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Information Processing in an Animal Model of Absence Epilepsy: Characteristics of Spike-Wave Discharges in WAG/Rij Rats

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Information Processing in an Animal Model of Absence Epilepsy: Characteristics of Spike-Wave Discharges in WAG/Rij rats.

INFORMATION PROCESSING IN AN ANIMAL MODEL OF ABSENCE EPILEPSY: CHARACTERISTICS OF SPIKE-WAVE DISCHARGES IN WAG/RIJ RATS.

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op het gebied van de Sociale Wetenschappen

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To my parents, Ank and Jacques

For Miekie, Stijn and Mees

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„WHETHER AN EXPERIMENTAL CONDITION TRULY MODELS A HUMAN DISEASE ALWAYS REMAINS OPEN TO QUESTION, BUT CONFIDENCE IN THE VALIDITY OF A MODEL GROWS WITH THE NUMBER OF OBJECTIVELY VERIFIABLE FEATURES IT SHARES WITH ITS PRESUMED HUMAN COUNTERPART."

DEBORAH TAYLOR-COURVAL AND PIERRE GLOOR, 1984

CHAPTER 1

INTRODUCTION TO PAROXYSMAL ELECTRICAL BRAIN ACTIVITY

Chapter 1 Introduction to paroxysmal electrical brain activity

1.1 Research on paroxysmal electrical brain activity

The first description of what later on appeared to be paroxysmal electrical brain activity dates back to about 3000 B.C., when the ancient Greeks provided a clinical picture of convulsive epilepsy with its present name (after Ἐπιλαμβάνω, meaning to grasp suddenly or to seize), which suggests a believe in an external causation of the disease, rather than in the brain as an internal source. In antiquity Hellenistic physicians considered trephination as a potent remedy of epilepsy, because it was thought to provide an outlet for pathogenic humours and vapours. As early as 175 B.C. Gaius Galenus distinguished seizures that originated in the brain from seizures originating in other parts of the body (Temkin 1971). In contrast to such considerations of a few bright scientists, during medieval times epilepsy was generally still not considered a brain disease, but merely a matter of demons and *'healing of the possessed'* (Heitmann 1991). Not too much progress was made until the first half of the nineteenth century, when the foundations of the controversies in classifying epilepsy (essential versus nonessential, idiopathic versus symptomatic) were laid (Reynolds 1986). In an attempt to crystallize the concept of aberrant electrical brain activity, Jackson defined epilepsy in 1873 as *'the name for occasional, sudden, excessive, rapid and local discharges of gray matter'*. As a consequence of Jackson's sharp definition, *'the borderland of epilepsy'* with its many differential diagnostic problems became paradoxically more and more pronounced (Gowers, 1907).

A milestone in the history of research on aberrant electrical brain activity is the availability of electroencephalographic techniques [EEG], which was first developed in Europe during the turn of the century (Caton 1875; Beck 1890; Berger 1929; for a review see Grass 1984). From the mid-thirties on, application of the EEG technique on epileptics made it possible to distinguish generalized seizures from partial seizures (Jasper and Droogleever-Fortuyn 1947; Penfield and Jasper 1947); numerous aberrant EEG patterns such as the spike-wave discharge pattern were found to be specific to a certain ('petit

mal') clinical picture (Gibbs, Davis and Lennox 1935). Now clinical EEG was established in man, it became rapidly clear that paroxysms are most appropriately described in terms of excessive electrical discharges, whereby these phenomena can have many clinical appearances in overt as well as in covert behaviour. From that point on research on epileptic brain activity has boomed; nowadays, next to the clinical features, the EEG is still the preferred diagnostic tool for studying epileptic patients (Chadwick 1990).

As a consequence of the application of EEG techniques a further differentiation of types of aberrant electrical brain activity in patients took place. This differentiation resulted in a classification system (Table I), the International Classification of Epilepsies, Epileptic Syndromes, and Related Seizure Disorders [ICES], which is controlled by the International League Against Epilepsy [ILAE] (Commission on classification 1981; 1985; 1989). This ICES classification is primarily based on two major distinctions (Dreifuss 1990): the first is a separation of epilepsies characterized by seizures that are generalized from those characterized by partial seizures that imply a focal cortical localization. The second distinction separates epilepsies that are primary or idiopathic (i.e. no demonstrable other neurological pathology present) from those that are secondary or symptomatic (associated with underlying cerebral disease) and from those whose cause is completely unknown (cryptogenic). Additional differentiations are made on the base of the degree of impairment of consciousness, the character of the concomitant convulsions (mostly defined in terms of muscle activity, such as clonic, tonic, or atonic), and etiology (known or unknown). Regularly, proposed updates and extensions are published; other factors such as age at onset, family history, and prognosis are integrated and results of recently developed brain research techniques are added to differentiate between epileptic syndromes (e.g. childhood epileptic syndromes: Wallace 1990). To date, the classification of epilepsy indicates that the epilepsies should be considered a heterogeneous collection of sets of symptoms, whose common feature still is the recurrent pathological neuronal discharge.

Table I International classification of epilepsies, epileptic syndromes, and related seizure disorders [ICES]. Adapted from CCT-ILAE, *Epilepsia* 30.389-399;1989.

1. Localization-related (focal, local, partial) epilepsies and syndromes

1 1 Idiopathic (with age-related onset)
- Benign childhood epilepsy with centrotemporal spike
- Childhood epilepsy with occipital paroxysms
- Primary reading epilepsy

1 2 Symptomatic (secondary)
- Chronic progressive epilepsia partialis continua of childhood

Syndromes characterized by seizures with specific modes of precipitation
- Temporal lobe epilepsies
- Frontal lobe epilepsies
- Parietal lobe epilepsies
- Occipital lobe epilepsies

1 3 Cryptogenic — Defined by

Seizure type (see ICES) / Clinical features / Etiology / Anatomical localization

2. Generalized epilepsies and syndromes

2 1 Idiopathic (with age-related onset)
- Benign neonatal familial convulsions
- Benign neonatal convulsions
- Benign myoclonic epilepsy in infancy

⇨ ⇨ ⇨ *Childhood absence epilepsy (pyknolepsy)*
- Juvenile absence epilepsy
- Juvenile myoclonic epilepsy in infancy
- Epilepsy with grand mal seizures (GTCS) on awakening

Other generalized idiopathic epilepsies

Epilepsies with seizures precipitated by specific modes of activation

2 2 Cryptogenic or symptomatic
- West syndrome (infantile spasms, Blitz Nick-Salaam Krämpfe)
- Lennox-Gastaut syndrome
- Epilepsy with myoclonic-astatic seizures
- Epilepsy with myoclonic absences

2 3 1 Symptomatic (nonspecific etiology)
- Early myoclonic encephalopathy
- Early infantile epileptic encephalopathy with suppression bursts

Other symptomatic generalized epilepsies

2 3 2 Specific syndromes

Epileptic seizures complicating other disease states

3. Epilepsies and syndromes undetermined whether focal or generalized

3 1 (with both generalized and focal seizures)
- Neonatal seizures
- Severe myoclonic epilepsy in infancy
- Epilepsy with continuous spike-waves during slow wave sleep
- Acquired epileptic aphasia (Landau-Kleffner syndrome)

Other undetermined epilepsies

3 2 (without unequivocal generalized or focal features)

4. Special syndromes

4 1 (situation-related seizures, gelegenheitsanfälle)
- Febrile convulsions
- Isolated seizures or isolated status epilepticus
- Seizures occurring only when there is an acute or toxic event due to factors such as alcohol, drugs, eclampsia, nonketotic hyperglycemia

1.2 Research on spike-wave paroxysms

1.2.1 Spike-wave discharges in man: electrophysiology

Recurrent spike-wave discharges are found in man as well as in some animal species (Fisher 1989). In man the spike-wave discharges with an average frequency of about 3 Hertz are the electroencephalographic hallmark in human primary generalized epilepsies of the absence type and also in epilepsies with tonic-clonic and myoclonic seizures. The terms 'spike-wave discharges', 'spike-wave complex', 'spike-wave activity', 'high voltage spindles', and 'polyspiking activity' are leniently used to indicate a series of sequentially occurring discharges. Such a discharge consists of a surface positive deflection, followed by a surface negative spike of short duration; the next, second surface negative spike has a much larger amplitude and precedes a positive transient. Finally a prominent surface negative slow-wave ends up the spike-wave discharge (Weir 1965). A train of spike-wave discharges, which lasts from 5 to 20 seconds and may occur as often as 20 to 200 times during a 24 hours' day, is typically found in childhood absence epilepsy (Loiseau 1992). On the cortex bisynchronous, symmetrical discharges of sudden onset with a frequency of 4 Hertz at onset slowing to 2.5 Hertz at resolution can be registered during an ictal period, while normal (interictal) waking background activity exists (Drury and Dreifuss 1985). The amplitude of a single spike-wave complex ranges from 100 to 1200 μVolt (Gibbs, Davis and Lennox 1935; Kiloh et al 1981). Maximal amplitude of the epileptic paroxysms can be registered at the superior frontal electrodes, whereas in some children (30-40%) the generalized spike-wave activity is preceded by associated rhythmic bursts of 3-4 Hertz activity over the posterior head regions (Drury 1989). However, as is also the case with duration and frequency of occurrence, large inter- and intra-individual differences in maximal amplitude are found. Furthermore, spike-wave discharges per se can be accompanied by different clinical appearances and can be differentially sensitive for activation procedures. Because of these differentiations, typical and atypical forms of absence epilepsy are defined (Drury 1989; Gomez and Westmoreland 1987). A comprehensive overview of other factors (inclusion criteria, clinical expressions, genetics, prognosis, etcetera) related to absence epilepsy can be found in Gomez and Westmoreland (1987) or in Sandstedt (1990).

1.2.2 Spike-wave discharges in man: information processing

So, the typical clinical concomitant of generalized spike-wave discharges in man is the absence seizure. The term 'absence' is preferred for this type of seizures because its synonyms 'petit mal' or 'minor seizure' are too easily used to indicate mistakenly any seizure which is not convulsive ('grand mal'). Furthermore, the term 'absence' elegantly describes the behavioural status of the patient during a seizure: while only subtle postural changes can be seen, the patient shows a reduced responsiveness to external stimulation, that gives an observer the impression that although the patient is physically present, he is mentally 'away' or 'absent'. It should be realized that in patients with childhood absence epilepsy information processing capacities are within the normal range when assessed during non-paroxysmal EEG activity (Bourgeois et al 1983; Farwell et al 1985). Despite these normal interictal intellectual capacities, in research concerning information processing during spike-wave discharges some complicating factors should be acknowledged. Disturbances in cognitive functioning can be found during spike-wave discharges even without overt clinical signs, a phenomenon that is known as 'Transient Cognitive Impairment' (Aarts et al 1984; Siebelink et al 1988). Moreover, as was the case with amplitude and frequency of occurrence of the spike-wave paroxysms, also with respect to the severity of the cognitive disturbance large inter- as well as intra-individual differences exist (Browne, Penry and Porter 1974; Shimazono et al 1953). Finally, also the cognitive requirements of the task employed have been shown to influence the extent of the measured impairment (Bornstein et al 1988).

Although the unresponsiveness nowadays associated with spike-wave activity was well-known by anecdotal material already in the early 19th century (e.g. Esquirol 1838), the first study in which ictal information processing was systematically studied, stems from the late-thirties. Because of the availability of the newly developed technique for electroencephalography, sensory stimuli could be presented during actual spike-wave activity and truly ictal reaction times could be determined (Schwab 1939). Ever since, experiments were carried out that tried to specifically investigate sensory, attentional, mnemonic, motoric, and central information processing of the brain during spike-wave paroxysms. Simple motoric responses (e.g. fingertapping) largely stay intact, while more

complex motoric response patterns or shifts in response patterns are impaired (Yeager and Guerrant 1957; Davidoff and Johnson 1964; Sengoku et al 1990). Many experiments have used decision tasks to obtain ictal response accuracy values and response latency values: during spike-wave discharges responses were absent or incorrect, while reaction times increased (Browne, Penry and Porter 1974; Sellden 1971; Tizard and Margerison 1963). In other reports, the disturbance in information processing during spike-wave discharges has been attributed to impairments of attention (Davidoff and Johnson 1964; Goode, Penry and Dreifuss 1970; Mirsky and Duncan 1990; Opp, Wenzel and Brandl 1992), to impairments of mental chronometry (Hutt, Newton and Fairweather 1977; Van Luijtelaar et al 1991), or to impairments of memory (Goldie and Green 1961; Hutt and Gilbert 1980; Jus and Jus 1962; Provinciali et al 1991). In general, during spike-wave activity performance on tasks with high demands (e.g. information load, attention, complexity) was more likely found to be disturbed than performance on less demanding tasks. However, no consistent model has been proposed which integrates all findings and accounts for the differences between spike-wave paroxysms as well as the differences within spike-wave paroxysms.

1.2.3 Spike-wave discharges in animals: electrophysiology

Whereas spike-wave discharges in man are considered a hallmark for epilepsy, the epileptic nature of spike-wave discharges which can be recorded in animals is not yet unanimously accepted (Kaplan 1985; Fisher 1989). The scepticism is likely to be derived from the fact that no known animal model mimics the conditions of human spike-wave discharges completely, thus encouraging alternative hypotheses. It has been suggested for example, that rodent spike-wave activity was an equivalent of feline sensorimotor rhythm or of human μ-rhythm, which both are nonpathological rhythms generated by thalamocortical pathways and caused by periodical decreased afferent activity (Semba, Szechtman and Komisaruk 1980). However, some authors have legitimately argued that even if the epileptic nature cannot yet be proven, the spike-wave discharges are valuable as a 'genetically determined electroencephalographic characteristic' with much potential to study nervous system functioning (Ryan and Sharpless 1979).

Several models approximate the human spike-wave discharges in a scientifically and

clinically useful manner: 3 Hertz bilateral symmetric spike-wave discharges can be recorded from rhesus monkeys, when cobalt powder is bilaterally placed on the premotor cortex (Marcus and Watson 1968). Also in the cat focal application of convulsants, such as penicillin, pentylenetetrazol, and estrogen, has been used to induce 3 Hertz spike-waves (Fisher and Prince 1977; Gloor, Quesney and Zumstein 1977; Marcus, Watson and Goldman 1966). However, this procedure to evoke the spike-wave discharges is very laborious and its effect is largely limited to the time during and just after application. This disadvantage also holds for the thalamic stimulation model, which, although of a considerable heuristic value with respect to pathophysiology, is limited in practical use: by micro-electrode electrical stimulation of the midline and intralaminar thalamus -the thalamic reticular system- cortical spike-waves with behavioural abnormalities could be registered (Hunter and Jasper 1949). Moreover, it was the thalamic stimulation model that showed in cats that the spike-components were associated with intracellular depolarizing shifts and synchronous neuron firing, while the wave components can be associated with inhibitory neuronal processes (Pollen, Reid and Perot, 1964). Of pragmatic value is the feline generalized penicillin epilepsy model [FGPE] because in this model cortical generalized spike-waves with concomitant clinical absence-like behaviour can be recorded during several hours after a systemic injection (intra-muscular) of a large dose of penicillin (Gloor 1979; Taylor-Courval and Gloor 1984). Despite the many pathophysiological hypotheses (see paragraph 1.2.3) which were postulated on the base of this penicillin model, its application appears to be restricted to cats, as penicillin in rats induces seizures that are different from the absence-like feline seizures (Fisher 1989). A model that appears less species-specific is the γ-hydroxybutyric acid [GHB] model (Snead 1988). Upon treatment with GHB, which is synthesized primarily from γ-aminobutyric acid [GABA] and which occurs naturally in the mammalian brain, rats showed 4-6 Hertz spike-wave discharges together with absence-like behaviour. In fact, many compounds (e.g. THIP, a partial $GABA_A$ receptor agonist; Fariello and Golden 1987) have been reported to produce bilaterally synchronous spike-wave discharges and absence-like behaviour in otherwise normal rats, effects that last for hours after peripheral injection. As a last category the genetic models should be mentioned, because many of these models exhibit 6-10 Hertz

spike-wave discharge phenomena without being handicapped by the practical and theoretical disadvantages of experimentally induced models (Löscher 1984). Chapter two of this thesis will discuss in more detail the genetic models, with emphasis on the WAG/Rij strain of rats.

Despite this rich variety of animal models, which all show some kind of spike-wave discharges with a broad range of peak frequencies, no evidence was yet accepted as to be so conclusive that the spike-wave discharges should be interpreted as epileptic phenomena. In this line of reasoning, Kaplan (1985) suggested that „ *one factor that would clarify this issue would be evidence showing whether or not rodents were conscious or unconscious while PSA (spike-wave discharges) were recorded* " p.433.

1.2.4 Spike-wave discharges in animals: information processing

In contrast to the large number of studies on information processing during spike-wave activity in humans, this question has been addressed in only a limited number of animal studies. In 1984 Taylor-Courval and Gloor used the feline generalized penicillin epilepsy model to study the cat's ability to respond to auditory and visual stimuli during spike-wave discharges . They found that, while a learned response was given correctly upon interictal stimulus presentations, a stimulus presentation during generalized spike-wave activity most often resulted in a complete lack of responding or in a significant increase in response latency. This response failure was attributed to '*a cognitive defect or to motor impairment associated with temporary amnesia*' (Taylor-Courval and Gloor 1984). As sometimes responses were registered, that appeared to be delayed until spike-wave activity had ended, Taylor-Courval and Gloor recognized the possibility of motor impairment not associated with amnesia or cognitive defect during spike-wave paroxysms. Vergnes et al (1991) used a strain of Wistar rats with spontaneous spike-wave discharges in a battery of behavioural tasks: spontaneous locomotion, open field behaviour, social interactions, and mouse killing behaviour were all found similar to those of control Wistars without paroxysms. Furthermore, they found that avoidance learning (shuttle box) and instrumental learning in a skinnerbox (FR-1, FR-2, FR-5) were unimpaired or had even improved. Comparable to Taylor-Courval and Gloor's experiment Vergnes et al (1991) also tested responding after

ictal and interictal stimulus presentations in a conditioned sound-bar pressing task. However, their outcomes were interpreted without suggestions about the possible underlying mechanisms: Vergnes et al (1991) indistinctly concluded that „ *only in borderline states, when motivation to act is low, the occurrence of spike-wave discharges may suppress perception of information and behavioral responsiveness* " p.103. A study by Van Luijtelaar et al (1991) completes the collection of studies on information processing and spike-wave discharges in animals: rats of the WAG/Rij strain, whose EEG spontaneously shows generalized spike-wave discharges (see chapter 2 for more details) were extensively trained to press for food in a fixed interval (FI-60). It was found that the post-reinforcement-pause was enlarged in trials with spike-wave discharges compared to trials without spike-wave discharges, whereas the additional duration of such an enlarged post-reinforcement pause was longer than the duration of the concomitant spike-wave discharges. The authors hypothesize that this disturbance in time estimation is caused by a diminishment of information processing during spike-wave discharges (Van Luijtelaar et al 1991). In conclusion, it should be noted that up to now, animal studies have not made it possible to draw conclusions concerning the characteristics, the extent, and especially the causes of the disturbances caused by generalized spike-wave discharges.

1.2.5 Spike-wave discharges: pathophysiology

After the initial description of the human electroencephalographic spike-wave pattern, attempts were made to locate the source of this phenomenon, that was at the outset regarded a consequence of a diffuse cortical disturbance (Gibbs, Davis and Lennox 1935; Gibbs, Lennox and Gibbs 1936). However, because electrocorticographic expression of the onset of the paroxysms was found to be rather variable, the origin of spike-wave discharges was soon thought to be located in subcortical structures (Gibbs, Lennox and Gibbs 1936). A pivotal role in the development of spike-wave discharges was suggested for the upper brain stem, the thalamus and the reciprocal thalamocortical projections (Lewy and Gammon 1940, Jasper and Kershman 1941; Morison and Dempsey 1942; Nieuwenhuys et al 1988). This theory, which became known as the centrencephalic hypothesis, emphasized the involvement of the medial, intralaminar thalamic structures and

the diffuse thalamo-cortical projections in regulating the symmetrical cerebral EEG spike-wave patterns (Jasper and Droogleever-Fortuyn 1947; Hunter and Jasper 1949; Penfield and Jasper 1954). Williams used both cortical and thalamic electrodes to study subcortical dynamics of spike-wave discharges in epileptic children and concluded that initiation of spike-wave discharges is dependent on thalamic mode and propagation depends on thalamo-cortical projections (Williams 1953).

In the late sixties Gloor proposed the hypothesis of 'generalized corticoreticular epilepsies', based on studies in his feline generalized penicillin epilepsy model. This hypothesis stated that diffuse moderate cortical neuronal hyperexcitability is responsible for the transformation of otherwise normal spindles into spike-wave discharges (Gloor 1969). Gloor argued that the central nervous system contributes to the genesis of generalized spike-wave discharges at three levels: first, the cortex, which must possess an increased excitability; second, the thalamus, especially the thalamic midline and intralaminar nuclei, which Gloor believed to provide a potent trigger for cortical spike-wave discharges; third, the reticular formation of the brainstem, whose inactivity is thought to favour the facilitation of spike-wave activity (Gloor 1979). As a fundamental cause for the increased cortical excitability a structural decreases in activity of inhibitory or excitatory neurotransmitters as well as minor morphological changes -generally referred to as 'microdysgenesis'- have been proposed; to substantiate these suggestions further research (e.g. genetic linkage or candidate gene analysis) is needed (Avoli and Gloor 1994; Berkovic et al 1987; Gardiner 1995).

Because to date, neurophysiological and neuroanatomical studies have led to a revision of the involvement of the reticular thalamic nuclei, as the assumed presence of afferents from the brainstem could not be clearly determined. Presently, the thalamus is thought to play a role in both the dorsal and the ventral activating reticular system (e.g. cholinergic and non-cholinergic afferents from nucleus basalis of Meynert), while the reticular thalamic nuclei appear to be connected to dorsal thalamic areas and to thalamocortical and corticothalamic pathways (Steriade and Buzsáki 1990). Steriade et al (1985) transected the connections between the reticular thalamic nuclei and the other of the thalamus of cats in order to determine their functional involvement in oscillatory activity. The authors

conclude that the rhythmical oscillations originate in the reticular thalamic nuclei, which should therefore be considered as a pacemaker. Recently, Buzsáki (1991) criticized the experiments of Steriade and postulated an alternative 'thalamic clock' hypothesis. He assumes that a network of dorsal thalamic neurons and reticular thalamic nuclei is able to generate oscillations due to its structure per se, whereby cells of the reticular thalamic nuclei are believed to hyperpolarize large numbers of thalamic relay cells. Inoue et al (1993) recorded multiple unit activity in the frontal cortex en various thalamic nuclei of WAG/Rij rats: rhythmic unit firing concurrent with the spike component of the cortical spike-wave discharge was observed in deep layers of the cortex, in the specific thalamic nuclei (ventroposterolateral, ventroposteromedial, and ventrolateral nuclei), and in the mediodorsal and the reticular thalamic nucleus, whereas a wave concurrent thalamic firing pattern was observed in the centrolateral nucleus and the paracentral nucleus. Inoue et al's findings further suggest (1993) that the paracentral and centrolateral nucleus as well as the interanteromedial nucleus have a function in the expression or maintenance rather than in the genesis of spike-wave discharges.

In short, it can be concluded that cortical spike-wave discharges represent oscillations in the thalamocortical and corticothalamic circuits with an important collateral involvement of the nucleus reticularis thalami (Avanzini, De Curtis and Spreafico 1993; Inoue et al 1993; Snead 1995). Although the thalamus is a nuclear complex within an extensive network of afferents and efferents (e.g. limbicohypothalamic complex), the rhythmic activity of the thalamic structures is modulated and controlled directly or via the intralaminar cell group by the ascending reticular activating system (Jones 1985; Steriade and Buzsáki 1990; Steriade, McCormick and Sejnowski 1993). In conjunction with neural structures located in hypothalamus and basal forebrain, this from the brainstem ascending system is thought to control level of vigilance (Bremer 1977; Steriade and McCarley 1990). Because of this neurophysiologically confirmed involvement of activating systems, the level of vigilance that favours the occurrence of spike-wave discharges, should be taken into account when the relationship between spike-wave activity and information processing is investigated.

1.3 Rationale and outline of the thesis

In all experiments presented in this thesis the WAG/Rij rat, which reliably mimics several aspects of human childhood epilepsy (see chapter 2), has been used to study the pathogenesis of spike-wave discharges and their effects on information processing. Some experiments were carried out as a consequence of questions which emerged from clinical studies; other experiments tried to answer questions of a more fundamental nature. The focus of this thesis is on the electroencephalographic phenomena of spike-wave discharges and their interaction with vigilance and cognition. It is to insight into paroxysmal electrical brain activity with a spike-wave morphology that this thesis primarily wants to contribute. An answer to the question whether or not the outcomes of the experiments described in this thesis contribute to the validity of the WAG/Rij model as a model for human childhood absence epilepsy, can be found in the quotation of Taylor-Courval and Gloor (1984):

> *„ Whether an experimental condition truly models a human disease always remains open to question, but confidence in the validity of a model grows with the number of objectively verifiable features it shares with its presumed human counterpart "* p.168.

