD-related firing. It has been concluded that in TCR neurons, (1) GABA$_A$ receptor-mediated events are recruited with each SWD, (2) SWD-related activity can be evoked with no significant contribution
of GABA_B receptors, and (3) blockade of GABA_A receptors potentiates SWD-related activity, presumably through an indirect effect mediated through GABA_B receptors. These results vote against a predominant or even exclusive contribution of GABA_B receptors to spontaneous SWDs in thalamic relay nuclei in the WAG/Rij strain, but rather point to a critical role of GABA_A receptor activation. This conclusion is in support of the view that the two subtypes of GABA receptors play a differential role in fast (5-10 Hz) and slow (3 Hz) spike wave paroxysms observed during absence seizures (137).

Considering all electrophysiological, pharmacological, genetic and cognitive data, it can be concluded that the WAG/Rij strain of rats is a valid model (36, 93, 158) for absence epilepsy in man (Table 4.).

<table>
<thead>
<tr>
<th></th>
<th>Human IGE</th>
<th>WAG/Rij rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEG</td>
<td>3 Hz generalized SWD</td>
<td>7-11 Hz generalized SWD</td>
</tr>
<tr>
<td>Symptoms</td>
<td>mild, short cognitive deficits</td>
<td>Immobile behaviour, vibrissal twitching</td>
</tr>
<tr>
<td>Age</td>
<td>6-12 years</td>
<td>6-12 months</td>
</tr>
<tr>
<td>Genetic</td>
<td>Polygenetic</td>
<td>Autosomal, polygenetic, dominant inheritance</td>
</tr>
<tr>
<td>Vigilance</td>
<td>NREM1, NREM2</td>
<td>Passive wakefulness and SWS1</td>
</tr>
<tr>
<td>Antiepileptic drugs</td>
<td>VLP, LTG, ETS, PHN,</td>
<td>VLP, LTG, ETS, PHN,</td>
</tr>
</tbody>
</table>

Table 4. Comparison of human absence and genetic rat epilepsy model
Aim and scope of the thesis

The WAG/Rij rat strain, and inbred strain originating from the Wistar outbred strain, is an accepted genetic model of absence epilepsy. A high percentage of these rats exhibit spontaneous seizures accompanied by behavioral arrest, face twitching and 7-11 Hz EEG spike-wave discharges, a duration of 1-45 sec and an amplitude of 200-1000 μV, bilaterally symmetrical and generalized over the cortex. It is well recognized that synchronized burst-firing within a thalamocortical circuit generates SWDs, which underlie generalized absence epilepsy. The two main neurotransmitter pathways involved in these pathological thalamocortical projections are glutamate and GABA. On the other hand, there is abundant data concerning the influence of other neurotransmitters in this type of epilepsy. The role of other neurotransmitters, or neuromodulators, such as noradrenaline, dopamine or nitric oxide (NO) and the influence of vigilance levels in absence epilepsy is well documented. Furthermore, there is growing evidence that serotonergic neurotransmission is involved in the generation of SWDs in genetic absence epilepsy models.

In the present thesis the underlying mechanisms of absence epilepsy are studied in WAG/Rij model. The introduction of new EEG recording methods (long-term EEGs, computer EEG, video EEGs) made it possible to design (clinical and) experimental studies to examine factors modulating SWD activity. Studies investigating the regulation of the release of substances directly modulating excitation and inhibition in thalamocortical pathways may help to better understand the control mechanisms of the SWD expression.

The following questions were raised:

1. What is the role of 5-HT$_{2C}$ receptors in seizure generation in WAG/Rij model?

Extracellular and intracellular recordings revealed that 5-HT-induced inhibition of burst firing in NRT is mediated through 5-HT$_2$ receptors with possible involvement of 5-HT$_{2C}$ receptors (100). The possible involvement of 5-HT$_{2C}$ receptors has been suggested by the finding that mutant mice lacking this receptor subtype are extremely susceptible to audiogenic seizures and are prone to spontaneous death from seizures, suggesting that
serotonergic neurotransmission mediated by 5-HT$_{2C}$ receptors suppresses neuronal network hyperexcitability and seizure activity (22, 151). Therefore, in the first set of experiments, we have determined the effects of the 5-HT$_{2C}$ receptor-preferring agonist 1-$m$-chlorophenyl-piperazine (mCPP) and the selective 5-HT$_{2C}$ receptor antagonist SB-242084 in WAG/Rij rats. To find out more about the possible role of 5-HT$_{2C}$ receptors after increased synaptic and extrasynaptic 5-HT concentration in this type of epilepsy, the combination of an SSRI, citalopram and the 5-HT$_{2C}$ receptor antagonist SB-242084 were administered.

2. What is the role of 5-HT$_{7}$ receptors in seizure generation in WAG/Rij model?

5-HT$_{7}$ receptor expression is relatively high within the thalamus (14, 157), which brain region most probably represents the primary neuronal dysfunction underlying the generation of spike-wave discharges (36, 102, 123, 140). Furthermore, the role of both 5-HT$_{1A}$ and 5-HT$_{7}$ receptors has been suggested in serotonin-induced responses in thalamic neurons (26, 27, 108, 109).

Data, found in relevant literature, firmly imply that in addition to 5-HT$_{1A}$ receptors, 5-HT$_{7}$ receptors also play a role in regulating epileptiform activity in the WAG/Rij model and in human absence epilepsy. The purpose of our study was to explore the role of these receptors in the WAG/Rij rat model of absence epilepsy using novel, subtype-selective agents.

3. What are the effects of AMPA receptor modulators in WAG/Rij rats on SWD, vigilance and behavior?

Since studies with selective AMPA receptor antagonists in animal seizure models have indicated that AMPA receptors are potentially promising anticonvulsant drug targets, we studied the effects of two AMPA negative modulator 2,3-benzodiazepine GYKI 52466 and GYKI 53405 in WAG/Rij rats on SWD, vigilance and behavior.
4. How do the glycine transporter-1 inhibitors influence SWDs and vigilance in WAG/Rij rats?

An important breakthrough in understanding NMDA receptor-mediated glutamatergic neurotransmission was the recognition of glycine as a coagonist of glutamate at this ion channel-coupled receptor (16, 72, 80). Blocking of the glycine transporter-1 enzyme increases glycine concentrations in glutamatergic synapses, which may enhance the activation of NMDA receptors. In some studies NMDA antagonists reduced epileptic activity (54), other studies reported antiepileptic effects of NMDA receptor agonists (13, 36, 97, 155). Because of these controversial results our goal was to define the role of NMDA receptor activation by two glycine transporter-1 inhibitors in the WAG/Rij model of absence epilepsy.
Methods

Animals and surgery

Seven to eight month old (in the first and second experiments 12-15 month old), adult male Albino Glaxo rats from Rijsijk, Netherlands (WAG/Rij rats), body weights 320-420 g, bred in our laboratory, (ancestors purchased from Charles River, Hungary) were used. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Rats were kept four/cage before surgery and individually in glass cages (measuring 40 x 40 x 50 cm) after surgery, with food and water available ad libitum, maintained on a 12 h light 12 h dark cycle (lights from 08:00 to 20:00 h; daylight-type fluorescent tubes, 18 W, approximately 300 lux), and at an ambient temperature of 21°C. Animals were chronically equipped with EEG, EMG electrodes. Surgery was performed under anesthesia with 2% halothane in oxygen (Fluotec 3) using a Kopf stereotaxic instrument. Stainless steel screw electrodes were implanted on the dura mater over the cortex: two in the frontal region (co-ordinates with skull surface flat and bregma zero-zero: A 2.0, L 3.0) and two in the parieto-occipital region (A –6.0, L 3.0) (115). The ground electrode was placed over the cerebellum. In addition stainless steel coil spring electrodes encased in silicone rubber tubing similar to pacemaker leads (Subcutaneous Electrode Wire, Plastics One Inc., Roanoke U.S.A.) were sewn into the neck muscles for EMG recording. The leads of the electrodes were soldered to a miniature connector, which was head mounted with cranioplastic cement (Plastics One Inc., Roanoke U.S.A.) and mounting screws. An electromagnetic transducer activated by cable movements was used to record motor activity. Recovery after surgery was at least 10 days.
**Electrophysiological recordings:**

In order to habituate the animals to the recording conditions, the rats were connected to the recording cables, and received intraperitonial (i.p.) injections of physiological saline for at least 3 days before the experiments. The animals were attached to a multichannel amplifier by a flexible recording cable and an electric swivel, fixed above the cages, permitting free movement for the animals. EEG, EMG and motor activity were recorded for 24-hour periods, starting at light onset. EEG, EMG and motor activity signals were amplified (amplification factor approx. 5000 for EEG and motor activity, 20000 for EMG respectively), conditioned by analog filters (filtering: below 0.53 Hz and above 30 Hz at 6 dB/octave) and subjected to an analogue-to digital conversion with a sampling rate of 64 Hz. The digitized signals were displayed on a PC monitor and stored on computer for further analysis.