Since the first publication by Van Luijtelaar and Coenen in 1986, various characteristics of the spike-wave discharges in the WAG/Rij model have been described and numerous hypotheses concerning many aspects of these discharges have been tested. As the occurrence of spike-wave paroxysms is not an exclusive property of the WAG/Rij strain, relevant results have been produced by studies in other animal 'spike-wave' models. Chapter two (2) of this thesis provides an extensive overview of almost a decade of research on spike-wave activity in the WAG/Rij model as well as in comparable animal models. In chapter three (3) a description is presented of the aberrant transients by means of spectral analysis of cortical EEG signals. Aim of this experiment was to differentiate between spike-wave discharges and other aberrant transients. In chapter four (4) the conditions of the brain with respect to sleep-wake states and the transitions, that are favourable for the occurrence of spike-wave discharges are determined. By describing

these conditions the vigilance-related prerequisites of the brain to generate spike-wave discharges are stipulated. Chapter five (5) deals with a provocation technique, derived from clinical practice, that is supposed to influence epileptogenesis by manipulating sleep-wake state dynamics, namely total sleep deprivation. By testing the hypothesis that changes in sleep-wake state distribution influence epileptogenesis, a twofold purpose is met: a further validation of the WAG/Rij model by applying a widely accepted, differential diagnostic tool for absence epilepsy, and a gain of knowledge with respect to the interaction of vigilance and spike-wave activity. To provide an overview of the relationship between vigilance and spike-wave discharges, in chapter six (6) a series of experiments is reported in which the interaction between the occurrence of spike-wave discharges and several other aspects of alertness have been studied (e.g. photic stimulation, REM sleep deprivation, circadian fluctuations in vigilance). Chapter seven (7) deals with the claim that information processing and spike-wave activity should be considered as mutually exclusive because during conditions favourable for the occurrence of spike-wave activity, level of vigilance is supposed to be so low that reliable cognitive testing should no longer be possible. An operant learning task was used to challenge this claim, whereby instrumental learning responses were studied in relation to the occurrence of spike-wave discharges. In chapter eight (8) an experiment is described in which information processing during spike-wave activity was tested by quantifying a physiological variable (ongoing EEG activity). A conditioning paradigm in a discrimination task was used to find out whether evaluation of the meaning of a stimulus is still possible during spike-wave activity in both cortex and thalamus. This discrimination experiment is elaborated in chapter nine (9) which presents an experiment in which an instrumental response (lever press) is required to obtain a food reward in a successive auditory discrimination task. Again, ictal information processing is studied, while in addition the ability to make an adequate motor response during as well as following spike-wave discharges was tested. Finally, in chapter ten (10) the results of the experiments and the 'state of the art' concerning spike-wave paroxysms, level of vigilance, and cognition are discussed.

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„Genetic animal models of epilepsy comprise genetically predisposed animal species in which seizures either occur spontaneously or in response to sensory stimulation. The major advantage of these naturally occurring epilepsies in animals as models of human epilepsy is that they simulate the clinical situation more closely than any other experimental epilepsy."

Wolfgang Löscher, 1984

CHAPTER 2

GENETIC MODELS OF ABSENCE EPILEPSY, WITH EMPHASIS ON THE WAG/RIJ STRAIN OF RATS[1]

[1] COENEN AML, DRINKENBURG WHIM, INOUE M, VAN LUIJTELAAR ELJM. GENETIC MODELS OF ABSENCE EPILEPSY, WITH EMPHASIS ON THE WAG/RIJ STRAIN OF RATS. *EPILEPSY RESEARCH* 12:75-86;1992.

Chapter 2 Introduction of animal models of absence epilepsy

2.1 Summary

In this chapter, the main characteristics of genetic models of absence epilepsy, in particular with respect to WAG/Rij rats, are presented. Genetic models are important and relevant, since evidence exists that these models mimic spontaneously occurring human epilepsy more than models in which epilepsy is artificially induced. Genetic models can be divided into models in which seizures are elicited and into those in which epilepsy appears without any sensory stimulation. The majority of genetic models show the absence type of epilepsy; during the last few years, we and others have noticed that rats of various strains exhibit spontaneously occurring spike-wave discharges in the EEG. Among the strains highly affected is the WAG/Rij strain, which is a fully inbred strain. Individuals are homozygous and because of this property, genetic studies are meaningful.

Electrophysiological studies have indicated that abnormal discharges in the cortical EEG are generalized and that the hippocampus is not involved. Parts of the thalamus, together with the thalamic reticular nucleus, apparently act as a pacemaker for the abnormal discharges. There is a circadian modulation in the number of spike-wave discharges. Discharges mainly occur during intermediate levels of vigilance such as passive wakefulness and light slow wave sleep and at transitions of sleep states. Pharmacological studies with clinically effective anti-epileptic drugs have shown a close agreement in seizure response between man and rat. Studies with new compounds have emphasized the role of the GABAergic and glutamatergic system in this type of epilepsy. Particularly striking is the role of the GABAergic system. GABA agonists enhance and GABA antagonists reduce the occurrence of spike-wave discharges, which deviates from the effects of GABAergic drugs in convulsive epilepsy. Even more striking is the role of the benzodiazepines, generally seen as GABA agonists; these drugs do not act as such in absence epilepsy since they reduce spike-wave discharges. Also good evidence for an involvement of other neurotransmitters such as noradrenaline, dopamine and opioid peptides exists in absence epilepsy.

Genetic data obtained from the WAG/Rij model for absence epilepsy, show a relatively simple pattern of inheritance with one gene determining whether an individual is epileptic or not, and with other genes regulating the number and duration of seizures. This is in good agreement with the more restricted human data. Cognitive studies have shown two important features of epilepsy in the WAG/Rij strain: modulation of the number of spike-wave discharges by mental or physical activity and on the other hand, the disruption of cognitive activity by spike-wave discharges. Finally, it is concluded from the aberrant shape of visual evoked potentials obtained during spike-wave activity, that sensory processing during spike-wave discharges is different from that occurring during normal states of vigilance. These differences may correlate with changes in cognitive functioning during absences.

2.2 Introduction

During the last decade, considerable progress has been made in the development and evaluation of new animal models of epilepsy. Löscher and Schmidt (1988) pointed out that an ideal model should have a type of seizure similar in its clinical phenomenology to those occurring in humans and that the seizure should be associated with abnormal activity in the EEG. A correspondence with respect to the age of seizure onset should be present and the response of seizures to anti-epileptic drugs should show a parallelism with epilepsy in man. Currently no single model meets all these criteria. Löscher (1984) strongly favours the use of genetic models for generalized epilepsies, including absences. Genetic factors play a role in absence epilepsy in man. Strong evidence for a genetic involvement was obtained from twin studies: among monozygotic twins, absence epilepsy is more likely to be present in both individuals compared to dizygotic twins. A multifactorial mode of inheritance has been suggested (Andermann 1982). Therefore, genetic animal models resemble absence epilepsy in man more closely than models in which absences are experimentally induced (Löscher 1984).

Genetic animal models can be subdivided into models in which the seizures are evoked by external stimuli and into those in which the seizures have a spontaneous nature. The first category includes the mongolian gerbil in which seizures can be elicited by diverse

stimuli such as a change in environment, handling or a blast of air (Frey et al 1983); the El strain of mice which shows seizures when repeatedly tossed up (Imaizuma 1964); the audiogenic seizure prone mice or rats which after an intense auditory stimulation often experience violent, sometimes fatal, generalized convulsions (Consroe 1979; Seyfried 1985) and the photo-sensitive baboon (Papio Papio) in which intermittent light stimulation provokes spike-wave discharges, myoclonus and tonic-clonic seizures (Naquet and Meldrum 1972). In models in which the paroxysmal activity is not triggered by a defined stimulus or treatment, the epilepsy is called spontaneous, meaning that the precipitating event is either nonexistent or unknown. Most genetic models with spontaneous epilepsy concern inbred lines of rodents in which absence-like epilepsy particularly occurs. However, in some models another type of epilepsy is present as well as absences. Maxson et al. (1983) described a mouse mutant (C57BL/10Bg) in which absences alternate with generalized tonic-clonic convulsions, whereas Serikawa and Yamada (1986) reported on the existence of a double mutant rat (SER), exhibiting both absence-like seizures and tonic convulsions.

2.3 Genetic models of absence epilepsy

Noebels (1984) described 14 single-gene mutations in mice that initiate spontaneous and non-convulsive patterns of epilepsy. These mice were initially identified by motor impairments and thus show abnormalities in addition to their seizures. One substrain is the 'tottering' mouse whose behaviour is characterized by a stereotyped triad of ataxia, motor seizures and generalized cortical spike-wave discharges, accompanied by behavioral arrest. This implies that two different types of seizures are present in the tottering mouse of which the motor seizures are not associated with abnormal EEG discharges. Pharmacological studies reveal a different profile for the two seizure types: spike-wave discharges can be blocked by ethosuximide, diazepam and phenobarbital and not by phenytoin, while the motor seizures are not affected by ethosuximide but are blocked by diazepam (Heller et al 1983). Brains of tottering mice have been compared to those of wild mice. It was found that the brain of the tottering mouse was characterized by hyperinnervation of regions containing noradrenaline as neurotransmitter. Newborn mice in

which the development of noradrenergic terminal density was prevented, did not develop seizures, indicating that noradrenergic innervation is involved in the pathogenesis of this type of epilepsy (Levitt and Noebels 1981).

Ryan (1984) reported that in some inbred strains of mice, such as DBA/2 and C57BL/6, short bursts of high amplitude spindles with an intraspindle frequency of 6.7 to 7.1 Hz, about 1.5 sec duration and an amplitude of 800 μV can be found. This description as well as a figure of the phenomena, clearly mimics what we and others have called a spike-wave discharge. These discharges are present during quiet waking in DBA/2 mice, but never during waking in the C57BL/6 strain. A rostropontine transsection which disconnects the locus coeruleus from the adjacent diencephalon, does release profuse spike-wave discharges. In preparations in which the transsection is made caudal to the locus coeruleus, the number of spike-wave discharges remains unchanged. These studies clearly emphasize the role of rostral parts of the brainstem, including the locus coeruleus. The locus coeruleus inhibits spike-wave discharges by exerting an arousing effect on higher parts (Ryan 1984).

Several laboratories have observed that a certain percentage of rats of their breeding colony shows spontaneous EEG seizures accompanied by behavioral arrest and vibrissal twitching. Klingberg and Pickenhain (1968) mentioned that 15 - 20 % of an unspecified inbred strain of rats, showed spike-wave discharges in a frequency between 7 and 10 Hz, although these authors used the term spindles. These spike-wave discharges were also described by Robinson and Gilmore (1980) in 10 out of 108 male Charles River CD adult rats, by Aldinio et al. (1985), by Kleinlogel (1985) and by Buzsáki et al. (1990) in Sprague Dawley rats. Aldinio et al. (1985) found that 15 % of Sprague Dawley rats at the age of 6 months showed spike-wave discharges, Buzsáki et al. (1990) reported no spike-wave discharges at an age of three months, but an incidence of 71.4 % at 26 months. Kleinlogel (1985) found that 20 % of the rats were affected at an age of six months. Semba and Komisaruk (1984) and Semba et al. (1980) noticed the presence of spike-wave discharges in 50 % of female Long Evans rats between 8 - 11 months. Also the third commonly used random bred albino rat, the Wistar rat, shows an age dependent increase in the number of spike-wave discharges; recently we found that at 22 months the incidence is

above 90 %. Vergnes et al. (1990) described EEG discharges in 24 out of 63 male and female Wistar rats which were 6 to 12 months old. Marescaux et al. (1984; 1984) selected and cross-bred their Strasbourg stock and managed to increase both the number of spike-wave discharges and the incidence. Generalized non-convulsive seizures appear at 40 - 120 days of age and last throughout life-time with an increase in number and duration with age (1989). In all strains studied so far, there is an age-dependent increase in the number of animals showing the phenomena, in both the number and the mean duration of the spike-wave discharges (Aldinio et al 1985; Buzsáki et al 1990; Coenen and Van Luijtelaar 1987; Vergnes et al 1986). Aldinio et al (1985) suggested that the age-related EEG abnormalities could even be used as a model for aging. It is clear that the development in man and rat does not run completely parallel: absence epilepsy is a childhood disease, which may disappear or transforms into a more serious type of epilepsy, while in rats spike-wave discharges appear around puberty and do not diminish throughout life.

Typical anti-absence drugs decrease spike-wave activity in a dose-dependent way, while drugs ineffective in absence epilepsy but effective in tonic-clonic epilepsy, have no decreasing effect or even aggravate absence seizures (Micheletti et al 1985[B]). Vergnes et al. (1982) found that in immobilized rats high amplitude spike-wave activity appeared in the lateral thalamic nuclei just before or together with cortical spike-wave discharges. No spike-wave discharges were recorded from the medial thalamus and from the cingulate cortex or the hippocampus, whereas spike-wave discharges of a lower amplitude were found in the striatum, the tegmentum and the substantia nigra. Injection of the GABA agonist muscimol in the latter structure, results in a dose-dependent decrease in spike-wave discharges in the cortex as well as in the thalamus, emphasizing the role of the substantia nigra (Depaulis et al 1988). The involvement of two output systems of the substantia nigra, the dopaminergic nigro-striatal pathway as well as the GABAergic nigro-thalamic and nigro-collicular pathway was studied. After lesions, hemisections or local injections, it appeared that this dopaminergic pathways has no effects on spike-wave discharges. In contrast, manipulations involving the superior colliculus showed that the GABAergic nigro-collicular pathway is important in the inhibitory control of the substantia nigra

(Depaulis et al 1988).

In studying the role of the corpus callosum in interhemispheric synchronization of spike-wave discharges, it was found that this pathway is important but not absolutely required for bilateral synchronization (Vergnes et al 1989).

Buzsáki (1991) and Buzsáki et al. (1988; 1990) used inbred Fischer 344 rats; spike-wave discharges were termed 'high voltage spindles', but the electroencephalographic, ontogenetic and pharmacological properties of these spindles seem identical to those of spike-wave discharges. Buzsáki (1991) correlated single and multiple unit activity of various limbic and thalamic structures in freely moving rats of the Fischer strain with comparable epileptic seizures and found that cortical spike-wave discharges are not correlated with unit activity in the hippocampus, but that units in various nuclei of the thalamus generate action potentials phase-locked with the peak of the cortical spike-wave discharge. In fact, thalamic unit activity precedes the cortical spike-wave discharge confirming the thalamic origin of the spike-wave discharges. Vergnes et al. (1982) found similar results with field potentials. Buzsáki et al. (1988) propose that properties of a thalamic network generate spike-wave discharges.

As already mentioned, an interesting rat with two mutations was recently described by Serikawa and Yamada (1986). By breeding the zitter-mutant of Sprague Dawley rats with tremor Wistar-rats, they obtained F-1 hybrids who could be homozygous for two mutations, zitter and tremor. After 8 weeks of age, these rats spontaneously exhibit staggering gate, vacuole formation, absence seizures and tonic convulsions. Therefore, these hybrids can be regarded as an animal model for both convulsive and non-convulsive epilepsy. Absence seizures are inhibited by trimethadione and ethosuximide, convulsions by phenytoin, and both types of seizures by phenobarbital and sodium valproate (Sasa et al 1988). The EEG characteristics of the seizures were low voltage fast spikes during tonic convulsions and 5-7 Hz spike-wave like discharges in both cortical and hippocampal EEG. The presence of spike-wave discharges in the hippocampus next to the cortex seems unique for these hybrids for the reason that this is not found in all other absence models (Sasa et al 1988).

2.4 The WAG/Rij model of absence epilepsy

In the last five years, we have described an additional rat model for absence epilepsy, the Wistar Albino Glaxo strain, bred in Rijswijk, The Netherlands (Altman and Katz 1979; Van Luijtelaar and Coenen 1986; Van Luijtelaar and Coenen 1989). The name of the strain is generally abbreviated as WAG/Rij. The model shares many features with, for instance, that of the Strasbourg group of Marescaux and Vergnes (e.g. Marescaux et al 1984; Vergnes et al 1990), but has some particular traits. The WAG/Rij 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. Therefore, rats from this strain offer an eminent possibility to study the genetic background and heredity of absence epilepsy (Inoue et al 1990; Peeters et al 1990[A]). Furthermore, the rats are fertile and show no signs of behavioral abnormalities. Recently, Frey and Voits (1991) also used another type of WAG rats, the WAG/Ola/Hsd strain, also as a model for absence epilepsy.

WAG/Rij rats show spike-wave discharges in the cortical EEG. These discharges have a frequency of 7-11 Hz, a duration of 1 to 45 sec and an amplitude of 200-1000 μV. The number of spike-wave discharges and their mean duration increase with age whereas sex differences are minimal (Coenen and Van Luijtelaar 1987). Rats of an age of six months show 16-18 spike-wave discharges per hour with a mean duration of 5 sec, adding up to approximately 300-400 spike-wave discharges and half an hour of abnormal EEG activity per day. Spike-wave discharges are bilaterally symmetrical and generalized over the cortex (Van Luijtelaar and Coenen 1989).

It was questioned whether spike-wave activity could also be registered in the hippocampus, a structure which is highly susceptible to epileptoformic activity. To that end, rats were equipped with hippocampal as well as cortical electrodes and hippocampal and cortical EEG were simultaneously recorded. Although spike-wave discharges were abundantly present in the cortical EEG, this was not the case in the simultaneously registered hippocampal EEG (Van Luijtelaar and Coenen 1989).

In addition to electrophysiological signs, behavioral concomitants of epilepsy were studied by videotaping the rat's behaviour just before, during, and after the occurrence of spike-wave discharges. As is the case in human absence epilepsy, spike-wave discharges

were preceded by immobile behaviour. In addition, minor changes in behaviour were generally seen during the presence of a spike-wave discharge. In our rats, 84 % of the seizures were accompanied by vibrissal twitching, 61 % by accelerated breathing, 48 % by head tilting and 12 % by eye twitching. Otherwise the animals were immobile. It was concluded that both electrophysiological and behavioral manifestations were reminiscent of human absence epilepsy (Van Luijtelaar and Coenen 1986).

The states of vigilance, such as wakefulness, REM and non-REM sleep, in which discharges by preference occur, were also established in WAG/Rij rats (Coenen et al 1991; Drinkenburg et al 1991). In 33 % of all cases, spike-wave discharges were preceded by passive wakefulness and 48 % by light slow-wave sleep. Deep slow-wave sleep had an intermediate score (13 %), while during active wakefulness and during REM sleep, spike-wave discharges rarely occurred. The prevalence for spike-wave discharges to occur at drowsiness and light slow-wave sleep is also found in man. The sleep-wake states preceding and following the discharges were different in the vast majority of discharges, indicating that at unstable periods such as transitions of vigilance, spike-wave discharges can easily break through. The similarity in occurrence with normal sleep spindles suggests that spike-wave discharges might belong to the same class of phenomena (see chapter 4).

It is also of interest to know that in rats of the WAG/Rij strain a certain sleep period, the intermediate stage, deviates from that of a non-epileptic strain. Intermediate stage occurs at the transition from slow wave sleep to REM sleep and may be considered as a trigger for REM sleep. In collaboration with researchers from the University of Nice, it was established that the intermediate stage in WAG/Rij rats is longer lasting and that WAG/Rij rats also have less REM sleep compared to Wistar rats (Gandolfo et al 1989). Whether the deviating characteristics of the intermediate stage are indeed related to epilepsy as such, should be further investigated. Finally, it was established that deprivation of REM sleep reduced the number of spike-wave discharges (Peeters, Van Luijtelaar and Coenen 1989). This result is in agreement with clinical data and is explained by the fact that this type of deprivation results in an increase of arousal. In contrast with this, are the effects of total sleep deprivation, inducing sleepiness and drowsiness. Preliminary data of our group indeed suggest that epileptic activity increases as total sleep deprivation

continues (see chapter 5). Sleep deprivation is clinically effective as an epilepsy provoking technique.

Important for a putative model is that the number and duration of seizures is reliable in the sense that there is a high correlation between two consecutive days. Nineteen adult male WAG/Rij rats were left undisturbed and continuous EEG recordings were made for 48 h. On an hour-to-hour basis, number and duration of spike-wave discharges were determined (Van Luijtelaar and Coenen 1988). Next, a cosine was fitted through the 48 h data in order to establish the presence of a circadian rhythm. This analysis revealed a clear circadian pattern for the number of spike-wave discharges with a maximum between the fourth and fifth hour of the dark period, whereas the minimum of the cosine just fell after the onset of light. Interestingly, this minimum coincided with the time of day that rats have the largest amounts of deep slow-wave sleep, also indicating that this type of sleep is not favourable for the occurrence of spike-wave discharges. Human data also contain indications for a 24 h rhythm, with a maximum likelihood for spike-wave discharges to occur at early morning awakenings (Martins da Silva et al 1984).

2.4.1 Pharmacological studies

An important issue for a model is its pharmacological profile. Ideally, anti-epileptic drugs specifically prescribed for generalized absence epilepsy should suppress spike-wave discharges. On the other hand, other anti-epileptics such as those for tonic-clonic convulsions, should have no effect upon, or should even aggravate, epileptic activity in this model. Ethosuximide and trimethadione were selected as anti-*absence* drugs and diphenylhydantoin and carbamazepine were used as examples of anti-*convulsant* drugs. The results are presented in Figure 2.1; only the anti-absence drugs caused a decrease in the number of spike-wave discharges, while the anti-convulsive drugs triggered a substantial increase in the number of spike-wave discharges (Peeters et al 1988). These results closely correspond to what others have found, both for rats (Micheletti et al 1985[B]) and for humans. Therefore, we consider the outcomes of this pharmacological evaluation as strong arguments for the specificity of the model for absence epilepsy in man (Micheletti et al 1985[B]; Peeters et al 1988). There is only one less positive report on the

pharmacological profile of the genetic models for absence epilepsy: Wahle and Frey (1990) found that rats treated with valproate showed only a temporary effect, with subsequent tolerance to the anti-convulsant effects of valproate.

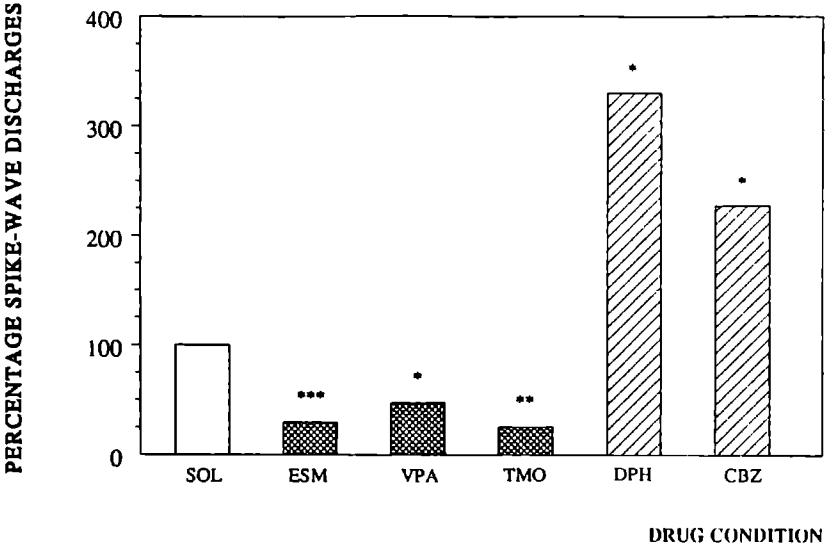

Figure 2.1 Effects of various anti-convulsants on the number of spike-wave discharges. Ethosuximide (ESM; 25 mg/kg), valproate (VPA; 100 mg/kg), trimethadion (TMO; 100 mg/kg), diphenylhydantoin (DPH; 40 mg/kg) and carbamazepine (CBZ; 40 mg/kg). Differences with control (sol) are indicated, *p<.05, **p<.01, ***p<.001.

Further pharmacological studies in collaboration with Schering AG at Berlin, were concentrated around a new type of benzodiazepine-like compounds, the beta-carbolines. These carbolines constitute an interesting new class of drugs which might be more specific compared to the classical benzodiazepines with their broad spectrum of activities: some beta-carbolines do not share all the side-effects of the benzodiazepines. Besides anxiolytic and anti-convulsive effects, benzodiazepines also have sleep promoting effects. Also well-known are the impairments of motor coordination and the induction of anterograde amnesia, together with the changes of the spectral content of the EEG. We tested the partial benzodiazepine agonist ZK 91296 and the inverse agonist FG 7142 in our model. ZK 91296 suppresses the number of spike-wave discharges in a dose-dependent way, without inducing sedation or sleep, and without altering the background activity of the

EEG (Coenen and Van Luijtelaar 1989). For this reason, it is thought that ZK 91296 is a putative anti-epileptic with fewer adverse effects (Coenen and Van Luijtelaar 1989). FG 7142 promotes spike-wave discharges as was anticipated. A newer beta-carboline as abecarnil (ZK 112 119), one of the first beta-carbolines with a strong anti-anxiety profile and adequate bioavailability in man, also suppresses the number of spike-wave discharges without inducing motor disturbances. Further studies towards the hypnotic effects of abecarnil showed that these effects are significantly smaller than those of the classical anxiolytic diazepam (Van Luijtelaar, Stephens and Coenen 1990).