The vigilance states were visually scored in 4 s epochs according to the conventional criteria. Active wakefulness (AW): EEG with high frequency low voltage activity (beta: 14-30 Hz and alpha: 8-13 Hz) and high EMG activity and movements; passive (or quiet) wakefulness (PW): with similar EEG but low EMG activity and without motor activity; light slow wave sleep (SWS1): high voltage slow cortical waves (0.5-4 Hz) interrupted by low voltage fast EEG activity (spindles: 6-15 Hz); deep slow wave sleep.
(SWS2): continuous high amplitude slow waves (0.5-4 Hz) with reduced EMG activity; intermediate stage of sleep (IS): a short lasting stage (mean 3 s) just prior to paradoxical sleep and sometimes just after it characterized by unusual association of high-amplitude spindles (mean 12.5 Hz) and low-frequency (5.4 Hz) theta rhythm; REM sleep: low voltage fast frontal waves with regular theta rhythm (about 6 Hz) over the occipital cortex and silent EMG with occasional twitching. Vigilance state of the SWD was defined as the vigilance level immediately preceding the SWD pattern.

Drugs:

**mCPP** (meta-chlorophenylpiperazine HCl, Research Biochemicals International, Natick, MA, USA) in doses 0.9 or 2.5 mg/kg (i.p.) and 0.05 or 0.1 mg/rat (i.c.v.), vehicles: saline in a volume of 1 ml/kg i.p. or artificial CSF in a volume of 5 ml i.c.v.

**SB-242084** (0.2 mg/kg, i.p., 6-chloro-5-methyl-1-[(2-[2-methylpyrid-3-yl oxy]pyrid-5-yl)carbamoyl]indoline dihydrochloride, Sigma-Adrich, Budapest, Hungary), vehicle (10% solution of 2-hydroxypropyl-β-cyclodextrin, HPCD, Research Biochemicals International, Natick, MA, USA) in a volume of 1 ml/kg i.p.

**Citalopram** (2.5 mg/kg, i.p., Citalopram HBr, kindly provided by H. Lundbeck A/S, Copenhagen, Denmark), vehicle: saline in a volume of 1 ml/kg i.p.

**WAY-100635** (0.2 mg/kg, i.p., N-[2-][4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl cyclohexanecarboxamide maleate, Research Biochemicals International, Natick, MA, USA); vehicle: saline in a volume of 1 ml/kg i.p.

**SB-258719** (10 mg/kg, i.p., (R)-3,N-Dimethyl-N-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzenesulfonamide, kindly donated by EGIS Pharmaceuticals Ltd., Budapest, Hungary); vehicle: saline in a volume of 1 ml/kg i.p.

**GYKI 52466** (3, 10, 30 mg/kg, i.p., 1-[4-aminophenyl]-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine, EGIS Ltd., Budapest, Hungary), vehicle (40% solution of 2-hydroxypropyl-β-cyclodextrin, HPCD, Research Biochemicals International, Natick, MA, USA) in a volume of 1 ml/kg i.p.
GYKI 53405 (16 mg/kg, i.p., 7-acetyl-5-(4-aminophenyl)-8-methyl-8,9-dihydro-7H-1,3-dioxolo[4,5-b][2,3]benzodiazepine, EGIS Ltd., Budapest, Hungary), vehicle: (40% solution of 2-hydroxypropyl-β-cyclodextrin, HPCD, Research Biochemicals International, Natick, MA, USA), in a volume of 1 ml/kg i.p.

8-OH-DPAT (0.2 mg/kg, i.p., 8-hydroxy-2-(di-n-propylamino)-tetralin, Research Biochemicals International, Natick, MA, USA), vehicle: saline in a volume of 1 ml/kg i.p.

NFPS (1, 3, 10 mg/kg, i.p., N[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)-propyl]sarcosine, EGIS Ltd., Budapest, Hungary, vehicle: (40% solution of 2-hydroxypropyl-β-cyclodextrin, HPCD, Research Biochemicals International, Natick, MA, USA), in a volume of 1 ml/kg i.p.

Org 24461 (1, 3, 10 mg/kg, i.p., R,S-(+/-)N-[(4-trifluoromethyl)phenoxy]-3-phenyl-propylglycine, EGIS Ltd., Budapest, Hungary, vehicle: (40% solution of 2-hydroxypropyl-β-cyclodextrin, HPCD, Research Biochemicals International, Natick, MA, USA), in a volume of 1 ml/kg i.p.

**Statistical analysis:**

Analysis of variance for repeated measures followed by Newman-Keuls post-hoc comparison was used for the statistical analysis. (STATISTICA for Windows, Stat Soft Inc., Tulsa, OK, USA). Mean (±SEM) values of groups (6-8 animals each) are reported on the figures.

**Protocols of experiments**

**Experiment 1.**

In the first set of experiments the effects of mCPP were characterized. In the first experiment 0.9 or 2.5 mg/kg doses of mCPP or vehicle (saline) were injected intraperitoneally (i.p.). In the second one, the animals were treated with 0.05 or 0.1 mg/rat mCPP or vehicle (artificial CSF) in a volume of 5 ml, i.c.v. In the third one, 2.5 mg/kg mCPP or vehicle was injected i.p. after pretreatment with SB-242084 (0.2 mg/kg,
i.p., or vehicle 10% HPCD. In the second set of experiments the effect of citalopram alone and in combination with subtype selective 5-HT receptor antagonists were performed. Citalopram (2.5 mg/kg, i.p) or vehicle (saline) were administered after pretreatment with SB-242084 (0.2 mg/kg, i.p.) or vehicle. Pretreatments preceded treatments by 5 minutes. All i.p. treatments were injected in a volume of 1 ml/kg.

To quantify the occurrence of SWD, namely, cumulative duration of SWD in seconds and number of SWD paroxysms (SWD frequency) were summarized in 30 min intervals for three hours, 2x30 min before and 4x30 min after drug or vehicle treatment.

**Experiment 2.**

The selective 5-HT$_{1A}$ receptor antagonist WAY-100635 (0.2 mg/kg, i.p.), and the selective 5-HT$_{7}$ receptor antagonist SB-258719 (10 mg/kg, i.p.) were administered intraperitoneally in a volume of 1 ml/kg, 60 minutes after starting the EEG recording. To quantify the occurrence of SWD, namely, cumulative duration of SWD in seconds and number of SWD paroxysms (SWD frequency) were summarized in 30 min intervals for six hours, 1h before and 5hs after drug or vehicle treatment, in thirty minutes intervals.

**Experiment 3.**

Three experiments were performed. In the first set of experiments effects of GYKI 52466 on spontaneously occurring SWD parallel with the vigilance states changes were studied. In these experiments animals were treated with GYKI 52466 (3, 10, 30 mg/kg, i.p., or vehicle, 40% HPCD, administered intraperitoneally (i.p.) 60 minutes after starting the EEG recording. In the second experiment animals were treated with GYKI 53405 (16 mg/kg, i.p., or vehicle 40% HPCD administered i.p. 60 minutes after starting the EEG recording. In the third set of experiments the effect of GYKI 52466 on SWD activated by 8-OH-DPAT and behavioral changes modified by this latter compound were studied. In these experiments, the animals were treated with 8-OH-DPAT (0.2 mg/kg, i.p.) 15 minutes after the pretreatment with GYKI 52466 (10 mg/kg, i.p.) or vehicle.
Time of different vigilance states as well as the number and total duration of spike-wave discharges (SWD) for each rat were summarized in 30 minutes intervals for one hour before and two hours after drug or vehicle treatments, scored by visual inspection of the EEG recordings. In addition, the amount of SWD during each vigilance state was also counted and calculated for each 30 min – or in the case of the short acting GYKI 52466 between 5-20 min post-injection – interval. The measure/proportion of SWD during a given period (amount of certain vigilance state) was expressed as a ratio, namely, the duration or the number of SWD divided by the duration of the given vigilance state in the same 15 or 30 min period.

Behavior of the animals was recorded on videotape and analyzed later by 3 persons, in parallel, all of them expert in rat behavior.

Experiment 4.

In the first set of experiments animals were treated with Org 24461 (1, 3, 10 mg/kg, i.p.) or vehicle, 40 % HPCD 60 min after starting the EEG recording.

In the second set of experiments animals were treated with NFPS (1, 3, 10 mg/kg, i.p.) or vehicle, 40 % HPCD 60 min after starting the EEG recording.

Time of different vigilance states as well as the cumulative duration of SWD in seconds and number of SWD paroxysms (SWD frequency) were summarized in 30 min intervals for three hours, 2x30 min before and 4x30 min after drug or vehicle treatment.
Results

1. Effects of the 5-HT$_{2C}$ receptor agonist mCPP, the selective 5-HT$_{2C}$ receptor antagonist SB-242084, the SSRI citalopram and their combinations on the number and cumulative duration of SWDs in WAG/Rij rats.

The 5-HT$_{2C}$ agonist mCPP caused marked, dose-dependent decreases in the cumulative duration and number of SWD administered either i.p. (0.9 and 2.5 mg/kg; Fig. 6.) or i.c.v. (0.05 and 0.1 mg/rat; Fig. 7.). These effects lasted up to 60 min after the treatment.

Figure 6. Effect of mCPP (0.9 and 2.5 mg/kg, i.p) on spike-wave discharges (SWD). The histograms show the cumulative duration of SWD (left) and the number of paroxysms (right) over 60 minutes. All data cited as means $\pm$ S.E.M., n=6-7. Significant ($p<0.05$) difference from the vehicle treated group is denoted by *.

Figure 7. Effect of mCPP (0.05 and 0.1 mg/rat, i.c.v) on spike-wave discharges (SWD). The histograms show the cumulative duration of SWD (left) and the number of paroxysms (right) over 60 minutes. All data cited as means $\pm$ S.E.M., n=6-7. Significant ($p<0.05$) difference from the vehicle treated group is denoted by *.
Treatment with SB-242084 (0.2 mg/kg, i.p.) alone failed to cause any significant change in SWD compared to vehicle. Neither wakefulness, nor light slow wave sleep (SWS1) were significantly altered by SB-242084 (0.3 mg/kg i.p.) (Fig. 9.,83.). Pretreatment with SB-242084 (0.2 mg/kg, i.p) eliminated the effects of mCPP on SWD (Fig. 8.).

Figure 8. Effects of SB-242084 (SB4; 0.2 mg/kg, i.p) or vehicle pretreatment on the effects of mCPP (2.5 mg/kg, i.p.) on spike-wave discharges (SWD). The histograms show the cumulative duration of SWD (left) and the number of paroxysms (right) over 60 minutes. The effect of Veh+mCPP was significant ($p<0.05$) compared to Veh+Veh, denoted by *, while SB4+mCPP vs. SB4+Veh was not.

Figure 9. The selective 5-HT$_{2C}$-receptor antagonist SB-242084 (0.3 mg/kg i.p.) failed to cause significant change in SWS1 duration compared to vehicle. All columns represent mean values (±SEM) in each hour within the first 4 h after treatment. ($p<0.01$)
Citalopram (2.5 mg/kg, i.p.) alone caused mild, non-significant increase in cumulative duration and number of SWD. The combination of SB-242084 (0.2 mg/kg, i.p.) and citalopram (2.5 mg/kg, i.p.) treatment caused an increase in cumulative duration and number of SWD compared to SB-242084+Vehicle treatment (Fig. 10.).

Figure 10. Effects of citalopram (2.5 mg/kg, i.p.) alone and after pretreatment with SB-242084 (SB4; 0.2 mg/kg, i.p) or vehicle on spike-wave discharges (SWD). Citalopram alone failed to cause any significant effect. After pretreatment with SB-242084, citalopram caused marked, significant increase in the cumulative duration of SWD (left) and number of paroxysms (right) over 90 minutes, compared to the control pretreatment. The effect of SB4+Cit was significant ($p<0.05$) compared to SB4+Veh, denoted by *.

2. Effects of the selective 5-HT$_{1A}$ receptor antagonist and 5-HT$_{7}$ receptor antagonist SB-258719 on epileptic activity in the WAG/Rij rat model of absence epilepsy.

The selective 5-HT$_{7}$ antagonist SB-258719 significantly reduced the number of paroxysms and the cumulative duration of spike-wave discharges (SWDs) compared to vehicle in the 90-120 min period after treatment. Furthermore, SB-258719 generally decreased average paroxysm duration during the 5 hours of recording after treatment. This effect was significant compared to vehicle in the 0-30, 150-180 and 180-210 min periods after the injection (Fig.11.).

The selective 5-HT$_{1A}$ receptor antagonist WAY-100635 caused a significant increase compared to vehicle in the number of paroxysms in the second 30 min period after
injection. However, in the following 4 hours we observed a general reduction in epileptic activity compared to vehicle. This effect was significant in the 90-120 and 270-300 min periods after treatment for the number of paroxysms, and in the 90-120 and 210-240 min periods after treatment for cumulative duration of SWDs. A significant reduction in average paroxysm duration was also observed in the 120-150 and 150-180 min periods (Fig. 11.).

Figure 11. Effects of SB-258719 (10 mg/kg, i.p.), WAY-100635 (0.2 mg/kg, i.p.) and vehicle (saline, 1 ml/kg, i.p.) on SWD in WAG/Rij rats. The histograms demonstrate the number (left) and cumulative duration of SWDs (right) and the average paroxysm duration (below) during 1 h hour baseline period and 5 hours after the injection. All data cited as means ± S.E.M., n=6-8. Significant (p<0.05) difference from the vehicle treated group is denoted by *.

3. 1. Effects of two AMPA negative modulator 2,3-benzodiazepine GYKI 52466 and GYKI 53405 in WAG/Rij rats on vigilance and behavior.
GYKI 52466 caused an immediate behavioral activation compared to control after 10 and 30 mg/kg. Animals treated with GYKI 52466 were almost continuously moving around, rearing and pushing the litter were very frequent in the first 8 minutes. Between 8-10 minutes clear signs of ataxia could be seen, loss of coordination, hindlimb abduction were evident in all animals after 30 mg/kg. By min 10, animals laid down, ataxia became complete, locomotor activity was absent, only a few small head and forepaw movement could be seen in a few animals. Some animals showed short periods of struggling after longer periods of resting. One animal died within an hour after the highest dose. GYKI 53405 (16 mg/kg i.p.) failed to cause similar changes. Self grooming was frequent in all animals treated with the drug compared to controls, and in three of the eight animals wet dog shakes were observed. Locomotor activity was somewhat reduced compared to controls, but no abnormal behavior or ataxia could be observed in these animals. Vigilance and SWD were analyzed between 5 and 20 min after the injection of GYKI 52466. This compound caused dose-dependent decrease in active wakefulness and increase in slow wave sleep and passive wakefulness (Fig. 12.).

![Graphs showing wakefulness, SWS1 duration, and SWS2 duration](https://via.placeholder.com/150)

<table>
<thead>
<tr>
<th>Group</th>
<th>Wakefulness (s/15 min)</th>
<th>SWS1 Duration (s/15 min)</th>
<th>SWS2 Duration (s/15 min)</th>
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<tr>
<td>VEH</td>
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<td>30 mg/kg</td>
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*Significant difference compared to control*
Figure 12. Effect of GYKI 52466 (3, 10, 30 mg/kg, i.p) on wake-sleep pattern compared to vehicle. The histograms demonstrate the duration of wakefulness and slow wave sleeps (SWS1 and SWS2) over 15 minutes beginning five minutes after injection. All data cited as means ± S.E.M., n=6. Significant \( p<0.05 \) difference from the vehicle treated group is denoted by *.

In the case of GYKI 53405, vigilance and SWD were analyzed in 30 min periods 1 hour before and 1 hour after the injection of this drug (16 mg/kg, i.p.). Neither wakefulness, nor slow wave sleep (SWS1, SWS2) were significantly altered by GYKI 53405 (Fig.13.).

Figure 13. Effect of GYKI 53405 (16 mg/kg, i. p.) on wake-sleep cycle compared to vehicle. The histograms show the duration of wakefulness (left) and light slow wave sleep (SWS1) (right) one hour before and one hour after injection in 30 minutes periods. There was no significant difference in vigilance changes compared to vehicle treated group. All data cited as means ± S.E.M., n=6.
3. 2. Effects of two AMPA negative modulator 2,3-benzodiazepine GYKI 52466 and GYKI 53405 in WAG/Rij rats on spike-wave discharges.

A dose-dependent increase in SWD was found between 5 and 20 min after the injection of GYKI 52466 (Fig. 14. a-b.). The average paroxysm duration during this period did not show significant changes (Fig. 14. c.).