Further pharmacological studies concern the GABA and glutamate systems. It is thought that seizures are generated when the excitatory glutamatergic system is in imbalance with the inhibitory GABAergic system. The glutamate antagonist, MK-801, decreases the number of spike-wave discharges in a dose-dependent way (Peeters et al 1989[A]). However, MK 801 gives rise to bizarre behavioral side-effects which makes this drug not useful for clinical applications (Peeters et al 1989[A]). Additionally and surprisingly, it was found that the GABA agonist muscimol enhances the number of spike-wave discharges (Micheletti et al 1985[A]; Peeters et al 1989[B]), while the GABA antagonists bicuculline reduces the number of spike-wave discharges in the WAG/Rij model (Peeters et al 1989[B]). Since it is known that the GABA agonist muscimol reduces convulsive epilepsy and the GABA antagonists bicuculline and picrotoxine enhances this type of epilepsy (Micheletti et al 1985[A]; Morita et al 1985; Olsen 1981), these results give further support to the differential pharmacological profile of convulsive and non-convulsive epilepsy (Micheletti et al 1985[B] ; Peeters et al 1988). These differential effects of GABAergic compounds on convulsive and non-convulsive epilepsy mean that the GABAergic system is differentially involved in these two types of epilepsy. The absence increasing properties of the agonists and the absence reducing properties of the antagonists, can only be explained by assuming a hyperactivation of the GABA system; a statement which can already be found in the literature (Myslobodsky 1984). On the other hand, the glutamatergic system plays an identical role in the two main kinds of epilepsy: agonists facilitate both convulsive and non-convulsive epilepsy and antagonists reduce convulsive as well as non-convulsive epilepsy. Table II gives a survey of the involvement

of the two neurotransmitter systems.

Table II Overview of GABA, benzodiazepine and glutamate systems in convulsive and non-convulsive epilepsy.

A decrease in epileptic activity is indicated with ↓, an increase with ↑ The numbers refer to the literature employed and listed at the bottom of the table Note the opposite profile of the GABAergic compounds for convulsive and non-convulsive epilepsy Note also that the profile of the benzodiazepine system, in particular that of non-convulsive epilepsy, is not identical to that of GABAergic compounds Finally, the profile of the glutamatergic compounds is similar for the two types of epilepsy

			Convulsive	Non-convulsive
GABA	agonist:	muscimol	↓ [10 11]	↑ [9 13]
	antagonist:	bicuculline	↑ [11]	↓ [9 13]
		picrotoxine	↑ [9,11]	↓ [8]
Benzodiazepine	agonist·	diazepam	↓ [5]	↓ [3 9]
		ZK 91296	↓ [15]	↓ [3,7]
	inverse agonist:	FG 7142	↑ [1]	↑ [3]
Glutamate	agonist:	NMDA	↑ [6]	↑ [14]
	antagonist:	APH	↓ [4 16]	↓ [14]
		MK 801	↓ [2]	↓ [12]

(1) Braestrup et al 1982, (2) Clineschmidt, Martin and Bunting 1982, (3) Coenen and Van Luijtelaar 1989, (4) Croucher, Collins and Meldrum 1982, (5) Gross and Kallenbach 1963, (6) Herron et al 1986, (7) Jensen et al 1984, (8) King 1979, (9) Micheletti et al 1985, (10) Morita et al 1985, (11) Olsen 1981, (12) Peeters et al 1989[A], (13) Peeters et al 1989[B], (14) Peeters et al 1990[B], (15) Petersen et al 1984, (16) Piredda and Gale 1986

From this table, a differential role of the GABA-ergic system in convulsive and non-convulsive epilepsy can be noticed. The same is the case with alpha-2-agonists: they facilitate spike-wave discharges but inhibit convulsive seizures (Buzsáki et al 1990). From Table II another intriguing phenomenon emerges: the action of benzodiazepine agonists corresponds with the action of GABA agonists in convulsive epilepsy, but the latter have opposite effects in non-convulsive epilepsy. It seems that benzodiazepines, commonly acting as GABA agonists, do not act as such in absence epilepsy!

Finally, there is both pharmacological and biochemical evidence for the involvement of opioid peptides and dopamine in this type of epilepsy. Dopaminergic drugs and opiates modulate the number of spike-wave discharges (Buzsáki et al 1990; Frey and Voits 1991;

Lasón et al 1990; Warter et al 1988). In addition, higher levels of regional dopaminergic activity in nigro-striatal parts of the brain were found in epileptic rats compared to a non-epileptic control strain (Buzsáki et al 1990). We found higher levels of pro-enkephalin in striatum and mesencephalon in WAG/Rij rats, compared to three age and strain matched controls (Lasón et al 1990). At first glance it might be striking that many compounds have an effect on the number of spike-wave discharges, but considering the intimate relationship between spike-wave discharges and the level of vigilance, it can be expected that any drug which influences vigilance or sleep, will alter the number of spike-wave discharges.

2.4.2 Genetic studies

In a recent study where various strains of inbred rats were compared, it was found that, besides the WAG/Rij strain, other inbred strains such as the BN/BiRij, G/Cpb, and B/Cpb, also show spike-wave discharges in the EEG, but to a much lesser extent than the WAG/Rij strain (Inoue et al 1990). This suggests that absence epilepsy in rats is a more common phenomenon than is often assumed. It seemed, therefore, difficult to trace a strain of rats which is completely free of spike-wave discharges. Fortunately, virtually no spike-wave discharges were detected during a 48 h recording session in the ACI strain (Inoue et al 1990; Peeters et al 1990[A]). This implies that we have available an epileptic (WAG/Rij) as well as a non-epileptic strain (ACI, also called AxC9935/Kun). Both strains are fully inbred, i.e. after 30 generations brother-sister mating, homozygous animals were obtained. Peeters et al. (1990[A]) initiated a Mendelian cross breeding study including backcrosses with both parental strains (ACI and WAG/Rij), in order to establish the heredity patterns of spike-wave discharges. The results in the F1 showed that all offspring had spike-wave discharges, whereas 79 % of the F2 showed spike-wave discharges. Furthermore, 37 % of the offspring of the combination of the F1 with the non-epileptic parental strain showed spike-wave discharges, and 95 % of the offspring of the F1 with the epileptic parental strain. Detailed quantitative genetic analyses revealed a simple pattern of heredity, suggesting that only one gene with dominant inheritance, determines whether an animal is epileptic or not, while other, modulating genes determine the absolute number of spike-wave discharges (Peeters et al 1990[A]; 1992). From the limited

literature on genetics of human absence epilepsy, the suggestion was obtained that dominance is also present and that relatively few genes determine the severity of this type of epilepsy.

2.4.3 Cognitive studies

In man, the abnormal brain activity characteristic for the various types of epilepsy is accompanied by impairments in cognitive functioning. This impairment ranges from a hardly noticeable interference with mental processes to a complete arrest of cognitive activity. The primary dysfunction of absence epilepsy is a brief lapse in the patient's ability to maintain contact with the environment.

An index for cognitive activity is derived from a temporal discrimination task: fixed-interval responding. In this task, food reward follows the first lever-press, but only after the passing of a specified amount of time. This means that lever presses made before this time has elapsed, remain without consequences. During training, a characteristic pattern of lever press responses develops: a period without any responding, the post-reinforcement pause, which is followed by an increased rate of lever-pressing. The post-reinforcement pause lasts about 50 % to 80 % of the interval. The hypothesis was that the presence of a spike-wave discharge will affect the timing of a fixed interval in that trial, compared with trials without such a discharge. It turned out that the post-reinforcement pause was significantly prolonged in trials with spike-wave discharges [58.4 ± 6.8 s, means and standard deviations] compared to trials without discharges [37.2 ± 7.1 s] (Van Luijtelaar et al 1991). The results show that spike-wave discharges prolong the duration of the post-reinforcement pause. Moreover, it was found that the prolongation exceeds the duration of the spike-wave discharge, suggesting that more time is 'missed' by the animal than the actual duration of the discharge.

A second remarkable result was that during the learning task, a significantly lower number of spike-wave discharges was found, compared to the preceding and succeeding base-line hour (Figure 2.2) This result is in line with the well documented relationship between vigilance, arousal and absence epilepsy in man and in rat (Clineschmidt, Martin and Bunting 1982). Finally, it was established that the outcomes of the learning study

could also be achieved in children suffering from primary generalized absence-epilepsy, in particular when the spike-wave discharges are short (Van Luijtelaar et al 1991). The common performance changes in man and rat contribute to the validity of the model.

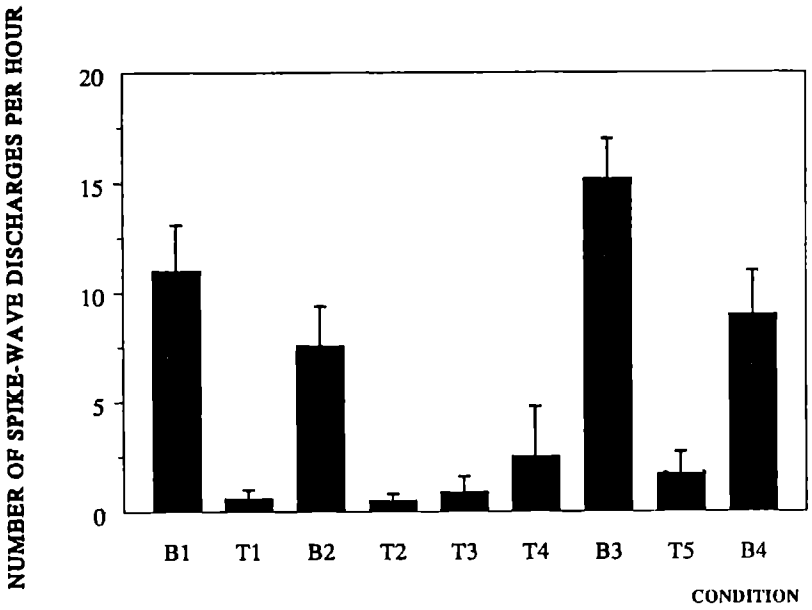

Figure 2.2 Means and standard error of means of the number of spike-wave discharges before (B1 and B3, base-line), during (T1-T5) and after (B2 and B4, base-line) the learning task. Note the large difference in the number of spike-wave discharges between the base-line and the task.

2.4.4 Visual evoked potentials studies

The mechanisms underlying the cognitive disturbances during spike-wave discharges remain a mystery. At first glance, brain functioning during these discharges shares some features with sleep, such as the lowering of responsiveness. In order to study this in further detail, visual evoked potentials were induced during spike-wave discharges and compared to those obtained during normal states of vigilance such as quiet wakefulness, slow wave sleep and REM sleep (Inoue et al 1992). Almost similar potentials were recorded during wakefulness and REM sleep, whereas during slow-wave sleep the P2 was considerably higher. In comparison to these normal sleep-wake states, visual evoked potentials during spike-wave discharges showed unique changes such as a decrease in the N1 amplitude, an

increase of the P4 amplitude and an enhanced afterdischarge (Figure 2.3). Other characteristics, such as the increase of P2 and the diminished P2-N3-P3 complex, were similar to those seen during slow-wave sleep. These findings indicate a hyper-synchronization in thalamus and cortex, which seems to be the result of a powerful

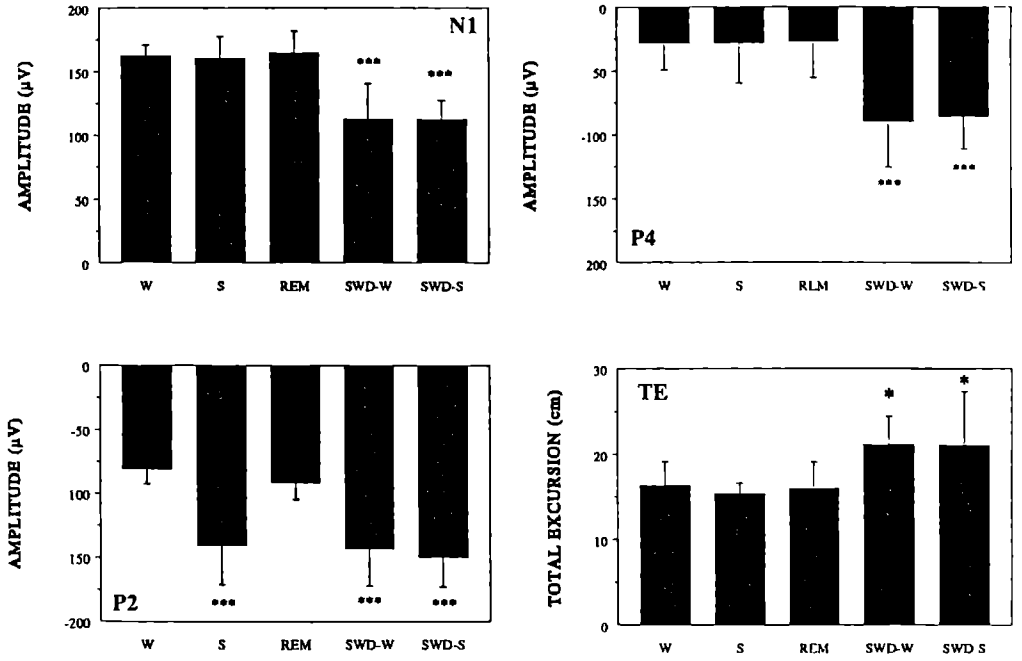

Figure 2.3 Means and standard deviations of the amplitudes of the various components of the visual evoked potential (N1, P4, P2, TE) during quiet wakefulness (W), slow wave sleep (S), REM sleep (REM), spike-wave discharges following wakefulness (SWD-W) and spike-wave discharges following sleep (SWD-S). * $p< 05$; *** $p<.001$). (TE is the total excursion of the afterdischarge)

recurrent inhibition together with a strong rebound excitation. As a result, excitation and inhibition are in imbalance. This agrees with the suggestion made earlier that the GABAergic inhibitory system is too active. As a consequence, the thalamus and cortex are in a deviant mode and sensory information cannot enter the thalamus (Inoue et al 1992). These aberrations may underlie the cognitive and sensory disturbances during absence epilepsy.

2.5 Epilogue

Considering all electrophysiological, pharmacological, genetic and cognitive data, it can be concluded that the WAG/Rij strain of rats is an interesting, additional model for absence epilepsy in man. It is striking that the various rat models described in the literature (Wistar Strasbourg rats [Marescaux et al 1984[A]; Marescaux et al 1984[B]; Vergnes et al 1986], Fischer 344 rats [Buzsáki et al 1988; Buzsáki et al 1990; Buzsáki 1991], Sprague-Dawley or Charles River rats [Aldinio et al 1979; Kleinlogel 1985], Long-Evans rats [Semba, Szechtmann and Komisaruk 1980; Semba and Komisaruk 1984]) share many phenomena with the WAG/Rij strain, including the EEG characteristics and the pharmacological profile. It is likely that all these strains can be regarded as identical models, which implies that data can be extrapolated from one strain to another. All models are profitable for gaining further insight into the genesis of human absence epilepsy. The WAG/Rij strain and the Fischer 344 have a unique feature; they are inbred strains meaning that all individuals are homozygous. This opens the way for studies into the genetic background of absence epilepsy and to the mechanisms by which genetic material causes this aberration.

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„THE CAUSAL CONNEXION BETWEEN SPIKE AND WAVE ACTIVITY AND ALTERATIONS IN CONSCIOUSNESS DESERVES SPECIAL INVESTIGATION, SINCE IT APPEARS THAT GROSS ELECTROENCEPHALOGRAPHIC CHANGES ARE RELATED TO MENTAL CHANGES BUT THAT THE POINTS IN TIME AT WHICH THESE EVENTS OCCUR DO NOT CORRELATE."

L. GOLDIE AND J.M. GREEN, 1961

CHAPTER 3

ABERRANT TRANSIENTS IN THE EEG OF EPILEPTIC RATS: A SPECTRAL ANALYTICAL APPROACH[1]

[1] DRINKENBURG WHIM, VAN LUIJTELAAR ELJM, VAN SCHAIJK WJ, COENEN AML. ABERRANT TRANSIENTS IN THE EEG OF EPILEPTIC RATS: A SPECTRAL ANALYTICAL APPROACH. *PHYSIOLOGY & BEHAVIOR* 54:779-783;1993.

Chapter 3 Aberrant transients in WAG/Rij rats

3.1 Summary

Aberrant transients in the cortical electroencephalogram of rats of the epileptic WAG/Rij strain were studied by means of spectral analysis. The EEG of rats of this strain contains, besides normal sleep spindles, high voltage spiky phenomena, epileptic spike-wave discharges, and deviant intermediate stage. Spectral analysis of these transient phenomena shows that some features, like their peak frequency, are alike, but that they differ in other spectral characteristics, as in the first harmonic of the peak frequency and in the domain of the high frequencies. The results provide arguments for the view that spike-wave discharges might be considered as unique aberrant phenomena, presumably related but dissimilar to the high voltage spiky phenomena and intermediate stage.

Next to this, spectral analysis was used to study the intra-phenomenal dynamics of spike-wave discharges. The peak frequency was found to decrease monotonously from about 10 Hz at the beginning of the spike-wave discharge to about 8 Hz at the end. Other spike-wave discharge frequency bands, showed an intra-phenomenal increase followed by a decrease. These time-variant EEG dynamics in spike-wave discharges might correlate with the cognitive disturbances during absence seizures in man.

3.2 Introduction

In the cortical EEG of rats, sleep spindles and intermediate stage can be distinguished as transient phenomena. Besides these physiological transients, several lines of in- and outbred strains of rats show also spontaneously occurring spike-wave discharges and high voltage spiky phenomena (Buzsáki et al 1988; Inoue et al 1990; Van Luijtelaar and Coenen 1986; Vergnes et al 1987). Among those strains is the WAG/Rij strain, whose members all show numerous spike-wave discharges. The behavioural, pharmacological, and genetic characteristics of these spike-wave discharges have been extensively studied and are thought to resemble seizures seen in human absence epilepsy (Coenen et al 1992; Van Luijtelaar and Coenen 1989). Furthermore, WAG/Rij animals show extended periods

of intermediate stage compared to random bred Wistar rats (Gandolfo et al 1990). Therefore, the WAG/Rij strain is a useful model for studying abnormal transient EEG phenomena, like the spike-wave discharges, the high voltage spiky phenomena, and the long-lasting intermediate stage. This was done with the aid of spectral analysis.

In addition, while studying visual evoked potentials (VEP) during spike-wave discharges, it was found that some components of these VEPs differed in a unique way from VEP components found during the normal sleep-wake states (Inoue et al 1992). Moreover, when studying the intra-phenomenal dynamics of a spike-wave discharge, it appeared that the largest changes in VEP components were present in the middle part of these discharges, suggesting a time-varying process. Since in rats intraseizure dynamics of this type are scarcely studied, the second purpose of the present study was to describe, also with the aid of spectral analysis, the intra-phenomenal spectral dynamics of a spike-wave discharge.

3.3 Methods

Subjects and surgery. Nine male WAG/Rij rats, bred in our laboratory, with an age of about 9 months and body weights between 340 and 390 g were used. Ancestors were purchased from the REPGO-institute of TNO at Rijswijk, The Netherlands. They were singly housed in Makrolon cages and received tap water and standard rat chow ad libitum. The animals were consistently kept at a 12-12 hour light-dark regime with bright lights on at 1700 h. A fortnight before the experiments started, all subjects were, under deep anesthesia (pentobarbital, 60 mg/kg, IP), chronically provided with a tripolar (Plastics One, MS 333/2-A) and a bipolar EEG electrode (Plastics One, MS 303/2). Two active electrodes were placed frontally in the cortex with tips 1 mm apart and the other two active electrodes in the parietal cortex, also with their tips 1 mm apart. The reference electrode was placed in the cerebellum. This set-up allowed registration of a frontal and a parietal EEG. Stereotaxic coordinates were, respectively, A 2.0, L 2.1; A -6.5, L 2.1 for frontal and parietal electrodes with skull surface flat and bregma zero-zero.

Polygraphic recording and spectral analysis. An Elema-Schönander polygraph was used to amplify and filter the EEG signals, which contained frequencies between 1 and 70 Hz. Recordings were made during 2 h on paper and on magnetic tape (SE 7000),

starting at 1000 hour. EEG transients were selected if agreement existed between two EEG analysts. Three types of transients were studied: intermediate stage, spike-wave discharges, and spiky phenomena (Fig. 3.1). Criteria for intermediate stage were: high amplitude

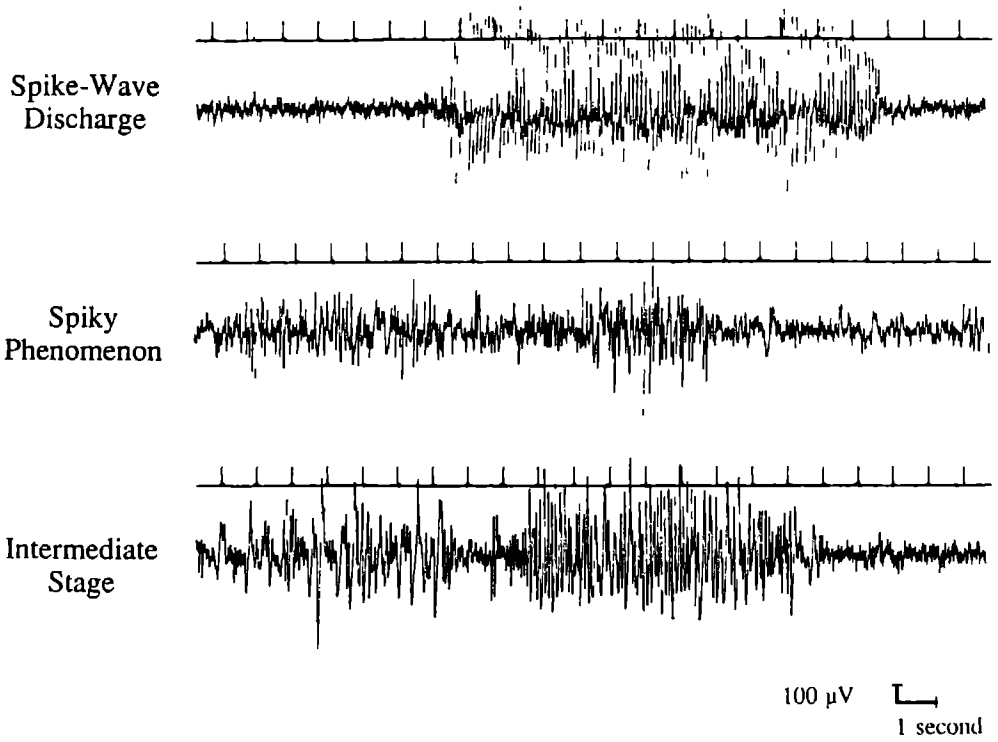

Figure 3.1 Representative examples of the three investigated aberrant transients as present in the frontal EEG of WAG/Rij rats. On top a spike-wave discharge (A), in the middle a typical spiky phenomenon (B), and at the bottom an intermediate stage phase (C) is shown.

frontal cortex spindles associated with low frequency parietal theta rhythm (as elaborated by Gandolfo et al 1990); for spike-wave discharges: high amplitude, frontal, asymmetric 7-10 Hz spike-waves, lasting at least 1 second [as elaborated by Van Luijtelaar and Coenen 1986); and for high voltage spiky phenomena: high amplitude, at least twice the background EEG, frontal, symmetrical, 8-14 Hz sharp phenomena, lasting at least 1 second. Representative epochs of each transient type were digitized with a sample rate of 256

samples per second. Subsequently, their spectral content was analyzed with a bin density of 1 Hz by means of a Fast Fourier Transformation (FFT) procedure. Afterwards, a mean spectrogram was calculated for each type of transient and the content of the following bands was determined: delta (2-4 Hz), theta (6-10 Hz), spindle (11-14 Hz), beta-1 (15-30 Hz), and beta-2 (31-70 Hz). To serve the second purpose of the study, spike-wave discharges were divided in three parts: the first second after spike wave activity onset, the last second before spike wave activity ending, and the middle portion. A spectrogram was made of each part. Differences between transients were analyzed by means of an ANOVA of Z-scores; differences within spike-wave discharges were analyzed with a *t*-test for dependent and repeated measurements.

3.4 Results

All three transients (Fig. 3.1) were predominantly present in the frontal EEG, while in the parietal recording the transients could also be found, but often in a less pronounced way. Spectral analysis was therefore performed on frontal signals only. Before comparing the transients, possible sleep-wake state effects on spike-wave discharge morphology were studied. During the 2 hours that were analyzed, spike-wave discharges occurred mainly during wakefulness (47.8%) and non-REM sleep (47.9%), but rarely during REM sleep (4%). Spectral analysis showed (Fig. 3.2) that wakefulness only promoted the 16-17 Hz frequencies compared to non-REM sleep, while no other frequencies differed significantly.

Subsequent spectral analysis of the frontal EEG of the three phenomena showed that they shared a peak frequency of about 9 Hz (Fig. 3.2), but that the amplitude of the first harmonic (18 Hz) of the spike-wave discharges was significantly more pronounced compared to the same harmonic of both spiky phenomena and intermediate stage, $F(2,30)=12.5$, $p<0.0001$. Moreover, spike-wave discharges were characterized by significantly more beta-1 activity (15-30 Hz) compared to intermediate stage and spiky spindles, $F(2,30)=83.4$, $p<0.0001$.