Figure 14. Effect of GYKI 52466 (3, 10, 30 mg/kg, i.p) on spike-wave discharges (SWD) compared to vehicle. The histograms demonstrate cumulative duration (a) and number of SWD (b), as well as the average paroxysm duration (c). All data cited as means ± S.E.M., n=6. Significant ($p<0.05$) difference from the vehicle treated group is denoted by *.
Proportion of SWD that started in a period, preceded by SWS1, the main vigilance state associated with SWD was also analyzed. Increase of this proportion of SWD during the analyzed period was clearly associated with the increase in the proportion of SWS1 itself. The rate of SWD associated to SWS1 compared to duration of SWS1 was not significantly affected among the doses of 30 mg/kg of GYKI 52466 (Fig. 15.)

![Graphs](image)

Figure 15. These histograms demonstrate the cumulative duration (a) and number of SWD (b), as well as the calculated measure of SWD (c, d) during light slow wave sleep (SWS1) over 15 minutes beginning 5 minutes after administration of GYKI 52466. All data cited as means ± S.E.M., n=6.
Beside vigilance, SWD were analyzed too, in 30 min periods 1 hour before and 1 hour after the injection of GYKI 53405 (16 mg/kg, i.p.). Similarly to the vigilance, neither number nor duration of SWD was significantly altered by GYKI 53405 (Fig.16.).

GYKI 52466 (10 mg/kg i.p.) was injected to the animals as pretreatment 15 min before 8-OH-DPAT (0.2 mg/kg). Effects of 8-OH-DPAT were measured for 2 hours after the drug. GYKI 52466 failed to affect number of SWD after 8-OH-DPAT (Fig.17.). In contrast, GYKI 52466 slightly, but significantly attenuated cumulative duration of SWD after the drug. (Fig.17.) A significant general treatment effect on average paroxysm duration (F1,7=12.76, P<0.01) was also found, but post hoc comparisons failed to show significant difference compared to the control at any time point (Figure 18.).

Figure 16. Effect of GYKI 53405 (16 mg/kg, i. p.) on spike-wave discharges (SWD) compared to vehicle. The histograms demonstrate cumulative duration (a) and number of SWD (b) during light slow wave sleep (SWS1) one hour before and one hour after injection in 30 minutes periods. There was no significant difference in the number and cumulative duration of SWD compared to vehicle treated group. All data cited as means ± S.E.M., n=6.
Figure 17. Effect of GYKI 52466 (10 mg/kg, i.p.) on spike-wave discharges (SWD) enhanced by 8-OH-DPAT (0.2 mg/kg i.p.) compared to control. The histograms show cumulative duration (a) and number of SWD (b). All data cited as means ± S.E.M., n=6. Significant (p<0.05) difference from the vehicle treated group is denoted by *.

![Graph showing effect of GYKI 52466 on SWD duration](image)

Figure 18. Effect of GYKI 52466 (10 mg/kg i.p.) pretreatment on the average paroxysm duration of spike-wave discharges (SWD) in combination with 8-OH-DPAT (0.2 mg/kg i.p.) compared to vehicle. All data cited as means ± S.E.M., n=6.

![Graph showing effect of GYKI 52466 on paroxysm duration](image)

4. Influence of two glycine transporter-1 inhibitors NFPS and Org 24461 on SWDs and vigilance.

The effects of the two compounds were very similar: Cumulative duration and number of SWDs in the first 30 min period after the drug administration showed a trend for decrease at the highest dose (10 mg/kg) of NFPS or Org 24461. Time effects were significant and there was a strong trend for time x treatment interaction in the case of duration [treatment effects (df=3, F= 0.9982, p=0.41 and df=3, F= 1.1632, p=0.345), time effects (df=5, F= 7.97, p< 0.0005 and df=5, F= 12.73, p< 0.0005) and time-treatment interactions (df=15, F=0.84, p=0.63 and df=15, F=1.68 p=0.06) for SWD frequency and cumulative duration, respectively] for Org 24461.
Figure 19. Effect of NFPS (1, 3, 10 mg/kg i.p.) on mean SWD frequency (up) and cumulative duration (below) during a 1-h baseline period and 2 h after the injection. Each data point represents the mean frequency or duration of SWD ±S.E.M for 6 rats in 30-min intervals.

Time effects and time x treatment interactions were significant [treatment effects (df=3, F= 1.34, p=0.28 and df=3, F= 1.04, p=0.395), time effects (df=5, F= 17.5, p< 0.0005 and df=5, F= 24.19, p< 0.0001) and time-treatment interactions (df=15, F=1.669, p=0.069]
and \( df=15, F=2.00 \ p=0.021 \) for frequency and duration, respectively] for NFPS (Fig.19.).

Org 24461 (1.0, 3.0 and 10 mg/kg) had little effect on vigilance. Neither the duration of active wake, SWS1, SWS2, nor REM were significantly altered by any dose of the drug. PW was decreased in the first 30 min period after the administration of the highest dose (10 mg/kg) but increased in the three later periods and thus, time-treatment interactions were significant (\( df=15, F=2.13, p=0.012 \)). This was accompanied by a small, albeit not significant increase in AW at the same time [treatment effect (\( df=3, F=0.5455, p=0.6561 \)), time effect (\( df=5, F=32.138, p<0.0005 \)) and time-treatment interaction (\( df=15, F=0.70, p=0.779 \)).

NFPS (1.0, 3.0 and 10 mg/kg) had also little effect on vigilance. Neither the duration of SWS2, nor REM were significantly altered by any dose of the drug. PW and SWS1 were transiently decreased in the first 30 min period after the administration of the highest dose (10 mg/kg) (Fig.20.). Time-treatment interactions were significant (\( df=15, F=1.979, p=0.0239; \ df=15, F=1.322, p=0.0203 \)) for PW and SWS1, respectively. This was accompanied by a significant increase in AW at the same time (\( df=15, F=2.204, p=0.0107 \)).
Figure 20. Effect of NFPS (1, 3, 10 mg/kg, i. p.) on passive and active wakefulness compared to vehicle. The upper histogram shows the duration of passive wakefulness, the lower histogram demonstrates the duration of active wakefulness one hour before and two hours after injection in 30 minutes periods. All data cited as means ± S.E.M., n=6. PW was transiently decreased in the first 30 min period after the administration of the highest dose (10 mg/kg), (p=0.0239) denoted by *. There was a parallel significant increase in AW at the same time. (p=0.0107), denoted by *.

When duration of vigilance states were taken into account, and SWDs were compared to the duration of PW and SWS1 neither duration nor number of SWDs showed any change after NFPS or Org 24461. Statistical data for Org 24461 [treatment effects (df=3, F= 0.794, p=0.509 and df=3, F= 0.8288, p=0.49), time effects (df=5, F= 4.999, p< 0.0005 and df=5, F= 5.22, p< 0.0005) and time-treatment interactions (df=15, F=0.998, p=0.461 and df=15, F=1.11, p=0.354) for SWD frequency and cumulative duration for SWD during PW/PW duration, respectively] and [treatment effects (df=3, F= 0.666, p=0.58 and df=3, F= 0.3928, p=0.759), time effects (df=5, F= 17.869, p< 0.0005 and df=5, F= 20.37, p< 0.0005) and time-treatment interactions (df=15, F=0.778, p=0.69 and df=15, F=0.83, p=0.641) for SWD frequency and cumulative duration of SWD during SWS1/SWS1 duration, respectively]. Statistical data for NFPS: treatment-time interactions were not significant (df=15, F= 0.553, p=0.903; df=15, F= 0.7369, p=0.741) for frequency and duration of SWD during PW/PW duration, respectively (Fig. 21.); (df=15, F= 1.0348, p=0.4269; df=15, F= 1.4081, p=0.158) for frequency and duration of SWD during SWS1/SWS1 duration, respectively. (Fig. 22.)
Figure 21. These histograms demonstrate the calculated measure of SWD frequency and cumulative duration during passive wakefulness after administration of NFPS (1, 3, 10 mg/g). All data cited as means ± S.E.M., n=6.
Figure 22. These histograms demonstrate the calculated measure of SWD frequency and cumulative duration during light slow wave sleep (SWS1) after administration of NFPS (1, 3, 10 mg/g). All data cited as means ± S.E.M., n=6.
Discussion

In our studies we examined the role of two serotonin receptors, 5-HT$_{2C}$ and 5-HT$_7$, the AMPA receptors and glycine transporter-1 in the generation of spike-wave discharges, vigilance and behavior in WAG/Rij model of absence epilepsy. Immunohistochemical analysis of the 5-HT$_{2C}$ receptor protein revealed that the most abundant 5-HT$_{2C}$-like immunoreactive cell bodies, beside the chorioid plexus, were in the anterior olfactory nucleus, medial and interrelated amygdaloid nuclei, hippocampus layers CA1 to CA3, laterodorsal and lateral geniculate thalamic nuclei, caudate-putamen and several areas of the cortex (including piriform and frontal), consistent with this receptor being located postsynaptic to serotonergic neurons. Immunopositive neurons were also found in the dorsal raphe, suggesting that 5-HT$_{2C}$ receptors may be on some serotonergic neurons (34, 90, 121). The abundance of 5-HT$_{2C}$ positive neurones in limbic areas, hippocampus, hypothalamus, thalamus, cortex and the striatum (162) are concordant with the role of this receptor in anxiogenesis, feeding, neuroendocrine function, locomotor activity, stereotype behavior and seizure generation, as suggested from the effect of 5-HT$_{2C}$ receptor compounds (9, 10, 11, 12, 17, 18, 56, 60, 83, 84, 161). The possible involvement of 5-HT$_{2C}$ receptors in epilepsy has been suggested by the finding that mutant mice lacking this receptor subtype are extremely susceptible to audiogenic seizures and are prone to spontaneous death from seizures, suggesting that serotonergic neurotransmission mediated by 5-HT$_{2C}$ receptors suppresses neuronal network hyperexcitability and seizure activity (22, 151).