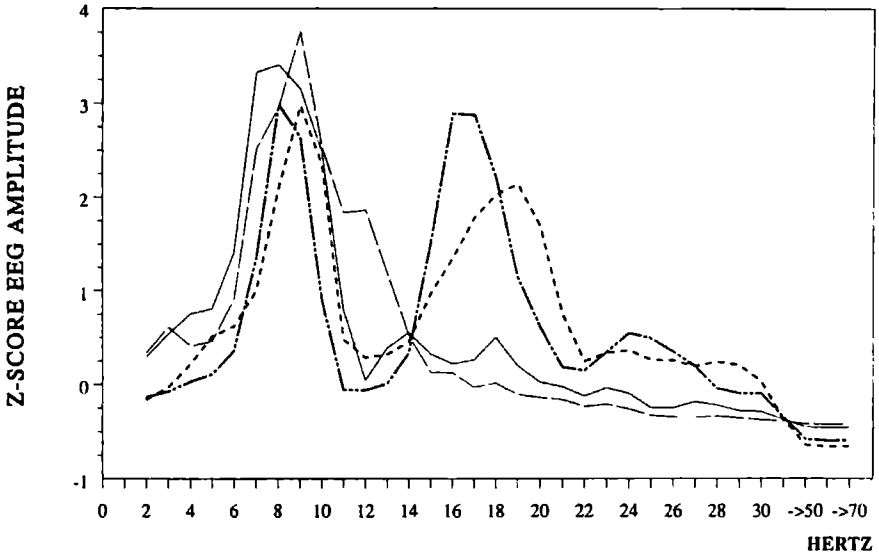

Figure 3.2 Mean spectrogram of the three investigated transients: spectrograms for intermediate stage (solid line), spiky phenomena (dashed line), and for spike-wave discharges occurring during wakefulness (dotted/dashed bold line) and during non-REM sleep (dotted bold line) are depicted. EEG amplitude expressed in Z-scores is given per 1 frequency bin (1 Hz) for frequencies between 2 and 30 Hz, and averaged over 10 frequency bins for frequencies between 31 and 70 Hz. Statistics can be found in the Results section.

Intraspike-wave discharge dynamics were analyzed by means of difference scores of the frontal EEG (begin-middle, begin-end, and middle-end) with regard to the dependency of the data. Intraspike-wave discharge spectral changes are indicated in Fig. 3.3. The peak frequency decreased monotonously, being 10 Hz at the beginning, 8-9 Hz at the middle part, and 8 Hz at the end. The amplitude of the 9 and 10 Hz peak frequencies was significantly higher in the beginning compared to the middle part (t=3.84, p<0.01) as well as to the end (t=3.63, p<0.05). A similar decrease was found, comparing the begin part to the middle and to the end for the first harmonic, the 20 Hz band (t=3.46, p<0.05 and t=2.66, p<0.05, respectively). On the contrary, a different course in frequency changes can be seen in the 14, 15, 16, and 17 Hz bands: their amplitudes were higher in the middle part compared to either the beginning (all p<0.5) or the end part. Finally, the energy in the delta band was larger in the beginning part compared to the middle and to the end part.

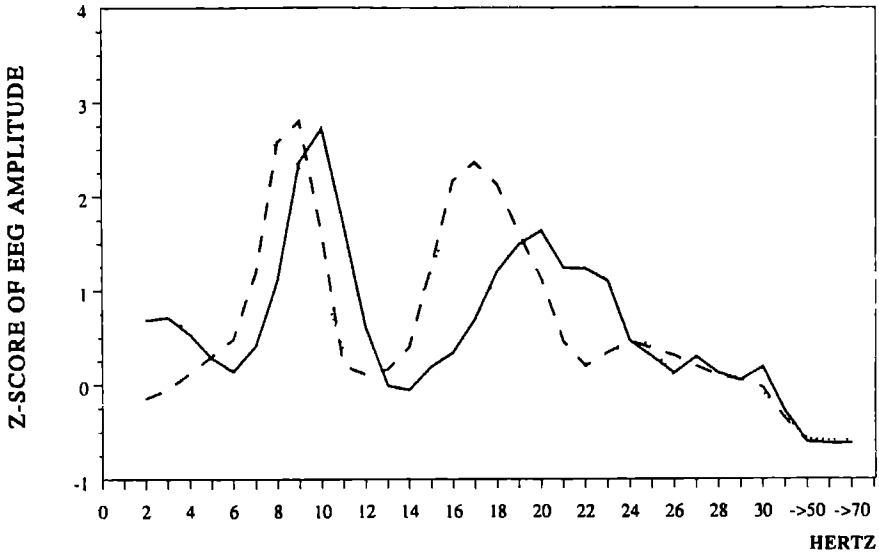

Figure 3.3 Mean spectrogram of the begin (solid line), the middle (dashed line), and the end (dotted line) part of spike-wave discharges EEG amplitude expressed in Z-scores is given per 1 frequency bin (1 Hz) for frequencies between 2 and 30 Hz, and averaged over 10 frequency bins for frequencies between 31 and 70 Hz. Statistics can be found in the Results section.

3.5 Discussion

In this study, it appeared that the three investigated transient phenomena shared spectral characteristics. Next to this, spike-wave discharges, intermediate stage, high voltage spiky phenomena and normal sleep spindles have other common characteristics. They all mainly occur during intermediate levels of vigilance and during transitional periods with changes in EEG synchronization, which lends arguments for their close relationship (Coenen et al 1991; Drinkenburg et al 1991[A]; Gandolfo et al 1990; Terzano et al 1989). Also the more pronounced expression of the transients on the frontal cortex reflects common underlying mechanisms (Kleinlogel 1990; 1991; Steriade and McCarley 1990). In the present study, it appeared that the transient phenomena shared an important feature of their morphology· peak frequency. Evidence exists that these oscillations are generated in a thalamo-cortical pathway. whereby the reticular thalamic nucleus fulfills a pacemaker function; this circuit is extensively involved in the genesis of both normal sleep spindles and epileptic spike-

wave discharges (Buzsáki et al 1990; Buzsáki 1991; Steriade and McCarley 1990). Moreover, by means of experimental manipulations, sleep spindles and spindle-like spiky phenomena are apt to transformation into spike-wave like discharges (Drinkenburg et al 1991[B]; Gloor 1988), suggesting that they share a mechanism of their genesis. These two arguments suggest a common origin of all these transients, but also that under certain circumstances one type of transient can be transformed into another. The same is known in man: spike-wave discharges can be seen as predecessors of generalized repetitive fast discharges (Halász 1991).

Intermediate stage is characterized by cortical spindle bursts together with hippocampal theta rhythm, whereby the reticular ascending influences reach their lowest level and the transmission level of the thalamic ventrobasal complex is the lowest of all sleep-wake states (Gottesmann 1988). The more pronounced presence and the morphology of intermediate stage in the WAG/Rij strain is likely to be modulated by REM sleep related neuronal processes, since intermediate stage is always preceded or followed by REM sleep. Also, in the present study, indications were found that ongoing sleep-wake state-related processes are slightly modulating the morphology of a transient, as is suggested in human studies (Declerck 1986; Kleinlogel 1990-1991; Ross et al 1966).

Although transient phenomena share many important qualities, they must be the product of partly different mechanisms as can be shown by pharmacological manipulation; while benzodiazepines may reduce the amount of spike-wave discharges, they induce spindles (Coenen and Van Luijtelaar 1989; Depoortere 1989). Furthermore, after injection of triazolam an increase of the mean duration of intermediate stage phases was found (Gandolfo and Gottesmann 1991). These studies suggest that one type of manipulation, administration of benzodiazepines, differentially influences the various transient phenomena.

The main outcome of the present study is that each investigated transient possesses, besides common characteristics, unique and characteristic features. This conclusion supports the view that, although the transients are based on common mechanisms, they should be considered as distinct events, because of differential influences of yet largely unknown mechanism, which modulate their morphological properties. Therefore, the

search for transient-modulating mechanisms is important and will undoubtedly contribute to the discovery of the genesis of spike-wave discharges.

Spectral analysis was used to describe the intra-spike-wave discharge dynamics, since it was shown in earlier visual evoked potential studies that in the middle part of the discharge the largest changes in VEP components were present, suggesting a time-varying phenomenon (Inoue et al 1992). From human studies it is known that one of the main characteristics of the generalized epilepsies is the decrease or loss of consciousness accompanying the epileptic EEG phenomena. The EEG phenomena of absence epilepsy are accompanied by a decrease in responsiveness to environmental stimulation, having a relatively mild decrease in performance at the beginning and at the end of the aberrant phenomenon and a more severe disturbance in the middle (Gloor 1988; Mirsky 1989; Shimazono 1953). During such an absence seizure, patients often report a reduction in the ability to process information, omissions in stimulus detection, and an inability to generate motor output (Mirsky 1989). The type and nature of the performance deterioration during a generalized absence seizure seems to vary in the course of the train of spike-wave discharges: the performance decrease is time-variant. This phenomenon has been called a trough of consciousness (Goldie and Green 1961; Shimazono 1953), and it is concluded that there is a rough relationship between the electroencephalic activity and cognitive changes in man. The results of the present study indeed suggest the middle part of the spike-wave discharge to be different from the beginning and end parts, especially with respect to the magnitude of the first harmonic. It is not yet clear whether these changes might be interpreted as functionally related to the EEG changes as found in the VEP study (Inoue et al 1992) or to the changes in responsiveness in man. Further experiments concerning the changes in responsiveness during spike-wave discharges are presently being carried out in WAG/Rij rats.

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„HENCE, THERE SEEMS TO EXIST AN OPTIMAL ZONE OF SUPERFICIALLY REDUCED VIGILANCE WHICH IS FAVORABLE FOR THE APPEARANCE OF SEIZURES WHILE STATES OF "EXTREME WAKING" AND "DEEPER SLEEP" ARE UNFAVORABLE."

PÉTER HALÁSZ, 1982

CHAPTER 4

SPIKE-WAVE DISCHARGES AND SLEEP-WAKE STATES IN RATS WITH ABSENCE EPILEPSY[1]

[1] DRINKENBURG WHIM, COENEN AML, VOSSEN JMH, VAN LUIJTELAAR ELJM. SPIKE-WAVE DISCHARGES AND SLEEP-WAKE STATES IN RATS WITH ABSENCE EPILEPSY. *EPILEPSY RESEARCH* 9:218-224;1991.

Chapter 4 Sleep-wake states and spike-wave discharges

4.1 Summary

The occurrence of spike-wave discharges was studied in relation to the daily fluctuations of vigilance level in rats. Eight rats of the WAG/Rij strain, an animal model for idiopathic generalized epilepsy of the absence type, which were equipped with cortical EEG and nuchal EMG electrodes, served as subjects. It was found that spike-wave discharges predominantly occur during light slow wave sleep and passive wakefulness. REM sleep, active wakefulness, and deep slow wave sleep are less susceptible to the occurrence of spike-wave discharges. Finally, spike-wave discharges tend to prevail in transitional states. A crucial role for the degree of stability of the level of vigilance in the genesis of absence seizures is suggested.

4.2 Introduction

For the past few decades, the influence of sleep-wake states on the occurrence of spike-wave discharges, which are symptoms of primary generalized, non-convulsive 'absence' epilepsy, has been studied. It is consistently found that absences mainly occur during periods in which a patient is involved in little or no activity (behavioural as well as mental), such as drowsiness or slow wave sleep (Kellaway 1985; Ross et al 1966; Sato et al 1973). Specifically stage 2 of slow wave sleep, characterized by transient, phasic phenomena, such as spindles or K-complexes, appears to be associated with the occurrence of spike-wave discharges (Beck et al 1977; Halász and Dévény 1974; Kellaway 1985; Offenbacher et al 1986). On the other hand, absences are less likely to occur when a person is active (Jung 1962; Vieth 1986). Less clear is the picture during REM sleep: while some studies report the presence of spike-wave discharges (Beck et al 1977; Nagao et al 1990), the majority rarely find discharges during REM sleep (Kellaway 1980; Ross et al 1966; Sato et al 1973; Vieth 1986). Considering the circadian time of occurrence it is found that spike-wave discharges tend to occur around sleep onset and around awakenings

(Burr et al 1986; Kellaway 1980; Martins da Silva et al 1984; Offenbacher et al 1986; Tomka 1985).

Until now, only a few animal studies have addressed this issue. Their results have showed several consistencies in condition and time of occurrence of paroxysmal discharges with clinical human data. In a feline model for generalized, myoclonic 'petit mal' epilepsy, induced by penicillin, spike-wave discharges increased during light slow wave sleep and were suppressed during REM sleep (Shouse 1987; Shouse et al 1989). In a study with spontaneously epileptic Wistar rats, spike-wave discharges occurred predominantly during quiet wakefulness and drowsiness, and rarely during REM sleep or active wakefulness (Lannes et al 1988).

We have been studying the inbred WAG/Rij strain of rats for several years. WAG/Rij rats exhibit spontaneously occurring spike-wave discharges together with associated behavioural phenomena (such as head-tilting, accelerated breathing, and vibrissal myoclonics), which add up to a characteristic image that is considered to be reminiscent of human absence epilepsy (Van Luijtelaar and Coenen 1986). The pharmacological profile of the spike-wave discharges also appears to be quite similar to the pharmacological profile of human absence epilepsy (Peeters et al 1988). The characteristics of this model seem largely to be in conformity with those of other models (Buzsáki et al 1990; Inoue et al 1990; Robinson and Gilmore 1980; Vergnes et al 1982). Since in the WAG/Rij model, spike-wave discharges occurred less frequently in the first hours of the light period and in view of the fact that in these hours deep slow wave sleep predominates, a relationship between the occurrence of spike-wave discharges and processes underlying the various vigilance levels was suggested (Coenen et al 1991; Van Luijtelaar and Coenen 1986; 1988).

The purpose of the present study is to describe the relationship between the occurrence of spike-wave discharges and the levels of vigilance, and, as a consequence, to determine the conditions in which the brain is susceptible to the occurrence of spike-wave discharges.

4.3 Methods

Subjects and surgery. Eight adult male members of the WAG/Rij strain (age about 6 months, weight 277-357 g with a mean of 314 g) served as subjects. Rats were singly housed in Makrolon cages and maintained on a 12-12 hour light-dark cycle with lights on at 08.00 hour. Access to food and water was ad lib. A month before testing started, each rat was, under complete anaesthesia (Nembutal, Abott Laboratories, 60 mg/kg i.p.), permanently implanted with a standard cortical tripolar EEG electrode (Plastics One MS-333/2-A) and a bipolar EMG electrode (Plastics One MS 303/71). EEG electrodes were placed in the frontal cortex and in the parietal region, respectively with coordinates[2]: A 2.0, L 3.5 and A -6.0, L 4.0 (with skull surface flat and bregma zero-zero), whereas a third earth electrode was placed in the cerebellum. The EMG electrode was subcutaneously placed in the dorsal neck muscles.

Polygraphic recordings and analysis. After a recovery period of 3 weeks and after 48 hours adaptation to the experimental setting, EEG- and EMG-recordings were made for 24 hours. The signals were amplified and filtered by an Elema-Schönander polygraph, which allowed frequencies between 1 and 70 Hz and 27 and 700 Hz respectively to pass, and recorded on magnetic tape (SE 7000). Subsequently, from these 24 hour recording periods, every second hour was analyzed off-line (thus for each animal 12 corresponding, non-consecutive samples of one hour were analyzed) with a fully automated sleep-wake classification system (Van Luijtelaar and Coenen 1984). This classification system discriminated between wakefulness, REM sleep, light slow wave sleep and deep slow wave sleep. For this purpose a mean EEG-spectrogram was constructed every 5 seconds, based on the output (sample-frequency 20 Hz) of 20 bandpass filters (2-28 Hz). From this mean spectrogram several EEG indices were obtained, which were, together with the mean amplitude of the EMG, subjected to decision rules for the identification of the different sleep-wake states. An additional distinction between active and passive wakefulness, based on the EMG, was made by hand. The signals as well as a classification code were written

[2] Stereotaxic coordinates were choosen to meet the requirements of the automated sleep-wake classification system as well as to reliably register spike-wave discharges.

out on polygraphic paper (speed 1.0 cm/sec). Spike-wave discharges were then visually scored according to criteria elaborated earlier (Van Luijtelaar and Coenen 1986).

The mean number, mean duration and total duration of spike-wave discharges as well as of the vigilance levels were determined for the analyzed hours. Furthermore, each spike-wave discharge was studied to note the pre and post vigilance states. In order to calculate a discharge rate for each of the vigilance levels, results were corrected for the distribution of sleep-wake states. Finally, the number and distribution of the transitions between the several sleep-wake states were quantified and analyzed.

4.4 Results

All results are based on eight subjects. For the twelve analyzed hours, an overall mean number of 13.0 (s.e.m. 1.3) spike-wave discharges per hour with a mean duration of 3.9 (s.e.m. 0.4) seconds was found. A total of 1244 spike-wave discharges were analyzed.

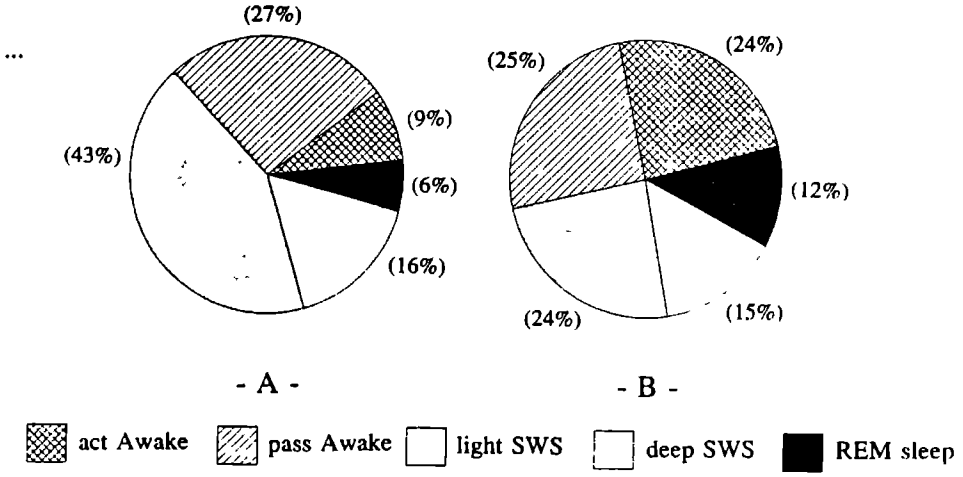

Figure 4.1 Two pies showing the distribution (in percentages) of sleep-wake states preceding spike-wave discharges (A), and following spike-wave discharges (B). act = active; pass = passive; SWS = slow wave sleep.

The distributions of the five levels of vigilance preceding and following the spike-wave discharges are presented in percentages in Figure 4.1.

In Figure 4.2 these distributions are also depicted, but now corrected for the distribution of the sleep-wake states over the analyzed hours.

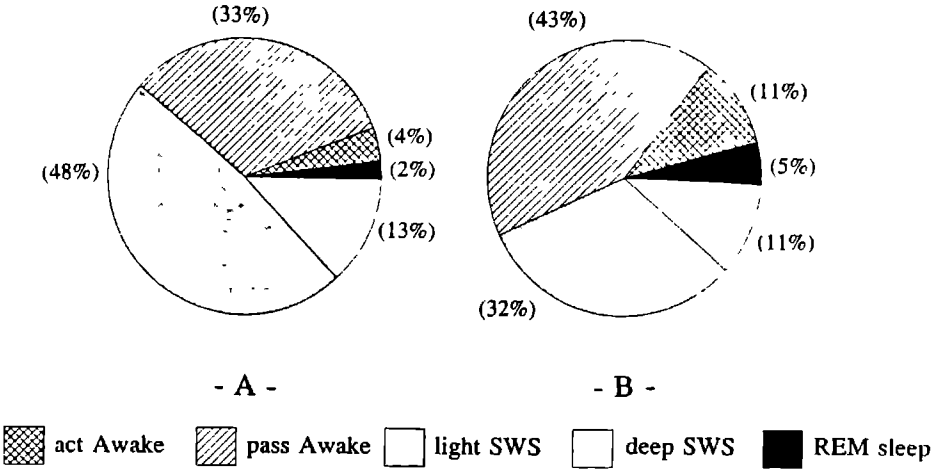

Figure 4.2 Two pies showing the distribution (in percentages) of sleep-wake states preceding spike-wave discharges (A), and following spike-wave discharges (B), corrected for the amounts of the various sleep-wake states. act = active; pass = passive; SWS = slow wave sleep.

For this correction, factors were calculated from the percentages of presence of each separate sleep-wake state (see Table III). Subsequently, the percentages of the distribution of Figure 4.1 were multiplied by the corresponding factor. After transformation of these 'corrected' distributions into percentages (transformation to 100 %), a comparison of discharge rates between sleep-wake states is legitimate. The dependence of the spike-wave discharges proportions (Chi^2 per subject) of the sleep-wake states was tested and the proportions were found to be different at an overall level of 0.05. As a consequence, these distributions can truly be interpreted as discharge rates: light slow wave sleep appears to be the most susceptible vigilance level for spike-wave discharges to occur. The opposite is true for REM sleep and active wakefulness, while deep slow wave sleep also can be considered unfavourable for the occurrence of spike-wave discharges. The distributions of vigilance levels following spike-wave discharges is not quite similar to those preceding

these discharges. Active wakefulness is more often found following spike-wave discharges. Analysis of the relationship between the sleep-wake states preceding and following spike-wave discharges showed that 57.7 % of all discharges had a different vigilance level before, in comparison with after their occurrence.

Table III Distribution of sleep-wake states

Total duration of the sleep-wake states expressed in seconds and as a percentage of total time, averaged over 12 hours and 8 subjects. In order to compare discharge rates, a correction factor for the percentage of presence was calculated of each sleep-wake state. (e.g. 100 : 8.1 = 12.4).

	duration (s)	% of total time	correction factor
act Awake	293.7	**8.1**	12.4
pass Awake	1283.8	**35.4**	2.8
light SWS	946.5	**26.0**	3.9
deep SWS	735.0	**20.5**	4.9
REM sleep	250.5	**7.0**	14.3

After excluding vigilance level transitions associated with the spike-wave discharges, the chance of an occurrence of a not-discharge-related transition in vigilance level appeared to be 22.8 %, this on the basis of classification-epochs of 5 seconds. Hence, spike-wave discharges appeared about 2.6 times as often on transitions as could be expected.

Table IV Distribution of transitions around spike-wave discharges in percentages

AFTER: / **BEFORE:**	act Awake	pass Awake	light SWS	deep SWS	REM sleep	
act Awake	1.0	1.0	1.2	0.4	0.0	**3.6**
pass Awake	4.9	16.5	8.7	2.7	0.3	**33.1**
light SWS	4.2	21.1	16.1	4.0	3.0	**48.4**
deep SWS	0.7	3.3	5.0	3.5	0.9	**13.4**
REM sleep	0.0	1.1	0.5	0.0	0.6	**2.2**
	10.8	**43.0**	**31.5**	**10.6**	**4.8**	

No circadian influences were found on the type of transitions over the analyzed hours, therefore all transitions around spike-wave discharges were pooled and presented in Table IV. It can be seen that spike-wave discharges mostly appear on transitions from light slow wave sleep to wakefulness, which was already indicated in Figures 4.1 and 4.2. The number of spike-wave discharges occurring on transitions having a vigilance enhancing direction (39.2 %), differed significantly at the 0.01 level (Wilcoxon Signed Rank test) from the number of spike-wave discharges on transitions with a vigilance lowering direction (18.0 %).

4.5 Discussion

The main outcome of this study was the identification of sleep-wake states during which spike-wave discharges predominantly occur: light slow wave sleep and passive wakefulness. Furthermore, it was detected that the sleep-wake states REM sleep, active wakefulness, and deep slow wave sleep are unfavourable for the occurrence of spike-wave discharges.

For REM sleep and active waking, similar results from human studies can be found (Vieth 1986). Both in active waking and in REM sleep, influences of a high degree of desynchronized neuronal activity appear to inhibit spike-wave discharges (Shouse et al 1989). Moreover, spike-wave discharges occur less frequently following deep slow wave sleep, a level of vigilance characterized by a high degree of neuronal synchronization, suggesting that there is no simple relationship between the level of synchronization and the number of spike-wave discharges. Nevertheless, these vigilance levels ought to posses some crucial qualities preventing the genesis of spike-wave discharges. The similarity in morphology of spike-wave discharges as found in human studies during REM sleep and wakefulness may be considered as a reflection of such shared qualities (Ross et al 1966; Sato et al 1973; Vieth 1986). Furthermore, this contributes to the argument for the existence of an optimum, intermediate level of vigilance with an intermediate synchronization for spike-wave discharges to occur and, as a consequence, for a link between the regulatory mechanisms of arousal and seizures (Miller et al 1989; Niedermeyer 1982; Terzano et al 1989; Vieth 1986).

When we consider stage 2 of slow wave sleep as the human counterpart of the rat's light slow wave sleep, our results suggest a further agreement between outcomes of studies in rats and in man (Offenbacher et al 1986). This type of sleep has the highest spike-wave discharge rate in rats. In man it has been recognized that the majority of generalized epileptic discharges occur during sleep with spindles, i.e. stage 2 of sleep, and even that nightly discharges often show a mixture of spike-waves and spindles (Gloor 1985; Kellaway 1985; Stevens et al 1971). The fact that in the course of the night in man, the increase in stage 2 sleep parallels a rise of the number of spike-wave discharges, can also be explained in this way. Nevertheless, the characteristics of stage 2 in man are somewhat different from those in rat. Besides spindles, some particular characteristic sleep transients as K-complexes or vertex waves can be found in the EEG of humans. The underlying mechanisms of these phasic sleep transients have been associated with the occurrence of spike-wave discharges in a number of theories (Gloor 1988; Halász 1984; Niedermeyer 1982; Terzano et al 1989). Although in rats only spindles can be recognized, a shared thalamo-cortical control has been extensively described and related to the occurrence of spike-wave discharges (Gloor 1988; Jasper and Drooglever-Fortuijn 1946; Vergnes et al 1990).