In our experiments, mCPP caused marked, dose-dependent decrease in the number and mean cumulative duration of spike-wave discharges administered either i.p or i.c.v. (mCPP is a potent partial agonist at the 5-HT$_{2C}$ receptor, it displays antagonist rather than agonist properties at the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors (38). The potency of mCPP was much higher after i.c.v. compared to i.p. administration. This fact indicates a central effect of the drug. In their previous study, Kantor and coworkers demonstrated that mCPP (0.9 and 2.5 mg/kg, i.p.) caused a dose-dependent decrease in the time of SWS2
and REM sleep latency, thus the effect of mCPP in our study on SWD was independent its effect on vigilance. (82).

Our findings are consistent with experiments demonstrating that mCPP weakly elevated seizure threshold in the mouse maximal electroshock seizure threshold test and also provided appreciable protection against pentylentetrazol-induced myoclonic and tonic seizures in mice and forelimb tonic seizures in rats (156). The blockade of 5-HT\textsubscript{2C} receptors with the subtype-selective antagonist SB-242084 was not associated with significant changes in the epileptiform activity, but pretreatment with this compound reversed the effects of mCPP on SWD. This finding is in agreement with results that antagonism of 5-HT\textsubscript{2B/2C} receptor subtype with SB-206553 alone did not lower the threshold to myoclonus, forelimb and/or hindlimb tonus in mice or rats (156), and that the highly selective 5-HT\textsubscript{2C} antagonist SB-242084 did not produce proconvulsant activity in the rat maximal electroshock seizure threshold test even after administration at very high acute dose (30 mg/kg, p.o.) (85). Thus, under normal physiological conditions basic serotonergic tone does not inhibit SWD mediated by 5-HT\textsubscript{2C} receptors. It has been also demonstrated, that neither wakefulness, nor light slow wave sleep (SWS1) were significantly altered by SB-242084 (83), thus the effect of the drug on SWD was independent of its effect on vigilance.

The inability of 5-HT\textsubscript{2C} receptor antagonists to reduce seizure threshold in adult rodents contrast with the observed characteristics of mutant mice lacking the 5-HT\textsubscript{2C} receptor (151). The mutant mice undergo spontaneous tonic-clonic convulsions and by 2-3 months of age exhibit enhanced susceptibility to pentylentetrazol and audiogenic-induced seizures (22, 151). Although other epilepsy models in addition to WAG/Rij rats have to be tested, our results together with the above mentioned studies (85, 156) suggest that pharmacological blockade of the receptor and “knock-out” of the receptor gene may result different effects. This might be explained by strain differences, developmental or neuroadaptive changes in the brain.

To find out more about the possible role of 5-HT\textsubscript{2C} receptors after increased synaptic and extrasynaptic 5-HT concentration in this type of epilepsy, the combination of SSRI, citalopram, and the 5-HT\textsubscript{2C} receptor antagonist SB-242084 were administered.
Citalopram alone had mild, non-significant effect, on SWD, but a marked increase of SWD was found when the SSRI was administered after pretreatment with SB-242084. Two studies (54, 58) demonstrated that the 5-HT1A agonist 8-OH-DPAT increases the number and cumulative duration of SWD in WAG/Rij rats, and thus, it might be assumed that the lack of inhibition of SSRIs may due to the summation of the inhibitory and excitatory effects of 5-HT at different 5-HT receptor subtypes. Indeed, SSRIs caused marked activation of SWD after pretreatment with the selective 5-HT2C antagonist SB-242084. These data provide evidence that increase in endogenous 5-HT produces a dual effect on SWD, and the inhibition is mediated by 5-HT2C receptors. Our findings are consistent with the known physiology of thalamic reticular cells, their depolarization by 5-HT (100, 101, 111, 112) and that serotonergic system, possibly through 5-HT2 receptors, modulates rat thalamocortical oscillations as measured by neocortical high-voltage spindle activity (79). To support our and the previously mentioned results, in his current review, Isaac M. (76) summarizes the recent advances in the understanding of the biology and function of the 5-HT2C receptor, along with the design and development of novel, potent and selective agonist ligands, which resis the possibility of of an entirely novel class of antiepileptic drugs.

Possible role of 5-HT1A and 5-HT2C rec. in generation of SWDs
1. Via 5-HT1A autoreceptor in raphe - ↓ 5-HT release
2. ↓ 5-HT release in RTN promotes rhythmic burst firing
3. Via postsynaptic 5-HT1A hyperpolarization of GABA interneurons - ↑ excitation in Cortex
4. Via postsynaptic 5-HT1A hyperpolarisation of GABA in SN- ↑ excitation in TCR
   But ↑ 5-HT (by SSRI or mCCP) through 5-HT2C rec. - ↓ rhythmic burst firing

Figure 23. Possible role of 5-HT1A and 5-HT2C receptors in generation of SWDs. (RTN-Reticular Thalamic Nucleus, CxTh-Cortico-thalamic cell, TCR-Thalamo-cortical Relay Cell, SN-Substantia Nigra)
In conclusion, our studies provide evidence that activation of 5-HT$_{2C}$ receptors by receptor agonists or by increase in endogenous 5-HT concentration inhibit SWD, although this inhibitory effect is not significant at basal 5-HT tone. In contrast, activation of 5-HT$_{1A}$ receptors by receptor agonists or increase in endogenous 5-HT concentration promotes SWD.

Our previous results and pharmacological data have suggested a possible role for 5-HT$_7$ receptors in absence seizures. As described above, 8-OH-DPAT increases the number and cumulative duration of SWDs in WAG/Rij rats (54, 55, 58). Aside from being a potent 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT is also an agonist at 5-HT$_7$ receptors (14, 20, 157). Thus, the effects induced by 8-OH-DPAT may also be partly 5-HT$_7$ receptor-mediated. The function of 5-HT$_7$ receptors has also been investigated in other animal epilepsy models as well. In DBA/2J mice, compounds with antagonistic properties at the 5-HT$_7$ receptor had a protective effect against sound-induced seizures (20). Whilst highly speculative, such studies may indicate a role for 5-HT$_7$ receptor antagonists in the treatment of epilepsy.

As described earlier absence epilepsy is characterized by the spontaneous occurrence of bilateral synchronous spike-wave discharges that involve the entire cortical mantle (36, 121, 159). Thalamus, as a pacemaker structure for the rhythmic cortical oscillations very likely represents the primary neuronal dysfunction underlying the generation of spike-wave discharges (36, 102, 121, 140). Within the thalamus, sleep spindles are generated as a recurrent interaction between thalamocortical and thalamic reticular cells (140). It has been suggested based on the resemblance in the EEG and the similar circadian pattern that spike-wave discharges are modified sleep spindles (102, 140, 159).

Sleep spindles and other rhythmic oscillations (e.g. delta waves) are produced by thalamic neurons by their ability to spontaneously generate rhythmic bursts of action potentials due to the interaction of the Ca$^{2+}$ current $I_T$ and the inward “pacemaker” current $I_h$. The amplitude, or voltage sensitivity of $I_h$ adjusts the rate at which the thalamic cells oscillate and this sensitivity is adjusted by the release of modulatory neurotransmitters (103). 5-HT has been shown to induce a shift in the voltage sensitivity of $I_h$ to more depolarized potentials in cat, monkey and rat thalamocortical cells in vitro.
The 5-HT$_7$ receptor was confirmed to mediate this effect through a cAMP-dependent, PKA-independent mechanism (26, 27, 109). Previous anatomical studies have shown that the thalamus expresses a relatively high density of 5-HT$_7$ receptors (14, 157) and single-cell RT-PCR analysis of thalamic anterodorsal nucleus neurons confirmed 5-HT$_7$ receptor mRNA expression in the cells producing 5-HT$_7$-like responses (26).