Spike-wave discharges are mostly found on transitions. This result has two possible implications. Firstly, absence seizures are likely to occur during transitional states: those states of vigilance where alterations in the level of vigilance, such as (micro-)arousals or sleep-wake state shifts, prevail (Declerck 1983; Offenbacher et al 1986; Terzano et al 1989). This implication is also supported by the findings that especially during the rat's slow wave sleep and during stage 2 of human slow wave sleep, periods with unstable EEG activity can be discriminated, which can be considered as transitional states (Depoortere et al 1991; Terzano et al 1985). However, the transitions on which spike-wave discharges are mostly found, possess a vigilance enhancing character. Therefore, a second implication cannot yet be ruled out: the spike-wave discharges themselves could modulate these transitions. Both implications are nonetheless in concordance with those hypotheses concerning the common mechanisms responsible for the (microstructural) organization of sleep and those involved in epileptogenesis (Broughton 1984; Burr et al 1986; Halász

1984; Niedermeyer 1982; Terzano et al 1989).

The sleep-architecture and the sleep-wake distribution, as well as the circadian rhythm of spike-wave discharges in this study with WAG/Rij rats, matches the results of earlier studies (Van Luijtelaar and Coenen 1984; 1988). Nevertheless, some deviations in the dynamic organization of sleep can be recognized in the WAG/Rij rat. Compared to Wistar rats, they show a longer lasting intermediate stage of sleep, which is less frequently followed by REM sleep and more frequently by arousals (Gandolfo 1990). This difficulty of entering REM sleep may reflect strain-specific aberrancies in vigilance controlling mechanisms, which possibly are related to spike-wave discharge genesis (Halász and Dévény 1974). This relationship is also reflected by the occasionally exhibited similarities in morphology between the intermediate stage and spike-wave discharges.

In conclusion, it is shown that light slow wave sleep and, to a lesser degree, passive wakefulness, are the most favourable vigilance levels for spike-wave discharges to occur, possibly because of the more instable, transitional nature of the cortical EEG activity during these states. In contrast, REM sleep, active wakefulness, and deep slow wave sleep, which are all accompanied by more stable cortical EEG activities, are less favourable for the occurrence of spike-wave discharges. These findings support the view that during periods with fluctuations in vigilance, presumably mediated by the degree of EEG stability caused by arousal-controlling structures, the occurrence of spike-wave discharges is favoured.

4.6 References

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„THOSE PROCEDURES WHICH TEND TO PRODUCE LARGE, SLOW WAVES IN THE NORMAL SUBJECT ALSO TEND TO PRODUCE SEIZURES IN PATIENTS WITH EPILEPSY."

FREDERICK GIBBS, HALLOWELL DAVIS, AND WILLIAM LENNOX, 1935

CHAPTER 5

SLEEP DEPRIVATION AND SPIKE-WAVE DISCHARGES IN EPILEPTIC RATS[1]

[1] DRINKENBURG WHIM, COENEN AML, VOSSEN JMH, VAN LUIJTELAAR ELJM. SLEEP DEPRIVATION AND SPIKE-WAVE DISCHARGES IN EPILEPTIC RATS. *SLEEP* 18:252-256;1995.

Chapter 5 Sleep deprivation and spike-wave discharges in epileptic rats

5.1 Summary

Effects of sleep deprivation were studied on the occurrence of spike-wave discharges in the electroencephalogram of rats of the epileptic WAG/Rij strain, a model for absence epilepsy. This was done before, during and after a period of twelve hours of near total sleep deprivation. A substantial increase in the number of spike-wave discharges was found during the first four hours of the deprivation period, while in the following deprivation hours epileptic activity returned to baseline values. Immediately after termination of deprivation, a decrease in the number of spike-wave discharges parallelled a rebound of REM sleep and deep non-REM sleep. An initial increase in epileptic activity has also been reported during sleep deprivation of humans. This initial increase as well as the epileptogenic effects during the course of the sleep deprivation and during the recovery period after sleep deprivation can be interpreted in terms of changes in sleep-wake states. Although the epilepsy provoking mechanisms are not yet understood, an explanation is suggested based on changes of transitions between sleep-wake states and shifts in level of synchronization.

5.2 Introduction

Sleep deprivation is an effective method of provoking epileptic discharges in patients (Broughton 1990, Logothetis et al 1986). A basic question, however, remains how sleep-wake regulation and epileptogenesis are linked. It is unclear whether the provocative effects of sleep deprivation are caused by changes in the amounts of sleep-wake states or by an intermediate factor that produces a lowering of the threshold for the occurrence of paroxysmal activity (Klingler et al 1991; Pratt et al 1968; Veldhuizen et al 1983). Examination of the changes in both epileptic activity and sleep-wake states as a result of sleep deprivation, may help to clarify the epileptogenic mechanisms of sleep deprivation.

Despite the apparent sensitivity of several types of generalized epilepsy to sleep deprivation, only a few studies have investigated the effects of sleep deprivation in animal

models. Shouse (1988) found in cats that a 24 hour period of sleep deprivation enhances experimentally induced myoclonic absence seizures in all sleep-wake states. She suggested that sleep loss induces brain hyperexcitability in all states of vigilance. Peeters et al (1989) selectively deprived epileptic rats of REM sleep and reported a decrease in the number of epileptic discharges during and after sleep deprivation. They held an increase in tonic arousal induced by REM sleep deprivation, responsible for the modulation of epileptic activity.

The present study describes the effects of depriving rats of both REM and non-REM sleep on epileptic activity. Sleep-wake states and epileptic discharges were analyzed before, during and following a 12-hour period of almost total sleep deprivation. This question was addressed in a genetic model for absence epilepsy, the WAG/Rij strain of rats, of which all animals spontaneously show trains of spike-wave discharges in the cortical electroencephalogram (EEG). These spike-wave discharges have been extensively evaluated and these studies showed that the WAG/Rij model could be regarded as a valid model for human absence epilepsy. Therefore, this model is useful for studying the relationship between sleep-wake states and spike-wave discharges (Coenen et al 1991; Coenen et al 1992).

5.3 Methods

Eight adult male rats of the WAG/Rij strain, with an age of about eight months and weights between 288 and 377 g were used. Under deep anaesthesia (Nembutal, 60 mg/kg, i.p.), rats were equipped with a tripolar EEG electrode set (Plastics One MS-333/2-A) and a bipolar electromyographic (EMG) electrode set (MS 303/71). With the skull surface placed horizontally, placement of the EEG electrodes was done at coordinates A 2.0, L 3.5 for the frontal electrode and A -6.0, L 4.0 for the parietal electrode[2]. The reference EEG electrode was located over the cerebellum and EMG electrodes were subcutaneously placed over the dorsal neck muscles. Following surgery, rats were singly housed and maintained on a 12-12 h light-dark cycle with bright white lights on at 8 a.m. Animals had ad libitum

[2] Stereotaxic coordinates were choosen to meet the requirements of the automated sleep-wake classification system as well as to reliably register spike-wave discharges.

access to standard laboratory food and water.

EEG and EMG signals were amplified and filtered by an Elema-Schönander polygraph, allowing EEG frequencies between 0.5 and 70 Hz and EMG frequencies between 27 and 700 Hz to pass. Signals were recorded on magnetic tape (SE 7000) and written out on chart paper with a paper speed of 1.0 cm/sec. In addition, signals were analyzed on line by means of an automatic sleep-wake classification system to determine the various sleep-wake states of the rat such as wakefulness, REM sleep, light non-REM sleep and deep non-REM sleep (Van Luijtelaar and Coenen 1984). The sleep-wake state of the animal was determined for subsequent epochs of five seconds duration. Spike-wave discharges were scored by visual inspection of the EEG, according to criteria elaborated earlier (Coenen et al 1992).

Animals were allowed to recover from the operation for two weeks and habituated to the experimental setting for 48 hours. Starting at 8 a.m., a baseline registration of EEG and EMG was made for 24 hours. Subsequently, animals were deprived of total sleep by shaking their cages as soon as sleep onset was detected. Determination of sleep onset was based on visual detection of slow waves of non-REM sleep or theta-activity associated with REM sleep. This was verified by observation of the animal. To awaken the animal upon detection of sleep onset, the experimenter started to shake the cage with a fixed intensity in a remote-controlled way from an adjacent room. Shaking continued till the animal was clearly aroused. Sleep deprivation was imposed for 12 hours from 8 a.m. till 8 p.m. during the light period; the main sleep period for rats. Thereafter, recording of the animals was continued for the next 12 hours till 8 a.m. In this period the animals could recover from the sleep deprivation.

The amounts of sleep-wake states, determined by the automatic sleep-wake classification system, were expressed as a percentage of recording time, during baseline, deprivation and recovery. The efficacy of the sleep deprivation procedure was analyzed by comparing amounts of sleep-wake states during successive 2-hour deprivation periods with amounts obtained from the corresponding 2-hour baseline periods. Furthermore, sleep-wake states occurring during the recovery and during the baseline period were also compared. In the same way, comparisons were made in the number of spike-wave discharges. Intra-

individual differences in sleep-wake state amounts and in epileptic activity between corresponding periods were statistically analyzed by means of the non-parametric Wilcoxon Matched-pairs Signed-ranks Test. All values are means of eight subjects and are indicated with standard errors of the means (SEM).

5.4 Results

The percentage of each sleep-wake state was calculated during baseline and deprivation treatment for periods of two hours. As compared to baseline values, all 2-hour deprivation periods contained significantly less non-REM and REM sleep. Results of the 2-hour periods of the baseline period, the deprivation period and the recovery period are presented in Fig. 5.1. In order to determine the overall efficacy of the sleep deprivation procedure, the total amounts of REM sleep and non-REM sleep were calculated.

Table V Sleep-wake state presence and epileptic activity.

Sleep-wake state presence and epileptic activity, both expressed as a percentage of the corresponding 2-hour baseline period (baseline values were set at 100 % for each subject). For subsequent two hour periods, percentages and standard error of means (between brackets) are given with respect to the mean number of spike-wave discharges (SWD) as well as with respect to the percentages of recording time for REM sleep (REM), light non-REM sleep (l-NREM), deep non-REM sleep (d-NREM), and waking (AWAKE). All conditions consist of 8 animals. [** $p<0.02$ and * $p<0.05$ are for Wilcoxon Matched-pairs Signed-ranks Test for within subject comparison of absolute percentages of the experimental two hour period with corresponding baseline two hour period].

Time	SWD	REM	l-NREM	d-NREM	AWAKE
DEPRIVATION PERIOD					
08-10	553.8 (107.3)**	0.0 (0.0)**	33.7 (9.1)**	0.8 (0.5)**	382.5 (57.3)**
10-12	246.1 (45.3)*	5.6 (5.6)*	24.0 (5.5)**	3.5 (2.4)**	228.7 (16.7)**
12-14	134.3 (30.0)	0.0 (0.0)**	26.6 (4.6)**	17.0 (6.8)**	268.0 (40.0)**
14-16	138.7 (39.5)	0.0 (0.0)**	29.7 (5.2)**	20.7 (5.4)**	205.5 (14.3)**
16-18	61.3 (19.4)	1.8 (1.8)**	30.1 (6.7)**	42.8 (27.8)*	233.0 (31.6)**
18-20	111.1 (52.1)	11.5 (8.5)**	32.7 (6.5)**	57.7 (17.8)	173.0 (17.2)**
RECOVERY PERIOD					
20-22	35.2 (8.2)**	203.7 (40.0)**	110.3 (2.0)	549.7 (112.4)**	60.0 (8.2)**
22-24	47.3 (12.8)*	206.1 (53.7)	154.4 (36.6)	396.6 (102.4)*	80.5 (11.6)*
00-02	64.3 (21.0)	137.0 (21.1)	119.7 (15.4)	353.8 (102.8)**	75.5 (10.4)*
02-04	88.5 (23.6)	164.1 (35.7)	124.6 (13.8)	236.3 (45.0)	81.1 (7.3)*
04-06	119.8 (37.3)	124.7 (17.3)	109.6 (16.5)	166.1 (32.7)	91.1 (12.1)
06-08	242.2 (100.0)	143.2 (21.8)	131.4 (12.7)	167.3 (31.2)	73.2 (13.3)

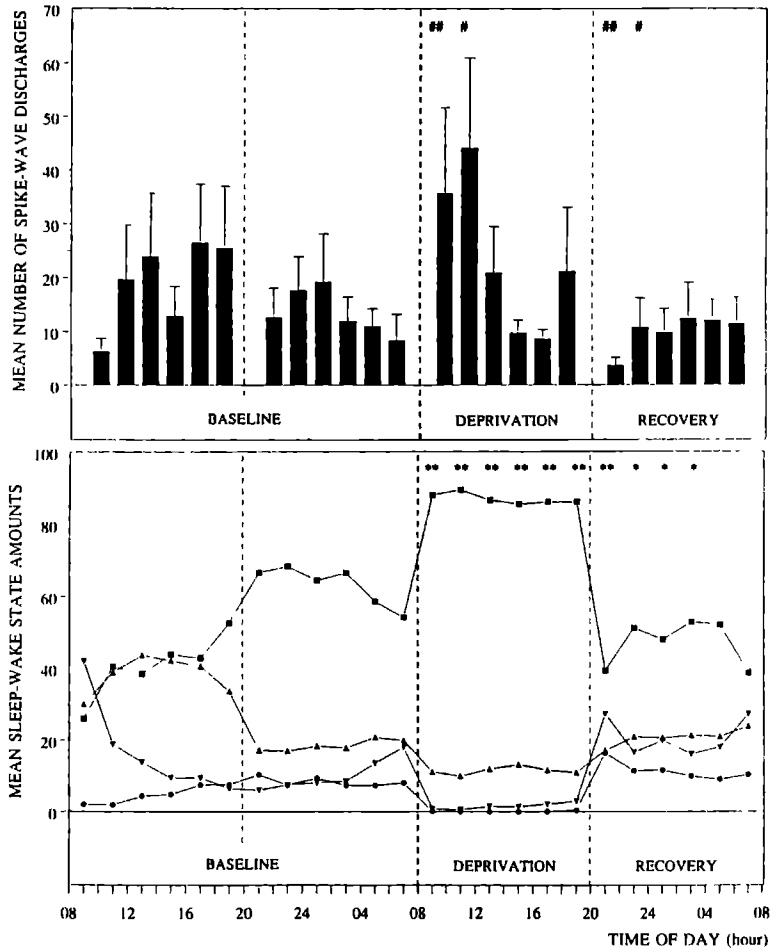

Figure 5.1 Mean number of spike-wave discharges (top window) and mean sleep-wake state amounts in percentage of total recording time (bottom window) are given for successive 2-hour periods of the baseline period, the deprivation period, and the recovery period, respectively. The mean number of spike-wave discharges and standard error of mean are indicated in the bar graph. In the line graph light non-REM sleep is indicated by ▲, deep non-REM sleep by ▼, REM sleep by ●, and wakefulness by ■.

$p<0.02$ and # $p<0.05$ are for Wilcoxon Matched-pairs Signed-ranks Test for differences in number between experimental and corresponding baseline two hour periods; ** $p<0.02$ and * $p<0.05$ are for Wilcoxon Matched-pairs Signed-ranks Test for differences in percentage of total sleep (combined REM sleep, light non-REM sleep and deep non-REM sleep) between experimental and corresponding baseline two hour periods.

Sleep was registered for 59% of total time during the light period of the baseline, while the amount of sleep decreased till 13% of the light period of the deprivation phase. This reduction is highly significant ($Z= -2.37$, $p<0.018$).

In the recovery period, a marked rebound of REM sleep and in particular of deep non-REM sleep was found during the first two hours (Table V: $Z= -2.52$, $p<0.012$, $Z= -2.52$, $p<0.012$, respectively). The increase in deep non-REM sleep was also present during the second 2-hour period after termination of sleep deprivation ($Z=-2.37$, $p<0.018$). To facilitate the overview of the deprivation-induced effects in Table V, the values of the deprivation and recovery period are additionally expressed as a percentage of the corresponding 2-hour baseline period value, which were all set to 100 percent.

An increase in the number of spike-wave discharges compared to the corresponding baseline hours was evident during the first four hours of the deprivation period (Table V: $Z=-2.52$, $p<0.012$, $Z=-2.20$, $p<0.028$, successively). After this initial increase, the mean number of spike-wave discharges gradually returned to baseline levels (Fig. 5.1). During the recovery period, a decrease in the number of spike-wave discharges compared to corresponding baseline numbers was found for a period up to four hours after the end of the deprivation ($Z=-2.52$, $p<0.012$, $Z=-2.10$, $p<0.036$, successively).

5.5 Discussion

Sleep deprivation produced an increase in epileptic discharges during the first four hours of deprivation. After this initial increase, the number of spike-wave discharges gradually returned to baseline levels. The first hours of the post-deprivation recovery period showed a decrease in the occurrence of spike-wave discharges compared to baseline. The numbers of discharges again returned to baseline values during the remaining hours of the recovery period.

In contrast to studies in humans on the epileptogenic effects following sleep deprivation (Broughton 1990; Klingler et al 1991; Logothetis et al 1986; Pratt et al 1968; Veldhuizen et al 1983), data obtained in humans during sleep deprivation are scarce (Beck et al 1977; Rodin et al 1962). Rodin et al (1962) reported an increase in epileptic-like paroxysms during sleep deprivation and this increase was limited to the first part of the

sleep deprivation period. In the present study, also an increase of epileptic activity was found only during the first part of the deprivation period. Furthermore, a reduction of epileptic activity was initially found during the recovery period. The majority of clinical studies investigated the effects of sleep deprivation after its termination. Although debate is going on whether activation is most prominent during waking or during sleeping, activation of typical absence spike-waves has been reported to be most conspicuous after the initial sleep rebound, when the patient is inactive but awake (Jovanović 1991). Considering the differences between man and rat with respect to sleep organization, it is awkward to compare recovery results between both species.

The question towards the epilepsy provoking mechanisms has not yet been solved. It has been suggested, that sleep deprivation causes its epileptogenic effect by changing sleep-wake state amounts (Beck et al 1977). In earlier studies in spontaneously epileptic rats, it was indeed confirmed that epileptic activity and sleep-wake states have a distinct relationship. Spike-wave discharges preferably occur during passive wakefulness and light non-REM sleep, but seldom during active wakefulness, deep non-REM sleep and REM sleep (Drinkenburg et al 1991; Gralewicz et al 1994). As a consequence, changes in the duration and the distribution of the several sleep-wake states may predict the amount of spike-wave activity during sleep deprivation. In the first hours of sleep deprivation which starts at the beginning of the light or sleep period of the rat, a high occurrence of wakefulness is enforced. As at that time the rats seemed drowsy but had a waking EEG, the animals were most likely in a state of passive wakefulness where spike-wave activity is frequent. This increase in passive wakefulness may therefore account for the initial increase in spike-wave discharges during deprivation. Nevertheless, after this initial increase the amount of spike-wave activity drops to base-line levels, while an increased percentage of wakefulness is maintained. It is considered that cumulative waking time increases and this cumulation will increase sleep propensity and sleepiness (Veldhuizen et al 1983). When sleep propensity increases, it reaches a level at which animals are difficult to arouse. In that situation, the activation needs to be so fierce and intensive that rats become wide awake shortly, but nonetheless fall into a deep sleep again immediately thereafter. It is not surprising that the occurrence of spike-wave discharges is then no

longer favored. During the recovery period high percentages of sleep-wake states such as deep non-REM and REM sleep occur, which are not favorable for the occurrence of spike-wave discharges. This can explain the low incidence of epileptic activity after deprivation, which is even lower than during the corresponding base-line period.

In our opinion in all situations the same epilepsy modulating mechanism is working. From neurophysiological studies it appears that epilepsy susceptibility is maximal during transitions and shifts in vigilance (Speckmann and Elger 1991). Epileptic paroxysms have been found to occur preferentially in periods in which sleep-inducing and arousing mechanisms are in competition and unstable levels of brain synchronization prevail (Halász 1991; Terzano at al 1989). In addition, transitions during intermediate levels of vigilance have been reported to be favorable for the occurrence of spike-wave discharges (Drinkenburg et al 1991; Gralewicz et al 1994). We suggest that the epileptogenic effects of sleep deprivation can be adequately explained by changes in shifts between levels of brain synchronization. Although a detailed study of such transitions was beyond the scope of the present experiment, we hypothesize that in the first hours of deprivation the number of shifts sharply increases as a result of drowsiness. Furthermore, we hold the view that after this initial period sleep propensity becomes so high that rats when falling asleep pass the paroxysm-sensitive, intermediate states of vigilance so quickly that there is less opportunity for epileptic discharges to occur. Often, a short spindle-like phenomenon is then seen in this fast transition to the production of large slow sleep waves. The results of the recovery period can be interpreted in an analogous way. Evidence exists that during recovery sleep the level of synchronization is even higher than during normal slow wave sleep (Dijk et al 1991). Indeed, the decrease in epileptic activity is parallelled especially by rebound deep non-REM sleep. Future studies aimed at describing the shifts in synchronization during state-transitions, may substantiate this proposal.

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„THE CORRELATIONS BETWEEN VIGILANCE AND EPILEPSY ARE MANIFOLD. (...) MANY OF THE INFLUENCES TRIGGERING OR INHIBITING EPILEPTIC SEIZURES PRODUCE ALTERATIONS OF VIGILANCE OR ARE PRODUCED BY THEM."

JÜRGEN VIETH, 1986

CHAPTER 6

ABSENCE EPILEPSY AND THE LEVEL OF VIGILANCE IN RATS OF THE WAG/RIJ STRAIN[1]

[1] COENEN AML, DRINKENBURG WHIM, PEETERS BWMM, VOSSEN JMH, VAN LUIJTELAAR ELJM. ABSENCE EPILEPSY AND THE LEVEL OF VIGILANCE IN RATS OF THE WAG/RIJ STRAIN. *NEUROSCIENCE & BIOBEHAVIORAL REVIEWS* 15:259-263;1991.

Chapter 6 Vigilance changes and spike-wave discharges

6.1 Summary

In man, a relationship exists between sleep-wake states and absence epilepsy. During wakefulness, spike-wave discharges predominantly occur when the level of vigilance is not high, while during sleep they have a preference to occur during slow wave sleep. During this latter type of sleep, spike-wave discharges prevail in periods where slow wave sleep is light.

In a series of experiments, the WAG/Rij rat model for absence epilepsy was characterized with respect to the relationships between the level of vigilance, sleep-wake states and the occurrence of spike-wave discharges. In the first experiment, continuous recordings were made for a period of 48 hours and a clear circadian rhythm was established for the number of spike-wave discharges. A maximum appeared during the middle of the dark period of the rat, whereas a minimum was detected directly after the onset of the light period, the time period during which deep slow wave sleep predominates. The relationship of spike-wave discharges with states of vigilance was elaborated in a second study. Spike-wave discharges were mainly found during light slow wave sleep, during passive wakefulness and in transition phases from sleep to wakefulness. During REM sleep no spike-wave discharges were found. In the last three experiments, the level of alertness was enhanced by various procedures as photostimulation, a learning task and deprivation of REM sleep. In all cases, an increase of alertness decreased the amount of epilepsy.

It is concluded that discharges preferably occur when the level of vigilance of the brain is close to the level noticed at the transitions from sleep to wakefulness i.e. during passive wakefulness, drowsiness and light slow wave sleep. Furthermore, modulation of the level of vigilance influences the occurrence of spike-wave discharges in rats of the WAG/Rij strain.

6.2 Introduction

About half a century ago, Griffiths and Fox (1938) collected data on the incidence of various kinds of epileptic attacks over a 24-hour period and described a clear circadian variation. During sleep, the number of seizures showed a gradual increase from midnight till morning. The peak in the early morning was followed by a sharp reduction till noon. After the meal a smaller peak occurred, whereas a considerable increase in seizure incidence was again found in the early evening hours.

More recently, an almost similar pattern was found for generalized spike-wave activity, including absence epilepsy (Martins da Silva et al 1984). Absence or petit-mal epilepsy is a mild form of epilepsy with short-lasting drops in consciousness accompanied by generalized aberrant EEG phenomena. During the evening and night hours, the number of absences was greatest and the existence of nocturnal maxima of this kind of fits was confirmed by several other studies (Ross, Johnson and Walter 1966; Stevens, Lonsbury and Goel 1972). In addition, it appeared that nightly discharges were shorter than the diurnal paroxysms and that often a mixture of spike-waves and spindles occurred (Stevens et al 1971). In concordance with this, the large majority of generalized epileptic discharges occurred during sleep with spindles, i.e. stage 2 of sleep (Kellaway 1985). This may explain the fact that, in the course of the night, the increase in stage 2 sleep parallels a rise of the number of discharges. While some studies generally confirmed these points or found a maximum at the early morning hours (Beck, Wenzel and Sauer 1977, Offenbacher etal 1986), others noticed a preponderance of discharges during stages 3 and 4 of sleep (Ross, Johnson and Walter 1966; Sato, Dreifuss and Penry 1973). With a single exception (Beck, Wenzel and Sauer 1977), almost all studies agreed that the number of discharges was lowest during REM sleep.

General agreement exists with respect to the diurnal incidence of absences. In particular, this kind of epileptic activity appears when the level of vigilance is not high (see for review Vieth 1986). An absence is less likely to occur when a pycnoleptic child is engaged in high mental activity. On the contrary, during short periods of relaxation the chance for spike-wave discharges to break-through increases. This is also the case just before falling asleep and during short arousals from sleep (Tomka 1985).