In our second study we examined the role of 5-HT$_7$ receptors in generation of SWD in absence epilepsy. The 5-HT$_7$ receptor exhibits a distinct distribution in the CNS. In rat and guinea pig brain, both the mRNA and receptor binding sites display a similar distribution (59, 145), indicating that the receptor is expressed close to the site of synthesis. 5-HT$_7$ receptor expression is relatively high within regions of the thalamus, hypothalamus and hippocampus with generally lower levels in cerebral cortex and amygdala (59, 152, 145). In our study the selective 5-HT$_7$ receptor antagonist SB-258719 significantly reduced the cumulative duration of SWDs, as well as the number and average duration of paroxysms compared to vehicle in the WAG/Rij rat model of absence epilepsy. The results presented above suggest that SB-258719 exerts its in vivo actions by blocking the 5-HT$_7$ receptor-mediated effect of endogenous 5-HT on the “pacemaker” current $I_h$, thus inhibiting the generation of rhythmic bursts of action potentials in thalamocortical neurons, and as a consequence, reducing the emergence of SWDs.

The role of 5-HT$_{1A}$ receptors in absence epilepsy has been more extensively studied (54, 55). The previously mentioned actions of 8-OH-DPAT on SWDs could be attenuated by pretreatment with NAN-190, a 5-HT$_{1A}$ receptor antagonist (58).

In our present study we found that the selective 5-HT$_{1A}$ antagonist WAY-100635 significantly increased the number of paroxysms in the 30-60 min period after the injection. However, after this time point a general, long-lasting reduction was observed in SWD activity, with significant decreases compared to vehicle in the number, cumulative and average duration of SWDs. Our present results prove that epileptic activity can be reduced in this animal model by blocking the effects of endogenous 5-HT on 5-HT$_{1A}$ receptors. The mechanism of action mediating this effect is not yet fully understood, but there are more than one possible explanations.

Activation of 5-HT$_{1A}$ receptors was reported to cause a direct hyperpolarization of thalamocortical neurons in vitro in the ferret dorsal thalamus through an increase in
potassium conductance (107, 108). Hyperpolarization due to the activation of a potassium current results in an enhancement of burst firing in thalamocortical neurons, which enables the generation of intrinsic oscillations, e.g. sleep spindles and generalized spike-wave seizures (108, 109). Thus, WAY-100635 possibly decreases SWD through inhibition of 5-HT$_{1A}$ receptor-mediated hyperpolarization and a consequent decrease in burst firing in thalamocortical neurons.

Another way of action of WAY-100635 may be exerted through somatodendritic 5-HT$_{1A}$ autoreceptors on serotonergic neurons. The activation of these autoreceptors inhibits 5-HT synthesis and release (14). Thus, blocking the effect of endogenous 5-HT on 5-HT$_{1A}$ somatodendritic autoreceptors by WAY-100635 results in an increased 5-HT release from serotonergic neurons. An increase in endogenous 5-HT produces a dual effect on SWDs; the activation of 5-HT$_{1A}$ receptors enhances epileptiform activity, the activation of 5-HT$_{2C}$ receptors inhibits the generation of SWDs. Since WAY-100635 blocks somatodendritic as well as postsynaptic (e.g. thalamic) 5-HT$_{1A}$ receptors, increase in 5-HT release does not activate these receptors and the intensification of epileptiform activity does not occur. However, increased 5-HT release activates 5-HT$_{2C}$ receptors, which causes a reduction of SWD activity.

In addition to direct actions of 5-HT, other possible mechanisms involving NO and GABA must be considered as well. Nitric oxide (NO) reduces the emergence of SWD seizures in GAERS rats, which is another commonly used animal model of absence epilepsy (52). Moreover, activation of 5-HT$_{1A}$ receptors was reported to inhibit the NMDA receptor/NO/cyclic GMP pathway in human neocortex slices (98). Thus, the epilepsy-inducing effects of 8-OH-DPAT may be exerted through the inhibition of NO production in the cerebral cortex. In contrast, WAY-100635 may reduce epileptic activity by facilitating NO production through blocking 5-HT$_{1A}$ receptors. Further studies combining serotonergic and NOergic compounds are needed to understand the interaction of these neuromodulators in absence epilepsy.

Inhibition of cortical GABAergic interneurons through activation of 5-HT$_{1A}$ receptors was also suggested to explain the SWD-inducing effects of 8-OH-DPAT (54). Furthermore, in vivo and in vitro studies revealed that 5-HT excites thalamic inhibitory GABAergic neurons. The excitation of these cells was reported to facilitate the
emergence of SWDs (108, 109) This GABAergic mechanism (122, 157) is further supported by studies reporting GABA_B agonist-induced enhancement and GABA_B antagonist-induced reduction of SWDs in animal models (97, 81).

Studies with selective AMPA receptor antagonists in animal seizure models have indicated that AMPA receptors are potentially promising anticonvulsant drug targets. As we cited many times, thalamus, as a pacemaker structure for rhythmic cortical oscillations, is very likely responsible for the primary neuronal dysfunction underlying the generation of spike-wave discharges (6, 143). Regardless of the primary cause, synaptically released glutamate acting on ionotropic and metabotropic receptors appears to play a major role in the initiation and spread of seizure activity [27, 28, 29, 30, 32, 104, 105, 106]. The role of NMDA receptors has been widely investigated in the epileptogenesis (21, 73, 106, 130); several studies show that the NMDA antagonist MK-801 decreases the spontaneous epileptic activity in WAG/Rij rats (54, 55, 114, 118). Although, Peeters et al. (119) demonstrated that high doses of intracerebroventricularly administered AMPA increase the amount of SWD in this model, AMPA receptors had not been long considered seriously as a potential targets for antiepileptic drugs. Recently, there is abundant data concerning the AMPA receptor antagonists as highly effective anticonvulsant agents (32, 46, 47, 150). Kamiński et al. (81) found minor effects of LY 300164; only the highest dose (16 mg/kg) of this compound reduced significantly the number of spike-wave discharges in Wag/Rij rats. Although, in the same study, Kamiński and co-workers examined a GABA_B receptor antagonist, and found additive effects of the two types of receptor antagonists against the spike-wave discharges in WAG/Rij rats.

A number of studies provided evidence that in addition to the known GABAergic, glutamatergic and dopaminergic control, serotonergic mechanisms also play an important role in the triggering and maintenance of epileptic activity (54, 58), and there are strong interactions between serotonergic and glutamatergic receptor-mediated actions (54, 55, 94, 95). As we cited before, Filakovszky (54, 55) and co-workers in their previous studies demonstrated that the 5-HT_{1A} agonist 8-OH-DPAT, administered either i.p. or i.c.v., caused marked, dose-dependent activation of SWD. They also found that the non-competitive NMDA receptor antagonist MK-801 markedly decreased spontaneous SWD in WAG/Rij rats, and prevented SWD-activating effect of 8-OH-DPAT (54). All these
effects were caused by parallel changes in the cumulative duration and frequency (the number of paroxysms per hour), and the average duration of paroxysms was not affected by MK-801 either alone or when applied as a pretreatment before 8-OH-DPAT (54). The same authors found, that the 5-HT1A agonist and the NMDA antagonist-induced effects on spike-wave discharges were independent of the changes caused in sleep-wake patterns (55).

In our study we show that the non-competitive AMPA receptor antagonist GYKI 52466 caused dose-dependent decrease in active wakefulness and increase in slow wave sleep and passive wakefulness between 5 and 20 minutes after the drug. A dose-dependent increase in SWD was also found during this period. Proportion of SWD that started in a period preceded by SWS1, the main vigilance state associated with SWD was also analyzed. Increase of this proportion of SWD during the analyzed period was clearly associated with the increase in the proportion of SWS1 itself.