As a consequence, clinical or experimental manipulation of the level of vigilance should be able to influence the genesis of spike-wave discharges. A logical method to avoid absences from occurring, might therefore consist of increasing the level of alertness (see e.g. Vieth 1986), and neurologists have applied this therapy successfully (e.g. Meier-Ewert 1978). It is also well-known that total deprivation of sleep activates epileptic activity. In fact, this method is widely used for clinical diagnosis of epilepsy (Declerck 1983; Logothetis, Milonas, and Bostantzopoulou 1986; Montplaisir, Laverdière and Saint-Hilaire 1985). Beck et al (1977) studying the effects of slow-wave sleep deprivation in children suffering from pycnolepsy, found that in the five hours of waking after a night of deprivation, absences were more frequent and longer lasting than after a night of normal sleep.

This result seems to favour the alertness-reduction hypothesis, which states that the increase in epileptic activity is solely the result of a reduction in alertness leading to drowsiness and sleepiness. Alternative hypotheses, however, cannot yet be ruled out (Geller et al 1969; Montplaisir, Laverdière and Saint-Hilaire 1985): epileptic activity may not be elicited by a lowered level of vigilance only, but also by endogeneously or exogeneously generated, deviant arousal-fluctuations.

Some years ago, we discovered that in a particular strain of rats, the WAG/Rij strain, all adult members spontaneously show electrophysiological (for an example see Fig. 6.1), as well as the behavioural characteristics resembling those of human absence epilepsy (Van Luijtelaar and Coenen 1986). In many respects this animal model for absence epilepsy closely resembles that of Marescaux et al (1984) and Vergnes et al (1982).

The WAG/Rij model has been validated by behavioural and pharmacological studies and has shown to be a useful supplemental animal model for primary generalized absence epilepsy in man, in particular for the reason that WAG/Rij rats are homozygous (Coenen and Van Luijtelaar 1987; Coenen and Van Luijtelaar 1989, Peeters et al 1988; Van Luijtelaar and Coenen 1986).

Figure 6.1 Example of a typical 7-10 Hz spike-wave discharge.

To study the relationship between vigilance and epilepsy, several experiments were carried out in which the occurrence of spike-wave discharges was studied during various spontaneous or experimentally induced levels of vigilance.

In the first experiment, it was established whether or not a circadian rhythmicity in discharges could be detected in the present model and whether there exists a relationship with the daily variation as found in man. The second experiment dealt with the question in which of the spontaneously occurring levels of vigilance -i.e. sleep-wake states- discharges occur most frequently. In the final experiments, it was investigated in three distinct ways whether modulation of the level of vigilance had an effect on the number of spike-wave discharges. It can be safely assumed that during the execution of a task in which learning is involved, the vigilance level of the brain is higher than during a period of rest. Therefore, in the first of these experiments, the number of spike-wave discharges in a one hour lasting learning task was compared with the number occurring one hour before and after the completion of this task. The operant learning task was pressing a lever for food on a fixed-interval schedule of reinforcement. Following the same line of reasoning, in the second modulation-experiment, spike-wave discharges were quantified before, during and after photic stimulation (Doose et al 1969). Finally, in the last of this type of these experiments, vigilance was modulated by selective deprivation of REM sleep. Evidence exists that this type of deprivation produces an increase in the level of vigilance (Mogilnicka et al 1986; Van Hulzen and Coenen 1984) and whereas general consensus

exists about the facilitating effects of total sleep deprivation on the occurrence of spike-wave discharges, much less is known about the effects of selective sleep deprivation. Beck et al (1977) compared the effects of selective deep slow wave sleep deprivation with those of REM sleep deprivation on the number of spike-wave discharges in pycnoleptic children. They found the more often reported increase after slow wave sleep deprivation but, interestingly, they noticed an opposite effect after selective REM sleep deprivation. This prompted us to investigate the effects of REM sleep deprivation on epileptic activity of the WAG/Rij rats.

6.3 Studies on changes in vigilance

6.3.1 Circadian rhythmicity

Subjects were 19 adult male rats of the WAG/Rij strain (age between 6 and 18 months). Under complete Nembutal anesthesia (60 mg/kg body weight), animals were provided with permanent cortical electrodes (Plastic Products Company, MS 333/2-A) and maintained on a 12-12 LD cycle with lights on at 01.00 h. After extensive habituation to all experimental procedures, a continuous EEG recording, lasting 48 hours, was made of every rat. By visual inspection of these recordings, spike-wave discharges were counted per hour. Results are presented in Figure 6.2, together with compiled data of wakefulness occurring per hour (after Drinkenburg et al 1991).

Analysis of the data was carried out by a cosinor curve fitting program according to Monk and Fort (1983). This cosinor analysis on the number of spike-wave discharges of each subject showed that a cosine, with a period length of 24 hours, could be fitted in 18 of the 19 animals ($p<0.05$). This implies an explicit circadian variation in the incidence of spike-wave discharges was uncovered. An analysis of variance revealed a significantly higher incidence of spike-wave discharges during the dark than during the light period ($F(1,18)=23.5$, $p<0.001$). Between the two experimental days no differences were found. The maximum number of discharges (acrophase) was found between the fourth and fifth hour of the dark period, whereas the minimum (nadir) coincided with the early hours of the light period. This trough was noticed immediately after the onset of the light period,

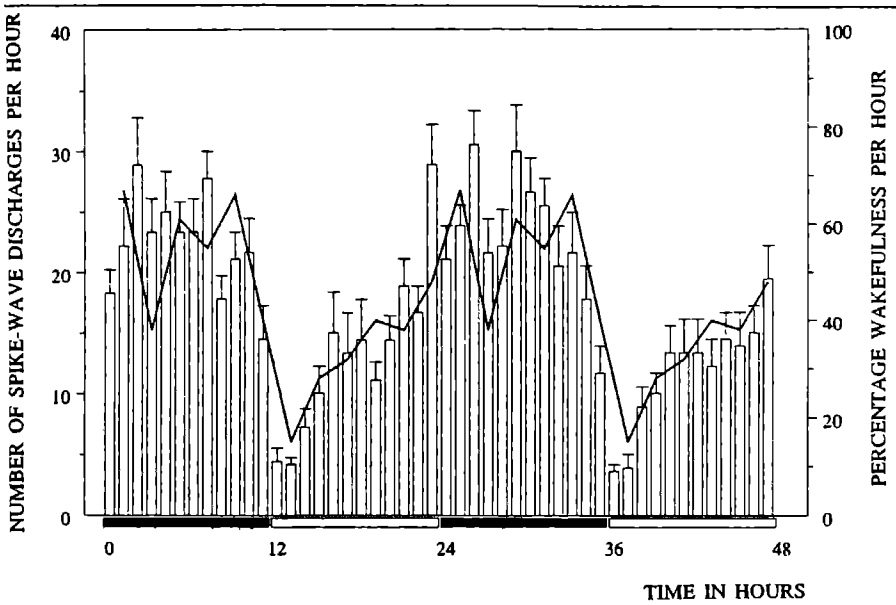

Figure 6.2 Compiled data of wakefulness occurring per hour in WAG/Rij rats (solid line; after Drinkenburg et al 1991 [see chapter 4]), and mean number (+ S.E.M.) of spike-wave discharges per hour per rat (bars: after Van Luijtelaar and Coenen 1988). Despite the fact of completely independent studies, there is a remarkable correspondence between the two parameters. Dark periods are indicated by black bars beneath the X-axis.

the major sleep period of the rat. This period is characterized by a high amount of specifically deep slow wave sleep (e.g. Van Luijtelaar and Coenen 1984; 1986). Finally, it appeared that also seizure activity gradually increased in the course of the light period.

6.3.2 Sleep-wake states

Eight male adult WAG/Rij rats in age varying between 7 and 28 months, with standard EEG and EMG electrodes, and maintained on a light-dark regime as used in the previous experiment, served as subjects. After extensive adaptation of the animals to the experimental conditions, electrophysiological registrations were made during one hour in the dark and one hour in the light period. Using common indices, wakefulness, slow-wave sleep (in the meaning of all non REM sleep) and REM sleep were distinguished (Van Hulzen and Coenen 1984).

All rats showed spike-wave discharges. During wakefulness 40% of the discharges occurred whereas the remaining 60% were seen during sleep. Spike-wave discharges were almost completely absent during REM sleep. It also appeared that a great number of paroxysms seen during sleep, were followed by a period of wakefulness of which the duration was often short (between 1 and 10 seconds). A significant positive correlation was found between the number of sleep stage shifts, mainly shifts to awakenings, and the number of spike-wave discharges. Furthermore, it is evident that they do rarely coincide with REM sleep. From previous research (Van Luijtelaar and Coenen 1986; 1988), it is known that fewer spike-wave discharges are apparent during active wakefulness when rats often show exploratory behavior. Moreover, spike-wave discharges are never seen when the animal is moving but always during immobile behaviour. This was further investigated in another study in which a distinction was made between active and passive wakefulness (Drinkenburg et al 1991). In that study, in which a similar distribution of spike-wave discharges occurring during wakefulness and slow wave sleep as in the before-mentioned experiment was found, it was confirmed that of the spike-wave discharges occurring during wakefulness only a small percentage was preceded by active wakefulness (6.8 %) and the vast majority by passive wakefulness (93.2 %). Moreover, a further distinction between light and deep slow wave sleep, based on delta-band power, was made; of all spike-wave discharges following slow wave sleep, 76.5 % was preceded by light slow wave sleep and 23.5 % by deep slow wave sleep (Drinkenburg et al 1991). Therefore, one may conclude that an active brain, as seen during active wakefulness and REM sleep, is not a favourable condition to generate spike-wave discharges. In all, evidence exists that the propensity for spike-wave activity to occur is greatest during wakefulness when the level of alertness is low, as also seems the case in humans (Vieth 1986).

6.3.3 FI-learning task

Eleven adult, male WAG/Rij rats (age 6-10 months) were trained in an operant learning task to press a lever for food, during at least 30 training sessions on a fixed interval schedule of reinforcement with an interval duration of 60 seconds. Next, they were implanted with permanent cortical EEG electrodes (as in experiment 1), allowed to recover

from surgery and retrained on the same operant task. Subsequently, their EEG's were recorded during three consecutive hours. In the base-line preceding training, rats had a mean of 11 (SEM=0.63) discharges per hour, during the one hour performance on the fixed interval schedule. This decreased to 0.6 (SEM=0.10) during the hour of training, while the amount of spike-wave discharges returned back to 7.6 (SEM=0.56) in the post-training base-line hour. These differences were significant (*t*-test for dependent groups) at the 0.01 level.

The most parsimonious interpretation is that the alertness level is increased during the hour in which the task is performed. This increased alertness is a consequence of the accompanying motor activity required to press the lever and of the cognitive activity implying the timing of the responses in order to obtain the food-rewards.

6.3.4 Photic stimulation

Subjects were seven male adult WAG/Rij rats (age 10-20 months) provided with permanently implanted EEG electrodes (see experiment 1). A range of stroboscopic stimulation frequencies (between 5 and 30 Hz.) was used in order to control any possible photosensitivity effects. Actually presented stimulation frequencies can be found in Figure 6.3. Each photic stimulation frequency was present during 1 minute and was followed by a rest period of 1 minute. Results are presented in Figure 6.3.

During periods of stroboscopic stimulation spike-wave discharges were seldom present (grand total over all rats over the nine stimulation periods 13), whereas in the periods between the various stimulation phases more paroxysms appeared (grand total over the nine rest periods 89). Since the effects of photic stimulation might be present beyond the period of stimulation, in another experiment 15 minutes of photic stimulation (25 Hertz) were preceded and followed by another 15 minutes EEG registration without any stimulation. Before photic stimulation, we noticed 8.6 (SEM=1.60) spike-wave discharges (n=9), during photic stimulation only 2.0 (SEM=1.05), while after stimulation a mean of 8.2 (SEM=0.99) spike-wave discharges per quarter of an hour was obtained. The pre- and post-stimulation period differed each from the stimulation period (*t*-test for dependent groups) at the 0.005 level. It was again thought that the external stimulation increases

arousal to such a level as the more active vigilance states. At its turn, this suppresses epileptic absence activity.

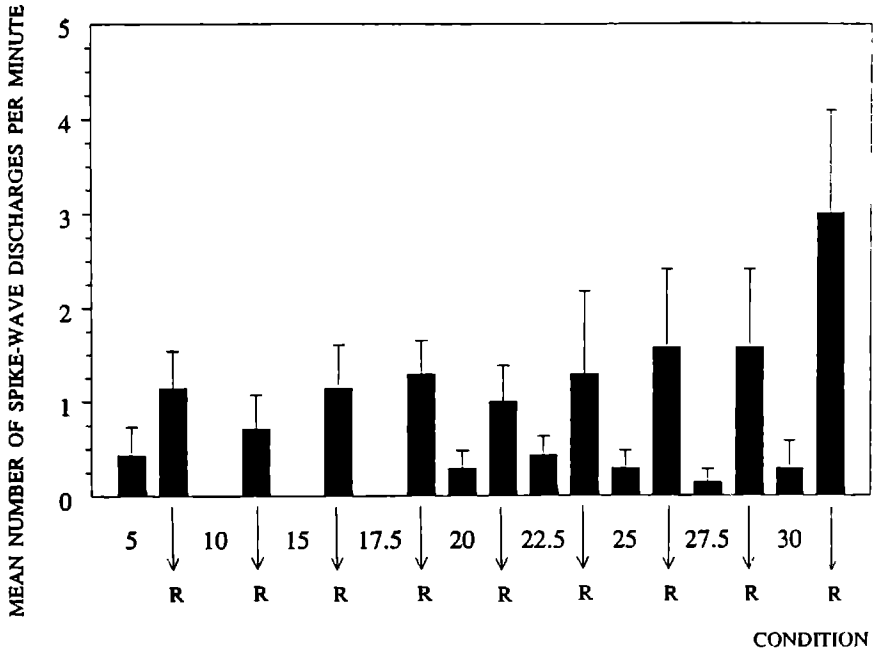

Figure 6.3 Mean number of spike-wave discharges per minute during photic stimulation and rest periods. Beneath the X-axis flash stimulation frequencies are given, R represents periods without stimulation. Spike-wave discharges predominate during periods of rest.

6.3.5 REM sleep deprivation

Nineteen adult male WAG/Rij rats with an age between 6 and 8 months, provided with standard cortical EEG electrodes (as in experiment 1), were used. After extensive adaptation to all recording conditions, ten rats were deprived for 72 hours of REM sleep using the pendulum technique (Van Hulzen and Coenen 1984), while nine others underwent a control treatment. In applying the pendulum technique, rats, while staying in their home-cage, are placed in a slowly moving swing which produces regularly postural imbalance and subsequent awakenings at the two extremes of oscillation of the pendulum. This permits slow wave sleep but prevents REM sleep. This technique has been electrophysiologically validated and it was found that sleep parameters during the

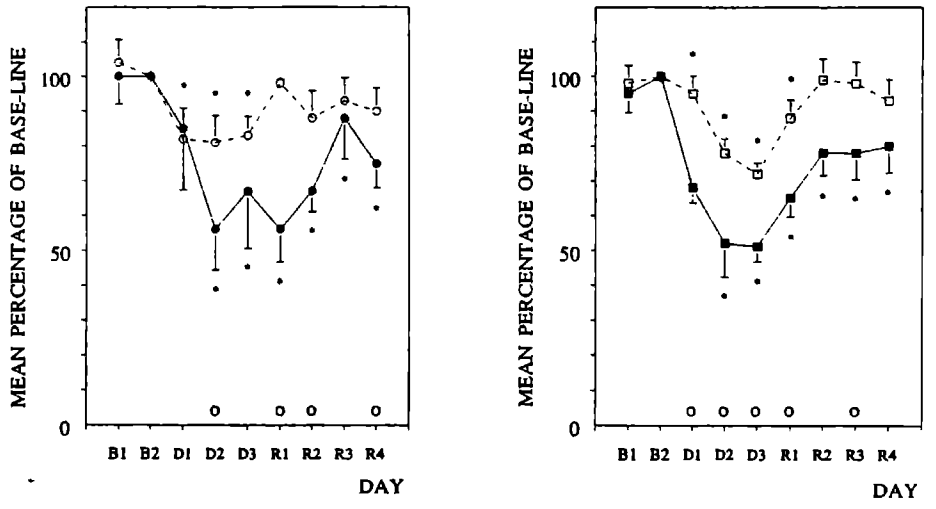

Figure 6.4 Left panel: the occurrence of spike-wave discharges as expressed by the mean hourly percentage of the second base-line day, during two base-line (B1, B2), three deprivation (D1, D2, D3), and four recovery days (R1, R2, R3, R4) in succession for the REM sleep deprived (filled circles) and the control group (unfilled circles). Right panel: the duration of spike-wave discharges as expressed by the mean hourly percentage of the second base-line day, during two base-line, three deprivation and four recovery days in succession for the REM sleep deprived (filled squares) and the control group (unfilled squares). O means significant differences between groups; * means significant differences with the second base-line day, $p<.05$)

deprivation and rebound period are not distinct from the commonly used, but criticized platform technique (Van Luijtelaar and Coenen 1986). Control rats are placed in an identical device which is so adjusted that no imbalance takes place. Consequently, REM sleep is untouched and there is no rebound (Van Hulzen and Coenen 1980). Before, during and after deprivation days, the number and mean duration of spike-wave discharges were determined.

The results of this experiment are depicted in Figure 6.4. Although both the REM sleep deprived and its control group showed a decrease in the number and mean duration of the discharges during the three deprivation days, the REM sleep deprived group had significantly less and shorter spike-wave discharges than the control group. After the

deprivation period, the control group quickly returned to pre-experimental values, which was not the case for the REM sleep deprived group. The number of spike-wave discharges remained significantly reduced during the recovery period and tended to return to base-line levels slowly. These results show that REM sleep deprivation reduces both the amount and duration of spike-wave discharges and these results are in agreement with the clinical data of Beck et al (1977). From earlier work, it is known that also after 72 hours of REM sleep deprivation with the pendulum technique the amplitude of the visual evoked potential is reduced (Van Hulzen and Coenen 1984). This was interpreted as an increase in tonic arousal. Therefore it is thought that REM sleep deprivation, in contrast to total or deep slow wave sleep deprivation which facilitates sleepiness and decreases alertness, enhances the level of vigilance. This increased alertness suppresses the number and duration of spike-wave discharges. The much smaller effects in the control group are explained by the supposition that also in this group, due to the treatment, there is some increase in the level of vigilance.

6.4 Discussion

Human data show, that although spike-wave discharges occur during all four stages of slow wave sleep, there is a prevalence of occurring during light sleep. The present data support the view that also in the rat spike-wave discharges preferably occur during light slow wave sleep. During the first few hours of the light period where deep slow wave sleep dominates in rats (Van Luijtelaar and Coenen 1984; 1986), there is a remarkable trough in the number of seizures. Furthermore, there is a gradual increase in the amount of spike-wave discharges during the light period. Both man and rat share the gradual increase in light slow wave sleep and seizure activity in the course of their sleeping period. During REM sleep paroxysms do rarely occur.

Although spike-wave discharges are more frequently found during sleep than during wakefulness, there are more of these discharges in the active period of the rats, which is the period with lights out, than in the passive period. This apparent discrepancy (more spike-wave discharges in the active period, while spike-wave discharges occur more often during drowsiness or light slow wave sleep) may be explained by the fact that deep slow

wave sleep and REM sleep are prevalent in the light period (Van Luijtelaar and Coenen 1984; 1986), during which states spike-wave discharges are less likely to occur, while during the dark period relatively more light slow wave sleep occurs. During the dark period also quiet wakefulness frequently occurs, a second state of vigilance with a rather high number of spike-wave discharges.

It is likely that more transitions from sleep to wakefulness occur in the dark than in the light period. A marked phenomenon is that when a spike-wave discharge occurs during sleep, this discharge is followed by an awakening in more than one-third of all cases. In all, in this way the intimate relationship between the number of spike-wave discharges per hour and the percentage of wakefulness in the same hour might be understood (Fig. 6.2).

Several data obtained in man show that the maximum number of spike-wave discharges occurs during the sleeping period, while the rat data show a maximum during the active period. The lack of a clear maximum during the daytime in man, may be the result from the fact that subjects are usually engaged in mental and physical activities. During these hours, the vigilance level is too high for absences to appear. During afternoon naps, or at periods of leisure, absences are more prevalent. Put into other words: the genuine distribution of spike-wave discharges over the 24 h period might be masked by several types of activities. This 'masking' hypothesis can be tested by having patients suffering from absence epilepsy lie in bed under constant conditions and instructing them to refrain from every type of activity. Then the rise and fall of the EEG paroxysms might reveal a different picture. A second possibility is to keep rats occupied during the dark portion of the light-dark cycle and to see whether a putative maximum is present during the sleeping period.

The relationship of spike-wave discharges with sleep spindles is another important topic. In man both spike-wave discharges and spindles occur predominantly during stage 2. In animals these two phenomena also seem closely linked. Kostopoulos and Gloor (1982) found that electrical stimulation of the thalamic nucleus centralis medialis in cats results in cortical spindles, which are transformed into spike-wave discharges after penicillin injection. We found that spike-wave activity in rats is often interspersed with spindles and that it is no exception that spike-wave discharges are preceded by spindling (Van

Luijtelaar and Coenen 1986). Recently Gandolfo et al (1990), found that the intermediate stage in epileptic WAG/Rij rats lasts much longer than in less epileptic Wistar rats. Among others, this stage is characterized by spindles and this suggests again a relationship between spindles and spike-wave discharges. As mentioned before, also other authors have emphasized this relationship. According to Halász (1982) there exists a close relationship between mechanisms responsible for sleep spindles and spike-wave discharges. The latter discharges might be the aberrant members of the same family of events to which sleep spindles belong. Halász (1982) concludes that there is an optimal zone of reduced vigilance or alertness which is favourable for the appearance of spindles and discharges, while states of extreme waking and REM-sleep, and, on the other hand, deep slow wave sleep are unfavourable, because of the lack of instability of the EEG.

This statement is quite close to our general conclusion from the five experiments: spike-wave discharges preferentially occur when the level of vigilance is not far from that characteristic of transitions from sleep to waking or vice versa. A high level of alertness either spontaneously present during active wakefulness or induced by REM sleep deprivation, by a learning task, or by photic stimulation, all lead to a reduction of spike-wave discharges. Also the active brain during REM sleep and, on the other hand, the brain condition during deep slow-wave sleep may also be less favourable.

In general, the present results contribute further to the validity of the WAG/Rij strain as a model for generalized absence epilepsy considering the close common results obtained in man and rat.

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„It remains to be established whether attention failures, (...), are due to disrupted sensory input, to impaired decision making, to impaired sensory-motor integration or reduced motor capacity, to temporary forgetting of the task instructions, or to some combination of these factors."

Allan Mirsky, 1988

CHAPTER 7

INTERACTION OF SPIKE-WAVE DISCHARGES AND OPERANT BEHAVIOUR IN EPILEPTIC RATS[1]

[1] DRINKENBURG WHIM, SONDAG HNPM, COENEN AML, VOSSEN JMH, VAN LUIJTELAAR ELJM. INTERACTION OF SPIKE-WAVE DISCHARGES AND OPERANT BEHAVIOUR IN EPILEPTIC RATS. *BEHAVIORAL BRAIN RESEARCH* (SHORT COMMUNICATION IN PREPARATION).

Chapter 7 Interaction of spike-wave discharges and operant behaviour in epileptic rats

7.1 Summary

To determine whether ictal information processing can be studied by means of test paradigms that require an operant response, the interaction of spike-wave discharges and operant behavior was assessed. WAG/Rij rats, whose cortical electroencephalogram spontaneously shows generalised spike-wave discharges, were equipped with electrodes for cortical electroencephalography (EEG). Subsequently, they were trained on an appetitively motivated visual detection task to press a lever upon presentation of a light stimulus, which was presented for a period of ten seconds on a variable-interval (VI 60) schedule. Responses, that were made within ten seconds after onset of the stimulus were rewarded with a food-pellet. During a three hours lasting test session of 180 trials both cortical EEG activity and operant performance were registered and analysed in blocks of 15 minutes. The occurrence of spike-wave discharges was largely restricted to the middle part of the test session, when animals responded on 75 till 90 percent of all stimulus presentations. Spike-wave discharges occurred less frequently during the periods with near maximal and low percentages of responded trials, which were mainly found at the beginning and at the end of the test session, respectively. It is therefore concluded that within a test session an optimal period exists during which the chance of occurrence of a spike-wave paroxysm as well as the chance of generation of an operant response are high. The present study underlines the feasibility of using operant behaviour to study information processing during epileptic activity in rats. Additionally it was noted that responses were never initiated during ongoing spike-wave discharges, while immediately after the disappearance of the spike-wave activity response performance was again normal.

7.2 Introduction

From studies in epileptics, it is since long known that spike-wave discharges are often accompanied by disturbances in cognitive functioning (Schwab 1939; Mirsky 1988). Such cognitive disturbances vary in severity, ranging from a less than a second lasting diminishment in alertness to many seconds lasting irresponsiveness to environmental stimulation (Browne et al 1974; Shimazono et al 1953). Disturbances in perception, in central information processing, in motor programming, and in motor execution have been suggested to explain these performance changes (Hutt and Gilbert 1980; Mirsky 1988; Provinciali et al 1991). The mechanisms in the brain, which underly this temporary and reversible cognitive defect are yet largely unknown and are considered a valuable object to study by means of genetic animal models; knowledge concerning both pathological and normal functioning of the brain can be gained (Coenen et al 1992; Löscher 1984).