Electrophysiological data suggest that seizures characterized by SWD are gradually generated from the spontaneous slow oscillations and sleep spindles with congruent power of cortical control upon thalamic oscillators (143, 136). Spike-wave discharge activity is known to be strongly influenced by the alteration of wakefulness and sleep in absence epilepsy. Observations in genetic epilepsy rat models of absence epilepsy showed predominant occurrence of SWD during passive wakefulness (PW) and light sleep (SWS1). Similarly, SWD were present almost exclusively in these vigilance stages in our studies. In vitro studies revealed that NMDA, AMPA and metabotropic receptors are involved in excitatory synaptic responses recorded from thalamocortical neurons (100). Considering the ubiquity of the of AMPA receptors in sensory thalamo-cortical cells, the involvement of sensory relay nuclei in this type of epilepsy, a stronger effect of the AMPA receptor antagonists would be suggested. Our results may raise doubts about the strong involvement of AMPA receptors in generating SWD, and may indicate that part of the effect of AMPA receptor antagonists in regulation of epileptic activity occur through vigilance effects in this model. GYKI 52466, like some other 2,3-benzodizepines, is short acting in rodents (45, 148, 149, 160). Several studies demonstrate that its maximal effect is at 10-15 min post-injection. Most convincingly Durmuller and co-workers (49) reported an extreme short time window in rats with
amygdala kindled seizures: GYKI 52466 at dose 10mg/kg i.p. caused significant effect on SWD only between 5 and 15 minutes after drug administration, but by 15 min the effect was not significant. At dose 20 mg/kg, they found slightly longer time window, the maximal effect of the AMPA antagonists could be seen at 15-20 min post-injection, but by 30 min the effect clearly declined.

Surprisingly, GYKI 53405 (16 mg/kg, i.p.) did not cause significant changes in the number and cumulative duration of SWD in our study. Neither wakefulness, nor slow wave sleep (SWS1, SWS2) were significantly altered by this compound. The significant antiepileptic effect reported by Kamiński and co-workers (81) about LY 300164, supports earlier findings that the racemate compound GYKI 53405 is a less effective AMPA antagonist compound compared to LY 300164 (81).

GYKI 52466, when applied as pretreatment - at dose 10 mg/kg, i.p. – 15 minutes before the selective 5-HT\textsubscript{1A} receptor agonist 8-OH-DPAT failed to affect the number of SWD enhanced by 8-OH-DPAT, although slightly, but significantly attenuated the cumulative duration of SWD. A significant general treatment effect on average paroxysm duration (F1,7=12.76, P<0.01) was also found, but post hoc comparisons failed to show significant difference compared to the control at any time point.

The doses applied in this study were based on data found in relevant literature (81, 150). The therapeutic index for the AMPA antagonists seems to be low. The reported most serious side effect is ataxia, which occurs at doses near to the anticonvulsive ED50 values [1, 31, 48, 94, 166], which may limit the practical utility of these compounds in epilepsy therapy. Correspondingly, analyzing the behavioral changes in WAG/Rij rats after administration of GYKI 52466 (10 and 30 mg/kg, i. p.) immediate behavioral activation could be observed compared to vehicle treated animals in our study. At 8-10 minutes post-injection signs of ataxia became obvious after 30 mg/kg. One animal died within an hour after the highest dose. In order to avoid the above described side effects, the lower dose (10 mg/kg, i.p.) of GYKI 52466 was applied as pretreatment before the 5-HT\textsubscript{1A} receptor agonist compound. Although not effective alone, GYKI 52466 caused small, but significant reduction in SWD duration promoting effect of 8-OH-DPAT. These data suggest that 8-OH-DPAT-induced effects on SWD include also mechanisms mediated by AMPA receptors.
In conclusion, our results suggest that AMPA receptors play a moderate role in the regulation of epileptic activity and some of these effects are connected to their effects on vigilance in WAG/Rij rats. That means that NMDA and AMPA receptors play a different role in this model.

The NMDA receptors have been extensively studied in rat models of absence epilepsy. Enhanced responses to NMDA were found in rats with petit mal-like seizures (126). The effects of MK-801, a non-competitive antagonist at the NMDA receptor, reduced the number and mean duration of SWD (54, 55, 119). Reviews agree that NMDA antagonists generally decrease epileptic activity in absence epilepsy. In contrast, the role of NMDA receptor agonists remains controversial. Based on experimental data, both inhibition and activation by direct or indirect agonists have been suggested (13, 36, 97, 159). An important breakthrough in understanding NMDA receptor-mediated glutamatergic neurotransmission was the recognition of glycine as a coagonist of glutamate at this ion channel-coupled receptor (16, 72, 80). Glycine and glutamate act as cotransmitters for opening NMDA-sensitive ionotropic glutamate receptors in a strychnine-insensitive manner (57) influencing the permeability of the receptor-coupled ion-channel for mono- and bivalent cations (129). NMDA receptors consist of NR1 and NR2 subunits, the former possesses binding site for glycine (glycineB binding site) and the latter binds glutamate to the agonist binding site (113). Activation of NMDA receptors requires occupancy of glycineB binding sites by endogenous glycine released from neighboring cells into the vicinity of NMDA receptors. A potent glycine transport mechanism assures that glycine concentrations below the level required to saturate glycine sites at NMDA receptors. Glycine transporters are members of the Na⁺- and Cl⁻-dependent neurotransmitter transporter family. Two glycine transporter genes (Gly1 and Gly2) have been identified and cloned (2, 87). In addition three isoforms for GlyT1 (GlyT1a, GlyT1b, GlyT1c) and two isoforms of GlyT2 (GlyT2a and GlyT2b) have been identified, cloned and characterized (19, 125) Glycine transporters, which regulate glycine concentrations in excitatory synapses, belong to GlyT1 transporters and transport proteins have been shown to be co-localized with NMDA receptors (53, 135). A number of compounds have been reported to have selective and high affinity to glycine transporters. Toth and Lajtha (153) and Toth and coworkers (154) have demonstrated
that glycine inhibits PCP-induced hyperactivity in mice and of the glycine derivatives, glycyldodecylamide (GDA) was found particularly active in this respect. Later, the glycine reuptake inhibitory effect of GDA has been demonstrated. In the glycine transporter inhibitor N[3-(4-fluorophenyl)-3-(4-phenylphenoxy)-propyl]sarcosine (NFPS) and R,S-(+/-)N-methyl-N-[(4-trifluoromethyl)phenoxy]-3-phenyl-propylglycine (Org 24461), the amino group of sarcosine was substituted with lipophilic heterocycles. Blocking of the glycine transporter-1 enzyme increases glycine concentrations in glutamatergic synapses, which may enhance the activation of NMDA receptors. In some studies NMDA antagonists reduced epileptic activity (54), other studies reported antiepileptic effects of NMDA receptor agonists (14, 36, 97, 159). Because of these controversial results we wanted to define the role of NMDA receptor activation by two glycine transporter-1 inhibitors in the WAG/Rij model of absence epilepsy.

The examined two glycine transporter-1 inhibitors, NFPS and Org 24461 had very similar effect. They had weak effect on vigilance. A trend for decrease only in PW and SWS1 accompanied by small increase in AW in the first 30 min period after the drug. A similar, small decrease in SWD was seen in the same period. This latter decrease was clearly caused by the decrease in PW and SWS1, since SWDs occur almost exclusively only in two vigilance states: PW and SWS1. Decrease in the duration of any of these leads to decrease in SWD. This conclusion was also confirmed by the lack of change in either duration or number of SWDs expressed as a ratio of time of either PW or SWS1. Transient antiepileptic activity of the drugs at the highest dose is clearly a result of their effects on vigilance.

**Conclusion**

The understanding of the pathophysiology of epilepsies is still incomplete. The idea that there may be a link between serotonin and the inhibition of epilepsy has been suggested as early as 1957 by Bonnycastle and coworkers (18). In their study they demonstrated that a series of anticonvulsants, including phenytoin, elevated brain serotonin levels. As we stated in the introduction despite the fact that a large number of 5-HT receptors with
different anatomical localizations and functions have been identified within the last two decades (14, 75) there are only few studies investigating the role of 5-HT receptor subtypes in the modulation of seizure activity, and the results are sometimes controversial depending on the used experimental epilepsy model. Most studies agree that elevation of serotonergic tone decrease generation of seizures, whereas a decrease in serotonergic function increases seizure susceptibility. Our studies provide evidences that 5-HT play a peculiar role in the generation of SWDs in WAG/Rij model of absence epilepsy. Our results show, that activation of different 5-HT receptors cause different, sometimes opposite effects.

Our experiments, examining the influence of glutamatergic neurotransmission in generation of SWD in absence epilepsy brought somewhat unexpected results. We demonstrated that AMPA receptors play moderate role in regulation of epileptic activity and some of these effects are connected to their effects on vigilance in WAG/Rij rats. That means that NMDA and AMPA receptors play a different role in this model. Similarly, the glycine transporter1-inhibitor compounds caused a transient antiepileptic effect only at their highest doses, what was clearly a result of their effects on vigilance.