The cortical electroencephalogram (EEG) of rats of the inbred WAG/Rij strain spontaneously show spike-wave discharges (Van Luijtelaar and Coenen 1986). Next to this EEG phenomenon, a behavioural arrest with some small postural changes, reminiscent of human absence epilepsy, can be found during the spike-wave discharges, while also the pharmacological and genetic characteristics of the spike-wave discharges resemble those found in human absence epilepsy (Coenen et al 1992). Therefore, the WAG/Rij model is regarded as a useful model for studying mechanisms underlying spike-wave paroxysms and consequential behavioural anomalies.

However, earlier studies found that the occurrence of spike-wave discharges is largely restricted to certain levels of vigilance; seizures most likely occur during intermediate levels of vigilance and sleep-wake states, such as light slow wave sleep and passive wakefulness (Coenen et al 1991; Drinkenburg et al 1991). As a consequence, some reports have argued that testing in epileptic rats of ictal information processing using reinforcement of behaviour is unfeasible because engagement in an operant behaviour is thought to heighten alertness to such a level that epileptic activity is suppressed (Marescaux et al 1991; Vergnes et al 1991). Therefore, the extent and course of interaction of the occurrence of spike-wave paroxysms and operant responding should be determined. The aim of the present study is to describe the effects of operant responding on the

occurrence of spike-wave discharges.

7.3 Methods

Eight, adult, male WAG/Rij rats (age about 10 months, free-feeding weight 200-375 grams: mean 271 grams) served as subjects. Rats were singly housed and were maintained on a 12-12 hour light-dark cycle with lights on at 7.00 p.m., while all experimentation took place during the first six hours of the dark period. A month before the training started, each rat was, under complete anaesthesia (Nembutal, Abott Laboratories, 60 mg/kg i.p.), permanently implanted with a standard cortical tripolar EEG electrode (Plastics One MS-333/2-A). EEG electrodes were placed in the frontal cortex and in the parietal region, respectively with coordinates[2]: A 2.0, L 3.5 and A -6.0, L 4.0 (skull surface flat and bregma zero-zero), while a third reference electrode was placed over the cerebellum. Animals were left undisturbed to recover for three weeks. Next, one week before training started, a mild food deprivation (down to 90 % of their free-feeding weights) was instated. Access to tap water remained ad lib.

For training and testing, two operant chambers (L27xW25xH24 cm) were used, equipped with one lever, a centrally placed food tray and pellet dispenser (delivering 45 mg pellets), red house lights, and a stimulus light above the lever. Chambers were connected to Skinner Box Controllers and controlled by an Apple Macintosh SE 30. The stimulus light consisted of 64 green LEDs (PD 1167, Siemens), together forming a 25,4 millimetres square light. Both boxes were adapted for recording of EEG in the operant chamber. EEG signals were amplified and filtered by an Elema-Schönander polygraph, which allowed frequencies between 0.5 and 70 Hertz to pass and were written out on chart paper (speed 1.0 cm/sec).

The following behavioural training protocol was used: rats were initially trained to press the lever on a continuous reinforcement (CRF) schedule using an autoshaping procedure. Subsequently, with the lever permanently available in the chamber, the stimulus light was presented for a ten seconds period. To obtain a food pellet and to progress to the

[2] Stereotaxic coordinates were choosen to meet the requirements of reliably scoring sleep-wake states as well as spike-wave discharges.

next trial, the rat had to press the lever within 10 seconds after stimulus onset. Upon pressing the lever, the stimulus light was switched off, the tray light illuminated and a food pellet delivered. The next trial followed after a variable interval of 60 seconds mean duration (VI-60 with range 30 - 90 s.). This rather long and variable inter-trial-interval was chosen to prevent the development of scalloping behaviour, thus promoting a more relaxed, spike-wave-prone vigilance level. If the rat failed to respond within 10 seconds the stimulus light was extinguished and no food pellet was delivered. Each session consisted of 180 trials and lasted 3 hours. Each animal received one session per day, five days a week. The task was considered to have been mastered if the number of rewarded responses was above 80 % for three consecutive days and both the latency to press the lever and the number of non-reinforced responses were stable (statistically not different) over the three days. After reaching this criterion and after 24 hours adaptation to the experimental setting, baseline EEG recordings were made during one hour preceding the final EEG-controlled testsession, that was started at 09.00 a.m. Task parameters of the training session were also used for the EEG-controlled test session, that also consisted of 180 trials and that lasted 3 hours. During the test session EEG activity was monitored not only to determine the occurrence of spike-wave discharges but also to detect the possible occurrence of sleep slow-waves.

Spike-wave discharges during the baseline and the test session were analysed for blocks of 15 minutes duration and scored independently by two experienced EEG-analysts according to the criteria elaborated earlier (van Luijtelaar and Coenen 1986).

Intra-individual differences were statistically analysed by means of the non-parametric Wilcoxon Matched-pairs Signed-ranks Test. All values are means of eight rats and are indicated with standard errors of the means (SEM).

7.4 Results

The mean number of spike-wave discharges during the test session was significantly below mean baseline hour value ($Z=-2.381$, $p=.017$). The distribution of the spike-wave discharges over the baseline hour and over the three testing hours is shown for 15 minutes periods in Fig. 7.1 (left Y-axis). It is clear that during the first two 15 minutes

blocks of the test session (respectively Z=-2.52, p=.012; Z=-2.521, p=.012) as well as during the last three 15 minutes blocks of the test session (respectively Z=-2.52, p=.012; Z=-2.10, p=.036; Z=-2.52, p=.012) spike-wave activity was suppressed as compared to the mean number of spike-wave dicharges during the base-line hour. The majority of spike-wave discharges appeared in the third till the ninth 15 minute period (between the 75th and 135th minute after the start) of the test session.

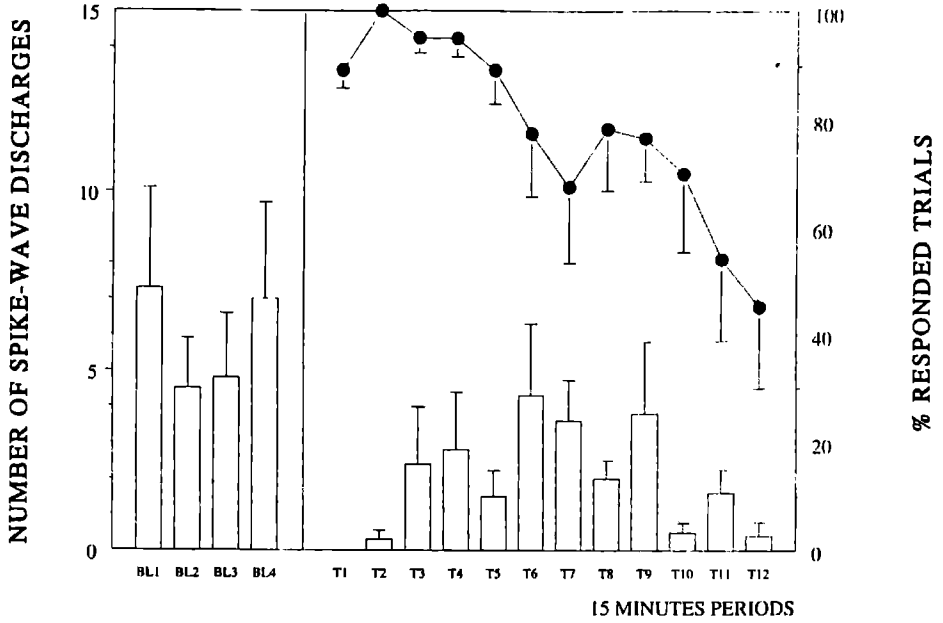

Figure 7.1 Mean number (± SEM) of spike-wave discharges (bars; left Y-axis) per block of 15 minutes duration is shown for the base-line hour (BL1 - BL4) and the three consecutive test hours (T1 - T12). Response behaviour as expressed by the percentage (± SEM) of trials in which a response was given (line; right Y-axis) is indicated per block of 15 minutes duration for the three test hours (T1 - T12). For statistical analysis see Results section.

The mean percentage of responses is also indicated in Fig. 7.1 (right Y-axis); starting from almost an optimal response rate (>90 %), the percentage of trials in which a response was given declined towards the end of the session. This reduction was parallelled by the appearance of slow waves in the EEG during the later part of the

testsession in six out of eight animals; from about the 9th 15-minute period onwards these animals progressively interrupted operant responding for minuts-lasting periods of sleep. Although the actual cause of these interruptions was beyond the scope of the present study and can only be speculated upon (e.g. satiation, fatigue), response rates then dropped below 80 percent and eventually were found just slightly above 40 percent on average.

An unexpected result, which was found as a spin-off of the experimental design, concerns the quality of responding: the onset of a small number of stimulus presentations happened to coincide with ongoing spike-wave discharges (on average 2.88 ± .095 per test session per animal). None of these ictal stimulus presentations was followed by a leverpress during ongoing spike-wave discharges, whereas immediately after the spike-wave activity had ended a leverpress was made. It should be noted that the stimulus presence usually extended into the again normalised EEG. Furthermore, in some cases (on average 3.88 ± .091 per test session per animal) the stimulus onset fell within 0.5 s till 10 s after spike-wave activity had ended; these post-ictal presentations were all responded to with a mean response latency (2.61 s ± 0.47) that did not differ (Z=-0.34, p=.74) from the mean response latency of stimulus presentation during non-spike-wave EEG (2.21 s ± 0.25).

7.5 Discussion

The number of spike-wave discharges during the baseline hour was comparable to results of earlier studies, and confirms that possible effects of food-deprivation per se on the occurrence of spike-wave discharges are minimal or absent (Coenen et al 1992; Vergnes et al 1991). The main outcome of the present study is that an optimal period for the occurrence of both spike-wave discharges and operant responses was established. Response rates declined from the 75th trial on, while at the same time the number of spike-wave discharges no longer differed from base-line frequencies. From about the 135th trial onward, the number of responses continued to decrease while spike-wave activity again became below baseline level.

The results stress the notion elaborated earlier (Coenen et al 1991) that a high level

of physical or mental activity, as can be assumed to exist when highly engaged in an attentionally demanding visual detection task, is correlated with a low incidence of spike-wave discharges. (Vergnes et al 1991).

During the next period of the session, rats got less involved in leverpressing. Earlier studies showed that short-lasting periods of relaxation and interruptions in task engagement favour the occurrence of spike-wave discharges (Coenen et al 1991; Van Luijtelaar et al 1991), whereby the appearance of spike-wave discharges is suggested to be related to the presence of transitional, intermediate levels of vigilance (Drinkenburg et al 1991; Gralewicz & Łuczak 1994). The lowering of both the response rate and the spike-wave activity during the last 45 minutes was accompanied by an clear-cut increase in drowsiness and even by short periods of sleep. The EEG of the animals then showed periods of sleep slow wave activity, that has been found unfavourable for the occurrence of spike-wave paroxysms (Drinkenburg et al 1991; 1995). These findings are keeping in with results of studies in both epileptic man and animals as they affirm the paroxysm-proneness of intermediate levels of vigilance (Halász 1991; Terzano et al 1989).

Although earlier studies questioned the feasibility of reliably studying information processing during spike-wave activity by means of analysis of operant behaviour (Marescaux et al 1991; Vergnes et al 1991), the present study shows that during a certain period the animals are at the same time sufficiently engaged in the task to reliably respond to a conditioned stimulus with response rates of about 75 percent as well as adequately (in)excited to generate spike-wave discharges.

Furthermore, leverpressing appeared to be inhibited only during actual spike-wave activity, suggesting impairment of perception, or attention, or of motor programming and execution (Orren 1978; Mirsky 1989). Immediately after the epileptic EEG signals had ended, the performance diminishment was no longer present. This latter finding underlines the truly ictal nature of the disturbance of responsiveness and stresses the need to study ictal information processing by using learning tasks, which investigate impairments specifically during ongoing spike-wave discharges without extending the presentation of the stimulus into post-ictal EEG. Such studies to further unravel the paroxysm-related impairment in information processing can be reliably undertaken in

WAG/Rij rats.

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Vergnes M, Marescaux C, Boehrer A, Depaulis A. Are rats with genetic absence epilepsy behaviorally impaired? *Epilepsy Res* 9:97-104;1991.

„THE QUESTION OF WHAT HAPPENS TO CONSCIOUSNESS DURING THE FRACTION OF A MINUTE OF THE LARVAL ATTACK IS IMPORTANT IN EVALUATING THE SERIOUSNESS OF THE CORTICAL DISTURBANCE AND ITS INFLUENCE ON OTHER CEREBRAL ACTIVITY."

ROBERT SCHWAB, 1938

CHAPTER 8

INFORMATION PROCESSING DURING SPIKE-WAVE DISCHARGES IN EPILEPTIC RATS: STIMULUS EVALUATION[1]

[1] DRINKENBURG WHIM, SCHUURMANS MLJE, COENEN AML, VOSSEN JMH, VAN LUIJTELAAR ELJM. INFORMATION PROCESSING DURING SPIKE-WAVE DISCHARGES IN EPILEPTIC RATS: STIMULUS EVALUATION. *ELECTROENCEPHALOGRAPHY AND CLINICAL NEUROPHYSIOLOGY* (SUBMITTED).

Chapter 8 Information processing during spike-wave discharges in epileptic rats: stimulus evaluation

8.1 Summary

In the present study it was investigated to what extent information processing is disturbed during the occurrence of generalised spike-wave discharges indicating non-convulsive absence epilepsy. To this end, WAG/Rij rats, which all spontaneously show trains of these discharges, were provided with electrodes for cortical electroencephalographic (EEG) registration. Next, the animals were trained in an appetitively motivated conditioning paradigm to discriminate between two auditory stimuli with equal duration and frequency, but with different intensities. Two experimental groups were formed: the first group of animals learned that the low intensity stimulus was always followed by a food reward, whereas the high intensity stimulus was never reinforced. In the second group reinforcement was given in a counterbalanced way. In the test phase, both stimuli were presented in pseudorandom order during spike-wave discharges and the reactivity of the ongoing EEG activity was analyzed. It was found that presentation of the reinforced stimulus had significantly more effect on ongoing EEG activity than the non-reinforced stimulus, regardless of the intensity of the stimuli. This shows that during generalised spike-wave discharges the brain is still capable of evaluating the meaning of an ictally presented stimulus; sensory and attentional processes are not completely disturbed. The results of the present study emphasize the heuristic value of rodent models for studying both ictal and normal information processing.

8.2 Introduction

Paroxysmal electrical brain activity has since long been associated with cognitive impairments (Esquirol 1838). In humans, cortically generalised spike-wave discharges constitute the hallmark of several non-convulsive types of epilepsy, such as childhood absence epilepsy (Drury and Dreifuss 1985; Gomez and Westmoreland 1987; Sandstedt 1990). Shortly after the introduction of electroencephalography (EEG), it was recognized that the electroencephalographic spike-wave activity in epileptics was accompanied by diminishments in responsiveness to external stimuli that during interictal EEG activity elicited responses in an adequate way (Gibbs, Davis and Lennox 1935; Schwab 1939). Since that time numerous studies in epileptics have tried to describe the impairments in information processing during the occurrence of spike-wave discharges in terms of disturbances in variables related to learning and memory (Hutt and Gilbert 1980; Jus and Jus 1962; Provinciali et al 1991), in mental chronometry (Hutt, Newton and Fairweather 1977; Van Luijtelaar et al 1991[B]), and also by studying more general variables such as attention or vigilance (Goode, Penry and Dreifuss 1970; Mirsky and Duncan 1990; Mirsky and Van Buren 1965; Opp, Wenzel and Brandl 1992). Decision tasks were used to study ictal response latency and response accuracy: during spike-wave discharges response latency increased, while response accuracy decreased (Browne et al 1974; Sengoku et al 1990; Tizard and Margerison 1963). Until now the various test paradigms used did not allow for a conclusive insight into the disturbances in information processing occurring during spike-wave activity.

Spontaneous spike-wave activity can also be recorded in the cortical EEG of several strains of rodents (Coenen et al 1992; Fisher 1989). These animal models offer ample possibilities to study underlying epileptogenic mechanisms and their consequences for the processing of information. In neurophysiological studies thalamo-cortical circuits were found to be pivotal in the genesis and continuation of cortical spike-wave discharges, whereas several thalamic relay nuclei were found to be differentially involved in the maintenance of the oscillatory neuronal activity (Buzsáki 1991; Inoue et al 1993; Vergnes, Marescaux and Depaulis 1990). Furthermore, animal studies stress the importance of vigilance and arousal-controlling systems in the genesis of spike-wave discharges (Coenen

et al 1991; Drinkenburg et al 1991; 1995; Gloor 1979; Steriade and McCarley 1990). Evoked potentials indicate that sensory processing is altered during spike-wave discharges (Inoue et al 1992). Only a few animal experiments have directly studied whether subsequent processing of the altered sensory input during spike-wave activity is adequate (e.g. Taylor-Courval and Gloor 1984). Other studies using test paradigms that were either designed to test interictal performance or to test ictal performance in an indirect way, do not report deterioration of inter-ictal performance: information processing diminishments during ictal performance were suggested but not investigated directly (Van Luijtelaar et al 1991[A]; Vergnes et al 1991).

In the present study a paradigm is introduced that directly studies information processing during spike-wave discharges. Rats of the WAG/Rij inbred strain were used as subjects. All rats of that strain spontaneously show numerous trains of spike-waves. In earlier experiments the interictal cognitive capacities of WAG/Rij rats were found to be within the normal range (Altman and Katz 1987; Van Luijtelaar, Van der Staay, and Kerbusch 1989). Furthermore, a period was ascertained during which adequate levels of alertness and engagement in operant response behaviour as well as ample spike-wave discharges were present (Drinkenburg et al submitted).

The purpose of the present study is to test in WAG/Rij rats whether ictal presentation of a stimulus associated with the presence of a reinforcer (a high impact stimulus) could be distinguished from ictal presentation of a stimulus associated with the absence of that reinforcer (low impact stimulus). This was done on the basis of electroencephalographic reactivity. By means of this paradigm, it can be established whether or not evaluation of a stimulus presented during spike-wave discharges takes place.

8.3 Methods

Subjects and surgery. Eight adult male WAG/Rij rats about 14 months old and with free-feeding weights between 316 and 383 g (mean 354 g) were used. The rats were singly housed and were maintained on a 12:12 hour light-dark cycle with lights on at 7.00 p.m.; all experimentation took place during the dark, active period. A month before training started, each rat was, while anaesthetized (Nembutal, Abott Laboratories, 60

mg/kg i.p.), implanted with a permanent tripolar EEG electrode unit (Plastics One MS-333/2-A). Two EEG electrodes were placed in the frontal cortex with 1 mm space between the tips at coordinates[2] A 2.0, L 2.1 with skull surface flat and bregma zero-zero according to Paxinos and Watson (1982). The third reference electrode was placed over the cerebellum. Animals were left undisturbed to recover for three weeks. One week before training started, a mild food deprivation down to 90 % of the free-feeding weights of the rats was instated. Access to tap water remained ad libitum.

Apparatus. Two conditioning chambers (L27xW25xH24 cm) were used for training and testing, each equipped with a centrally placed food tray and pellet dispenser (Campden Instruments, 45 mg precision pellets), red house lights, and two piezo loudspeakers placed symmetrically on both sides of the food tray. Through these loudspeakers the discriminative stimuli were presented: two auditory stimuli with an equal duration of 3 seconds and an equal frequency of 8 kHz, but with different intensities of 18 dB(A) and 30 dB(A). Auditory stimuli of 8 kHz were used, because at that frequency both sensitivity and discriminatory abilities of albino rats have been reported to be highest and because perception of auditory stimuli is largely independent of the position of the animal in the conditioning chamber (Hack 1971; Kelly and Masterton 1977). Chambers were connected to Skinner Box Controllers and controlled by an Apple Macintosh SE 30. Both Skinner boxes were adapted for recording of EEG of the freely moving animal in the conditioning chamber. EEG signals were amplified and filtered by an Elema-Schönander polygraph, which allowed frequencies between 0.5 and 70 Hz to pass. The EEG signals as well as the onset and the offset of the stimulus were written out on chart paper (speed 1.0 cm/sec). They were simultaneously stored in digitised form on magneto-optical disk by means of polygraphic registration software (Dataq Instruments Inc., CODAS-system) to allow off-line analysis on a millisecond time scale.

Behavioural protocol. After a magazine training of 60 trials, the behavioural protocol consisted of two phases: a conditioning phase and a test phase. In the conditioning phase

[2] Stereotaxic coordinates were choosen to optimize the detection of onset of spike-wave discharges.

the discriminative stimuli were associated with the presence (+) or the absence (-) of a reinforcer providing a stimulus with either a high impact or a low impact, respectively. Two experimental groups of four subjects each, matched on weight and number of spike-wave discharges recorded during 60 minutes post-surgery, were formed. In one group (the H+/L- group) a food pellet was always presented two seconds after onset of the high intensity stimulus (H+), while presentation of the low intensity stimulus was never followed by a food pellet (L-). In the other group (the H-/L+ group) a food pellet followed presentation of the low intensity stimulus (L+), whereas onset of the high intensity stimulus was never followed by a food pellet (H-). The conditioning phase took place on four consecutive days, each day consisting of one conditioning session of 120 trials. In the conditioning session 60 reinforced stimuli and 60 non-reinforced stimuli were presented in random order on a VI-40 schedule (range 20 - 60 s.). On the reinforced trials a food tray visit was necessary to progress to the next stimulus presentation. After four sessions, the number of food tray visits was less and the latency to visit the food tray was shorter for the reinforced (H+ and L+) stimuli as compared to the non-reinforced (L- and H-) stimuli (both parameters Wilcoxon Matched-pairs Signed-ranks Test; $Z = 2.52$, $p < 0.001$). The final test phase consisted of one session with a maximal duration of three hours.

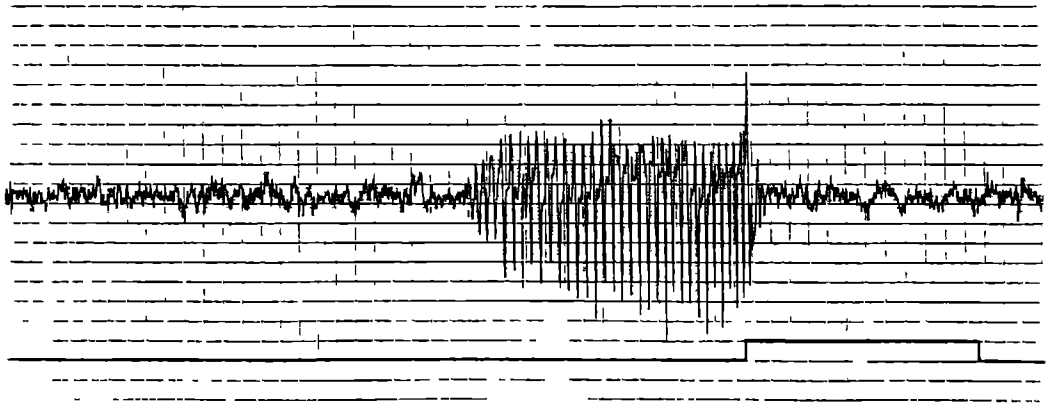
During this test phase a trained EEG analyst constantly monitored the EEG. This analyst presented to each animal at least twenty conditioned stimuli, with stimulus onset between the first and the third second after the onset of spike-wave activity in the EEG. A reinforced (H+, L+) stimulus or a non-reinforced stimulus (L-, H-), respectively, was presented in a pseudorandom order unknown by the EEG analyst; both stimuli occurred equally often in a sequence of six presentations.

Data analysis. In order to analyse effects of spike-wave discharges on information processing, the reactivity of the ongoing EEG was determined for each stimulus presentation during the test session. Two types of EEG reactivity were distinguished. If within 0.5 s (see Jung 1962) after stimulus onset the spike-wave activity had changed to such a degree that the classification criteria (Van Luijtelaar and Coenen 1986) were no longer met, and spike-wave activity had been replaced by any kind of non-paroxysmal,

interictal EEG activity then the spike-wave discharges were assumed to be aborted by the presentation of the stimulus. If, however, spike-wave activity remained unchanged or the spike-wave morphology was restored within 1 second after stimulus onset, then the presentation was considered as non-abortive. The percentages of stimulus presentations which resulted in abortion of ongoing spike-wave discharges were determined and differences between conditions were statistically analysed by means of the Wilcoxon Matched-pairs Signed-ranks Test. All values are means of eight animals (H+/L- group: n=4; H-/L+ group: n=4) and are given with the corresponding standard errors of the means (SEM).

8.4 Results

A typical example of an abortion of spike-wave activity that was brought about by presentation of a reinforced stimulus, is shown in Figure 8.1.



(Calibration horizontally one square corresponds to 250 mS and vertically one square indicates 75 μV)

Figure 8.1 An example of typical spike-wave discharges as recorded from the frontal cortex is shown in the upper trace. The lower trace indicates the onset and offset of the stimulus. Note that the ongoing spike-wave activity is aborted within 0.5 s after stimulus onset

It was found that 76.8 % ± 6.4 % of all previously reinforced stimulus presentations

resulted in abortion of ongoing spike-wave discharges. Of all previously non-reinforced stimulus presentations only 34.5 % ± 7.6 % led to abortions, which is significantly less than the abortion percentage of the reinforced stimulus presentations (*Z=2.52, p= 0.006*). In the H+/L- group presentation of the H+ stimulus aborted significantly more spike-wave discharges (75.8 % ± 5.0 %) than the L- stimulus presentations (32.5 % ± 7.6 %; *Z=1.83, p= 0.034*; see also Figure 8.2).