Over the years, there has been considerable success in the development of novel antiepileptic drugs (AED) along with new improved formulations. However the new drugs have brought considerable improvement in tolerability and seizure control, there is a continuing need for new AEDs. Our results draw attention to 5-HT receptors and demonstrate that the drugs acting on these receptors have influence on generation of SWD in absence epilepsy.
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Figure legends

Table 1. Generalized Epilepsies according to Classification and Terminology of the ILAE 2001. ABS: absence seizure; ATO: atonic seizure; MYO: myoclonic seizure; TCS: tonic-clonic seizure

Table 2. Classification of absence seizures according to Classification and Terminology of the ILAE 2001.

Table 3. Classification of 5-HT receptors (in brackets the previous name of the receptor, prior to the classification).

Table 4. Comparison of human absence and genetic rat epilepsy model.

Picture 1. Laboratory of Neurochemistry and Neuropsychopharmacology, National Institute of Psychiatry and Neurology, Budapest.

Figure 1. The figure represents a typical absence seizure with 3-Hz spike-wave discharges.

Figure 2. The basic thalamo-cortical circuit. (RTN-Reticular Thalamic Nucleus, TCR-Thalamo-cortical Relay Cell, CxTh-Cortico-thalamic cell). Schematic diagram of thalamo-cortical circuitry showing the relationship between neurons in the RTN, TCR and CxTh neurons in layer VI of the cerebral cortex. The figure illustrates the reciprocal excitation between TCR and cortical neurons (I), recurrent inhibition between RTN neurons (II), reciprocal inhibition between TCR and RTN neurons (III), and parallel excitation between CxTh neurons and RTN and TCR (IV). (Modification of a figure published by O. Carter Snead III. M.D., 1995)

Figure 3. Factors influencing SWD activity
Figure 4. The basic extrinsic neurotransmitter systems influencing the thalamus and cortex. Each of the neurotransmitter systems has the potential to modulate thalamo-cortical oscillatory activity and therefore SWD activity, too. (OB-olfactory bulb, SEP-septum, STR-striatum, NB-nucleus basalis, VTA-ventral tegmental area, SC-superior colliculus, LC-locus coeruleus, dlt-dorsolateral tegmental nucleus, TPP-pediculopon tine tegmental nucleus, SN-substantia nigra, PC-pars compacta, PR-pars reticulata). (Copy of a figure published by O. Carter Snead III. M.D., 1995)

Figure 5. A brief typical paroxysm in WAG/Rij rat. (from our study).

Figure 6. Effect of mCPP (0.9 and 2.5 mg/kg, i.p) on spike-wave discharges (SWD). The histograms show the cumulative duration of SWD (left) and the number of paroxysms (right) over 60 minutes. All data cited as means ± S.E.M., n=6-7. Significant (p<0.05) difference from the vehicle treated group is denoted by *.

Figure 7. Effect of mCPP (0.05 and 0.1 mg/rat, i.c.v) on spike-wave discharges (SWD). The histograms show the cumulative duration of SWD (left) and the number of paroxysms (right) over 60 minutes. All data cited as means ± S.E.M., n=6-7. Significant (p<0.05) difference from the vehicle treated group is denoted by *.

Figure 8. Effects of SB-242084 (SB4; 0.2 mg/kg, i.p) or vehicle pretreatment on the effects of mCPP (2.5 mg/kg, i.p.) on spike-wave discharges (SWD). The histograms show the cumulative duration of SWD (left) and the number of paroxysms (right) over 60 minutes. The effect of Veh+mCPP was significant (p<0.05) compared to Veh+Veh, denoted by *, while SB4+mCPP vs. SB4+Veh was not.

Figure 9. The selective 5-HT2c-receptor antagonist SB-242084 (0.3 mg/kg i.p.) failed to cause significant change in SWS1 duration compared to vehicle. All columns represent mean values (±SEM) in each hour within the first 4 h after treatment. (p<0.01).
Figure 10. Effects of citalopram (2.5 mg/kg, i.p.) alone and after pretreatment with SB-242084 (SB4; 0.2 mg/kg, i.p) or vehicle on spike-wave discharges (SWD). Citalopram alone failed to cause any significant effect. After pretreatment with SB-242084, citalopram caused marked, significant increase in the cumulative duration of SWD (left) and number of paroxysms (right) over 90 minutes, compared to the control pretreatment. The effect of SB4+Cit was significant ($p<0.05$) compared to SB4+Veh, denoted by *.

Figure 11. Effects of SB-258719 (10 mg/kg, i.p.), WAY-100635 (0.2 mg/kg, i.p.) and vehicle (saline, 1 ml/kg, i.p.) on SWD in WAG/Rij rats. The histograms demonstrate the number (left) and cumulative duration of SWDs (right) and the average paroxysm duration (below) during 1 hour baseline period and 5 hours after the injection. All data cited as means ± S.E.M., n=6-8. Significant ($p<0.05$) difference from the vehicle treated group is denoted by *.

Figure 12. Effect of GYKI 52466 (3, 10, 30 mg/kg, i.p) on wake-sleep pattern compared to vehicle. The histograms demonstrate the duration of wakefulness and slow wave sleeps (SWS1 and SWS2) over 15 minutes beginning five minutes after injection. All data cited as means ± S.E.M., n=6. Significant ($p<0.05$) difference from the vehicle treated group is denoted by *.

Figure 13. Effect of GYKI 53405 (16 mg/kg, i.p.) on wake-sleep cycle compared to vehicle. The histograms show the duration of wakefulness (left) and light slow wave sleep (SWS1) (right) one hour before and one hour after injection in 30 minutes periods. There was no significant difference in vigilance changes compared to vehicle treated group. All data cited as means ± S.E.M., n=6.

Figure 14. Effect of GYKI 52466 (3, 10, 30 mg/kg, i.p) on spike-wave discharges (SWD) compared to vehicle. The histograms demonstrate cumulative duration (a) and number of SWD (b), as well as the average paroxysm duration (c). All data cited as means ± S.E.M., n=6. Significant ($p<0.05$) difference from the vehicle treated group is denoted by *.
Figure 15. These histograms demonstrate the cumulative duration (a) and number of SWD (b), as well as the calculated measure of SWD (c, d) during light slow wave sleep (SWS1) over 15 minutes beginning 5 minutes after administration of GYKI 52466. All data cited as means ± S.E.M., n=6.

Figure 16. Effect of GYKI 53405 (16 mg/kg, i. p.) on spike-wave discharges (SWD) compared to vehicle. The histograms demonstrate cumulative duration (a) and number of SWD (b) during light slow wave sleep (SWS1) one hour before and one hour after injection in 30 minutes periods. There was no significant difference in the number and cumulative duration of SWD compared to vehicle treated group. All data cited as means ± S.E.M., n=6.

Figure 17. Effect of GYKI 52466 (10 mg/kg, i.p.) on spike-wave discharges (SWD) enhanced by 8-OH-DPAT (0.2 mg/kg i.p.) compared to control. The histograms show cumulative duration (a) and number of SWD (b). All data cited as means ± S.E.M., n=6. Significant (p<0.05) difference from the vehicle treated group is denoted by *.

Figure 18. Effect of GYKI 52466 (10 mg/kg i.p.) pretreatment on the average paroxysm duration of spike-wave discharges (SWD) in combination with 8-OH-DPAT (0.2 mg/kg i.p.) compared to vehicle. All data cited as means ± S.E.M., n=6.

Figure 19. Effect of NFPS (1, 3, 10 mg/kg i.p.) on mean SWD frequency (up) and cumulative duration (below) during a 1-h baseline period and 2 h after the injection. Each data point represents the mean frequency or duration of SWD ±S.E.M for 6 rats in 30-min intervals.

Figure 20. Effect of NFPS (1, 3, 10 mg/kg, i. p.) on passive and active wakefulness compared to vehicle. The upper histogram shows the duration of passive wakefulness, the lower histogram demonstrates the duration of active wakefulness one hour before and two hours after injection in 30 minutes periods. All data cited as means ± S.E.M., n=6. PW was transiently decreased in the first 30 min period after the administration of the
highest dose (10 mg/kg), (p=0.0239) denoted by *. There was a parallel significant increase in AW at the same time. (p=0.0107), denoted by *.

Figure 21. These histograms demonstrate the calculated measure of SWD frequency and cumulative duration during passive wakefulness after administration of NFPS (1, 3, 10 mg/g). All data cited as means ± S.E.M., n=6.

Figure 22. These histograms demonstrate the calculated measure of SWD frequency and cumulative duration during light slow wave sleep (SWS1) after administration of NFPS (1, 3, 10 mg/g). All data cited as means ± S.E.M., n=6.

Figure 23. Possible role of 5-HT₁A and 5-HT₂C receptors in generation of SWDs. (RTN-Reticular Thalamic Nucleus, CxTh-Cortico-thalamic cell, TCR-Thalamo-cortical Relay Cell, SN-Substantia Nigra).


**Other publications, lectures, posters**

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