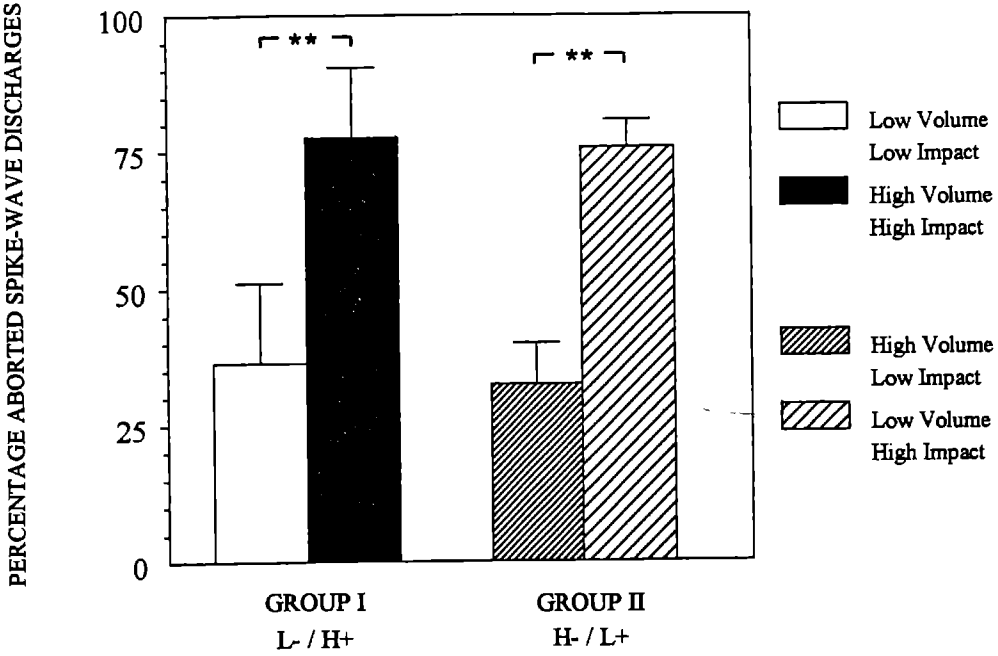


Figure 8.2 The mean percentage (± SEM) of aborted spike-wave discharges of all ictal stimulus presentation are depicted with respect to the L-/H+ group (for the low intensity non-reinforced stimulus L- and high intensity reinforced stimuli H+, respectively) on the left side of the graph, whereas on the right side of the graph these results are shown with respect to the H-/L+ group (for the high intensity non-reinforced stimulus H- and low intensity reinforced stimuli L+, respectively).

** $p<0.01$ are for Wilcoxon Matched-pairs Signed-ranks Test for differences in percentage of aborted spike-wave discharges within each group between the high impact stimulus and the low impact stimulus.

The results of the H+/L- group are in itself, however, not unequivocally interpretable

due to the physical supremacy of the high intensity stimulus. Therefore, the results of the counterbalanced H+/L- group, the L+/H- group, are more indicative of the effects of the impact of the stimulus per se. The results of the L+/H- group are depicted on the right side of the graph in Figure 2. The L+ stimulus was clearly more effective in aborting the ongoing spike-wave activity (*Z=-1.83, p=0 0034*) than the H- stimulus. To conclude: especially the impact of a stimulus determined whether or not EEG reactivity occurred.

8.5 Discussion

The main outcome of this study was the demonstration of a differential sensitivity for ictally presented stimuli with different impacts for the animal: reinforced 'high impact' stimuli were more effective in aborting ongoing spike-wave discharges than non-reinforced 'low impact' stimuli. The impact of a stimulus produced these effects regardless of differences in physical intensity of the stimulus. These findings indicate that during cortical spike-wave activity, the brain is still capable of evaluating the meaning of an externally presented stimulus.

Studies on ictal information processing in animal models of generalised spike-wave activity are scarce. Taylor-Courval and Gloor (1984) used the feline generalised penicillin epilepsy model to study its ability to press a lever after presentation of auditory or visual stimuli during spike-wave discharges. Most of the stimulus presentations were not followed by the required response or a significant increase in response latency occurred; this response deficit was attributed to „a cognitive defect or to motor impairment associated with temporary amnesia" (Taylor-Courval and Gloor 1984). A strain of Wistar rats with spontaneous spike-wave discharges (GAERS) was used by Vergnes et al (1991) to study responding after ictal and interictal stimulus presentations in a conditioned sound-barpressing task. These authors found that bar presses were absent when a conditioned sound was presented during a spike-wave discharge. No conclusions were drawn about the ictal disturbances in information processing. Vergnes et al (1991) suggested that the irresponsiveness was attributable to a lowered level of motivation to perform the task: as long as rats were working for reinforcement spike-wave discharges were suppressed, while spike-wave discharges reappeared when bar pressing was

suppressed (Vergnes et al 1991). Van Luijtelaar et al (1991[A]) trained WAG/Rij rats to press for food on a fixed interval schedule and reported that the post-reinforcement-pause was consistently enlarged in trials with spike-wave discharges compared to trials without spike-wave discharges. The authors suggest that the consistent error in time estimation is caused by a lowering in information processing during spike-wave discharges (Van Luijtelaar et al 1991[A]). In contrast to the above-mentioned studies, the present study did not use an instrumental response to assess ictal information processing capacity, but determined the disturbance more directly by quantifying EEG reactivity; stimulus evaluation turned out to be adequate in a vast majority of presentations. Clearly, sensory, attentional, and mnemonic processes are still functioning to a certain degree.

Sensory processing can be studied by recording evoked potentials during spike-wave discharges (Orren 1978). In WAG/Rij rats, the primary components (N1 and P2) of the visual evoked potential recorded during spike-wave discharges differed from the visual evoked potential during wakefulness and REM sleep, whereas other characteristics were found similar to the visual evoked potential during slow wave sleep (Inoue et al 1992). The authors suggest that during the oscillatory spike-wave mode the sensory afferent information that enters the thalamus and eventually the cortex, is probably reduced, but at least altered (Inoue et al 1992). The present results indicate that, despite sensory alterations and a reduced transfer of afferent activation by the thalamus, stimulus evaluation is possible (Coenen and Vendrik 1972; Coenen 1995; Inoue 1992). By recording multiple unit activity during cortical spike-wave discharges it was established in rats that the deep cortical layers, the specific thalamic relay nuclei, the reticular nucleus of the thalamus, and parts of the mesencephalon are firing in a rhythmical mode, whereas no spike-wave related activity was found in the anterior thalamic nuclei and in the limbic structures (Inoue et al 1993; Vergnes, Marescaux and Depaulis 1990). It looks as if limbic structures play a role in stimulus evaluation. Especially the hippocampus may have a function in stimulus evaluation because of its role in stimulus encoding, a mnemonic function, that is considered crucial for evaluation (Mirsky 1987; 1988).

In sum, this study affirms that stimulus evaluation occurs in the presence of spike-wave discharges, and that, consequently, information processing is not completely

disturbed during spike-wave activity.

8.6 References

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„FAILURE TO RESPOND TO STIMULI PRESENTED DURING SW DISCHARGE COULD BE DUE TO A COGNITIVE DEFECT, AN INABILITY TO PERCEIVE THE STIMULUS OR TO RECOGNIZE ITS SIGNIFICANCE. IT COULD ALSO BE DUE TO AN INABILITY TO CARRY OUT MOVEMENTS, ESPECIALLY THE TYPE AKIN TO HUMAN 'VOLUNTARY' MOVEMENTS, WHETHER THEY ARE PROMPTED BY A STIMULUS OR ARE PERFORMED SPONTANEOUSLY."

DEBORAH TAYLOR-COURVAL AND PIERRE GLOOR, 1984

CHAPTER 9

INFORMATION PROCESSING DURING SPIKE-WAVE DISCHARGES IN EPILEPTIC RATS: MOTOR RESPONSES[1]

[1] DRINKENBURG WHIM, SCHUURMANS MLJE, COENEN AML, VOSSEN JMH, VAN LUIJTELAAR ELJM. INFORMATION PROCESSING DURING SPIKE-WAVE DISCHARGES IN EPILEPTIC RATS: MOTOR RESPONSES. *PHYSIOLOGY & BEHAVIOR* (SUBMITTED).

Chapter 9 Information processing during spike-wave discharges in epileptic rats: motor responses

9.1 Summary

Spike-wave discharges in the cortical electroencephalogram (EEG) of epileptics are characteristically accompanied by a diminishment of responsiveness to external stimulation. A successive auditory discrimination task was used to study motor response organisation and execution during spike-wave paroxysms in rats. WAG/Rij rats, which all spontaneously show trains of spike-wave discharges, were provided with electrodes for cortical electroencephalographic registration. Next, a mild food deprivation was instated and the animals were trained in an operant conditioning paradigm to discriminate between two auditory stimuli with equal intensity and duration, but with different frequencies. A food reward was delivered if the animals pressed the right lever upon presentation of a low frequency tone or the left lever upon presentation of a high frequency tone. In the test phase, both reinforced stimuli were presented during spike-wave activity as well as during non-epileptic EEG activity; operant performance was analysed by means of the numbers of correct and incorrect lever presses and omissions (no response). It was found that lever presses were never generated during ongoing spike-wave activity, which affirms the incompatibility of spike-wave discharges and response initiation. Next to impairments in motor initiation, that are held responsible for the ictal irresponsiveness, a cognitive deficit was shown. Following paroxysms which were not aborted by the presentation of the stimulus, motor responses were mostly omitted, whereas after spike-wave activity that was aborted by the presentation of the stimulus, animals responded in a vast majority of stimulus presentations. The accuracy of the latter type of responses, however, declined to near chance level. As earlier studies in WAG/Rij rats showed that during spike-wave discharges sensory processing and stimulus evaluation function to a certain degree, the present findings lend support for the existence of a dysfunction in more advanced stages of information processing, such as a sensory-motor integration failure or amnesia, which causes the lower performance after the aborted spike-wave discharges.

9.2 Introduction

Generalised spike-wave discharges in the cortical electroencephalogram (EEG) are the hallmark of several types of non-convulsive epilepsy (Drury 1989). For instance, in childhood absence epilepsy bilaterally synchronous paroxysms with a spike-wave morphology are characteristically associated with an absence seizure (Gomez and Westmoreland 1987; Sandstedt 1990). Numerous studies in epileptics have demonstrated that during such spike-wave EEG activity a behavioural irresponsiveness to external stimulation exists (Mirsky, Duncan and Levav 1995; Van Luijtelaar 1991). This irresponsiveness has been confirmed in tests, that were considered to measure sensory, attentional, mnemonic, or motor processes (Hutt, Newton and Fairweather 1977; Opp, Wenzel and Brandl 1992; Provinciali et al 1991; Sengoku et al 1990). From experiments with detection tasks it has been suggested that during spike-wave activity a motor blockade may exist (e.g. Tizard and Margerison 1963). However, simple repetitive, motor responding, such as tapping non-complex rhythms or repetitively pulling a wire, were found to be rather undisturbed (Shimazono 1953; Yeager and Guerrant 1957). This implies that the irresponsiveness can not be explained solely by a blockade of motor execution. Therefore, despite the fact that earlier studies found ample evidence that information processing is disturbed during spike-wave discharges, it still remains to be elucidated what the extent and causes of this disturbance are.

Spontaneously occurring spike-wave discharges can also be found in the EEG of several strains of rodents. Some of these in- and outbred strains have been validated as animal models of absence epilepsy (Fisher 1989). Likewise, rats of the WAG/Rij inbred strain spontaneously show numerous trains of spike-waves each hour and are considered a useful model for studying the genesis of this epileptic activity and its behavioural consequences (Coenen et al 1992; Van Luijtelaar and Coenen 1986). In earlier experiments the interictal cognitive capacities of WAG/Rij rats were found to be within the normal range (Altman and Katz 1987; Van Luijtelaar, Van der Staay, and Kerbusch 1989), indicating that behavioural training and testing of epileptic rats is not beforehand awkward. Moreover, earlier experiments showed that during spike-wave activity sensory processing was altered but not blocked and that the evaluation of the impact of an ictally presented

stimulus is still possible (Drinkenburg et al submitted; Inoue et al 1992). The generation of conditional motor responses during spike-wave discharges was investigated in only a small number of animal studies (Taylor-Courval and Gloor 1984; Vergnes et al 1991).

In the present study an operant conditioning paradigm was used to determine whether during the presence of EEG spike-wave activity the brain is capable of a correct initiation and execution of a conditional motor response. A testing paradigm was used that establishes whether WAG/Rij rats are still able to choose and press a lever upon the ictal presentation of a discriminatory stimulus. Also the probability and the adequacy of lever presses that were delayed till after the end of the spike-wave discharges, were analysed.

9.3 Methods

Subjects and surgery. Six, adult, male WAG/Rij rats with ages about 18 months each and with free-feeding weights between 305 and 420 g (mean 388 g) were used. Rats were singly housed and were maintained on a 12:12 hour light-dark cycle with lights on at 7.00 p.m. At the start of the experiment all rats were anaesthetised (Nembutal, Abott Laboratories, 60 mg/kg i.p.) and then implanted with a permanent tripolar EEG electrode unit (Plastics One MS-333/2-A). Two EEG electrodes were placed in the frontal cortex with 1 mm space between the tips at co-ordinates A 2.0, L 2.1 with skull surface flat and bregma zero-zero according to Paxinos and Watson (1982). The third reference electrode was placed over the cerebellum. After operation a recovery period of at least four weeks followed, during which animals were left undisturbed. Two weeks before the conditioning sessions started, the EEG of each rat was tested during a one hour recording. Next, the animal was placed on a mild food deprivation schedule down to 90 % of his free-feeding weight. Access to tap water remained ad libitum in the home cage and in the conditioning and testing settings.

Apparatus. For conditioning and testing, two conditioning chambers (L27xW25xH24 cm) were used, each equipped with red house lights, a centrally placed food tray, and pellet dispenser (Campden Instruments, 45 mg precision pellets). A retractable lever was situated at each side of the food tray with a piezo loudspeaker above it. Through these loudspeakers the discriminatory stimuli were presented: two auditory stimuli with an

equal duration (1 s) and an equal intensity (18 dB[A]), but with different frequencies of 7.4 kHz and 11.5 kHz. These frequencies were used, because around frequencies of 8 kHz both the sensitivity and the discriminatory abilities of albino rats have been reported to be highest and perception of these stimuli is largely independent on the animal's position in the conditioning chamber (Hack 1971; Kelly and Masterton 1977). Chambers were connected to Skinner Box Controllers and controlled by an Apple Macintosh SE 30. Both Skinner boxes were adapted for recording of EEG of the freely moving animal in the conditioning chamber. EEG signals were amplified and filtered by an Elema-Schönander polygraph, which allowed frequencies between 0.5 and 70 Hz to pass. The EEG signals, the onset and the offset of the stimulus, as well as the responses of the animal were stored in digitised form on magneto-optical disk by means of polygraphic registration software (Dataq Instruments Inc., CODAS-system) to allow off-line analysis on a millisecond time scale.

Behavioural protocol. The operant conditioning procedure consisted of several phases (for details see Table VI): magazine training, lever press training, stimulus discrimination training, and finally an EEG-controlled test phase. After two magazine training sessions, the instrumental response (to press a lever) was reinforced by presentation of a food pellet. To this end, three sessions were presented wherein the right lever was inserted and reinforced on a Continuous Reinforcement Schedule, followed by three likewise sessions with the left lever inserted. To conclude this phase, the left or the right lever was ad random inserted during two sessions and reinforcement was again given upon leverpress. During all training phases a leverpress was required to progress to the next trial. In the next phase, it was learned that reinforcement of a leverpress was conditional upon presentation of an auditory stimulus. At first, only one lever was presented on a single trial always followed by the presentation of only its corresponding stimulus (11.5 kHz stimulus for left lever, 7.5 kHz stimulus for right lever); reinforcement was conditional upon the presentation of the stimulus. The subsequent phase differed from the preceding with respect to the presence of the levers: now both levers were simultaneously inserted and one of both stimuli was presented after a variable interval (10-20 s). If a response was given before stimulus presentation, or if the animal failed to respond during stimulus

presence or pressed the wrong lever, then both levers were withdrawn and a time-out period (15 s) followed before the next trial started. In the final phase of the training both levers were permanently inserted at the start of the session and the stimuli were presented on a variable inter-stimulus-interval of 60 s average (range 30-90 s) in a quasi-random order: it was controlled for that both stimuli appeared equally often in a series of 100 presentation. Animals were trained in this final phase until they reached a criterion of 75% correct responses on each of three consecutive sessions (one session of 100 trials each day: Table VI).

Table VI Chronological overview of the phases of the conditioning and testing protocol.

Type indicates experimental phase; *Aim* indicates the targeted behaviour; *Sessions* indicates the number of presented sessions; *Trials* indicates the number of presented trials per session; *LPP* indicates whether the insertion of the lever(s) was done each session or each trial; *I-T-I* indicates the length (in s) of the inter-stimulus-interval if present; *TO* indicates the length (in s) of the time-out period if present; *LISO* indicates the duration (in s) between of insertion of the lever(s) and the onset of the stimulus. #: individually determined.

Type	Aim	Sessions	Trials	LPP	I-T-I	TO	LISO
training	magazine	2	60	-	30-90	-	-
training	CRF left lever	3	20	-	-	-	-
training	CRF right lever	3	20	-	-	-	-
training	CRF right/left alter	2	50	no	3	-	0
training	stim.-lever reinfor.	20	300	no	4	15	10-20
training	stimulus discrim.	18	300	no	4	15	10-20
training	stimulus discrim.	10	300	yes	30-60	-	-
training	stimulus discrim.	4#	100	yes	30-90	-	-
test	discrim + EEG	≥1#	200	yes	30-90	-	-

After criterion was reached the animal progressed into the test phase. This test phase was identical to the final training phase, except that the EEG of the animal was constantly monitored by an experienced EEG-analyst, who had the possibility to interrupt the ongoing inter-stimulus-interval; if the analyst pressed a button, then the stimulus next in order was immediately presented. The analyst was unaware of the quasi-random order and was instructed to press the button after visual confirmation of at least 1 s and at most 3 s of spike-wave activity according to the criteria as elaborated by Van Luijtelaar and Coenen (1986). After such an interruption the normal inter-stimulus-interval was

reinstated. A minimum of 20 ictal presentations was required to end the test session of 200 trials. If this minimum number of presentations was not accomplished within one session, then the next day an additional testsession was presented.

Data analysis. To control for possible response omissions because of reduced motivation, fatigue, or satiation, only those ictal stimulus presentations were analysed, which were preceded as well as followed by a stimulus presentation that was responded to. In the same way, interictal presentations were analyzed only if they were preceded and followed by a stimulus presentation that was responded to. Ictal response behaviour was compared to interictal response behaviour with respect to number of presentations that was followed by a correct leverpress, an incorrect leverpress or no leverpress. Furthermore, it was determined whether or not the presentation of the stimulus aborted ongoing spike-wave activity. If within 0.5 s after stimulus onset the spike-wave activity was changed to such a degree that the classification criteria were no longer met, the spike-wave dicharges were assumed to be aborted by the presentation of the stimulus (see Jung 1962). If, however, spike-wave activity remained unchanged or the spike-wave morphology was restored within 1 second after stimulus onset, then the presentation was considered as non-abortive. For each of the two types of presentations (abortive and non-abortive) it was again determined whether or not the leverpress was given (omissions) and if so, whether the correct lever was chosen corresponding to the presented discriminatory stimulus. Comparisons between conditions with respect to percentages correct and incorrect responses and to percentages errors of omissions were analysed by means of T-tests for dependent samples. The dependence of the behavioural response proportions of the EEG condition was tested by means of a χ^2-test per subject. All values are means of six animals and are given with the corresponding standard errors of the means (SEM).

9.4 Results

A total of 329 presentations was presented during spike-wave discharges. In none of these ictal presentations, a leverpress response was registered during ongoing spike-wave activity (see Table VII). Nonetheless, when spike-wave activity was ended, a 'delayed'

response could be found in about 65% of all ictal presentations, whereas in the remaining cases (35%) no response was given. It was found that the type of response depended on whether or not the EEG was epileptic (χ^2=87.35, p<.001). When compared to interictal response behaviour a significantly higher percentage omissions (t_5=2.98, p<.04) and lower percentage correct responses (t_5=-4.37, p<.01) was found after ictal presentations (Table VII). In contrast, the percentage of incorrect responses did not differ between ictal and interictal presentations.

Table VII Ictal, post-ictal and interictal motor response behaviour.

Mean number with SEM and percentages with SEM are given for all presentations (*Total*), and in dependence of the time of response (*Ictal, Post-ictal, and Inter-ictal)*, and the type of response registered (*Omission, Correct*, and *Incorrect*).
[a]: percentage of all presentations, both ictal and interictal
[b]: percentage of all ictal presentations
[c]: percentage of all interictal presentations

	Ictal Responses		*Post-ictal Responses*		*Interictal Responses*	
	Mean Number	*Percentage*	*Mean Number*	*Percentage*	*Mean Number*	*Percentage*
Total	0.0 ± 0.0	0.0[a] ± 0.0	54.83 ± 8.92	31.47[a] ± 5.65	137.00 ± 27.85	68.53[a] ± 5.65
Omission	0.0 ± 0.0	0.0[b] ± 0.0	19.50 ± 5.81	35.02[b] ± 8.50	10.17 ± 2.24	7.48[c] ± 1.53
Correct	0.0 ± 0.0	0.0[b] ± 0.0	26.00 ± 6.85	45.48[b] ± 8.26	101.17 ± 19.55	74.64[c] ± 2.47
Incorrect	0.0 ± 0.0	0.0[b] ± 0.0	9.33 ± 1.17	19.50[b] ± 3.89	5.67 ± 7.83	17.88[c] ± 3.40

Figure 9.1 presents the distribution of the different motor behaviours if the abortive effects of the ictal presentations on the ongoing spike-wave discharges are taken into account. Abortion of ongoing spike-wave activity was found in a majority (65 %) of ictal presentations. After such an abortive presentation nor the percentage of correct responses, neither the percentage of incorrect responses, nor the percentage of omissions did differ from the percentage of corresponding interictal responses. In figure 8.1 a typical example of an abortion of spike-wave activity that was brought about by an ictal stimulus presentation, is shown.

In contrast, if stimulus presentation did not abort spike-wave discharges, in most cases no leverpress was found after the spontaneous ending of the epileptic activity. In these

non-abortive cases a significantly lower percentage of correct responses was found as compared to inter-ictal percentages (t_5=-5.69, p<.002). Indeed, the response behaviour was

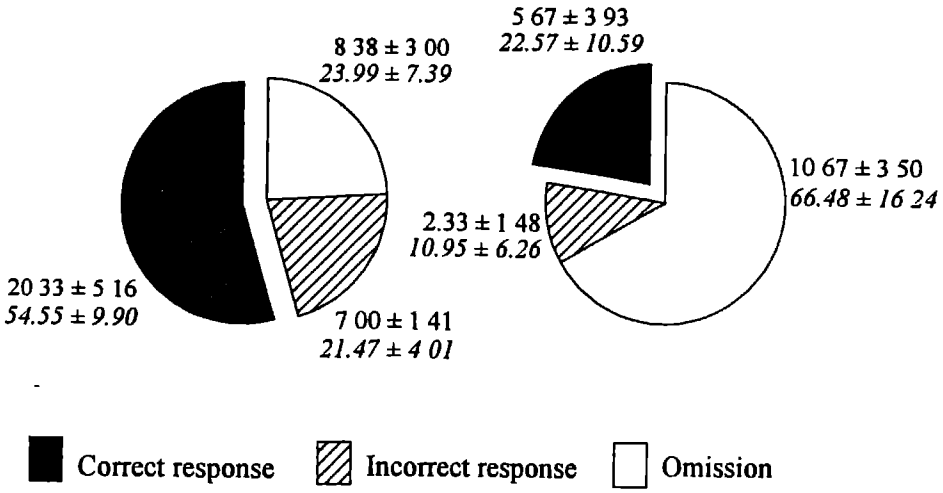

Figure 9.1 Distribution of types of responses (omission, correct responses, and incorrect responses) after abortive (left pie) and non-abortive (right pie) ictal presentations Mean number (normal font) with SEM and percentages (italic font) with SEM of all ictal presentations are given (totals 36 17 ± 6 33, *65 75 ± 6 38* and 18 67 ± 5 02, *34 25 ± 6 38* for abortive and non-abortive presentations, respectively).

found dependent of the abortive capacities of the stimulus presentation (χ^2=127 58, p<.0001).

9.5 Discussion

The main result of the present experiment was the complete absence of motor responses during ongoing spike-wave discharges. Furthermore, whether or not the ictal EEG was aborted by the stimulus presentation turned out to be crucial for the occurrence of post-ictal motor responses. If post-ictal responses were given, then their accuracy was reduced. These findings suggest that during cortical spike-wave activity, the brain is incapable of initiating an adequate motor response.

Up to date, motor response behaviour after presentation of a conditioned stimulus was tested in only a small number of animal studies; a conditioned sound-bar pressing task was used in epileptic rats (GAERS) to study responding after ictal and interictal stimulus presentations (Vergnes et al 1991). It was found that when a sound was presented during spike-wave discharges no bar pressing occurred, while a later sound in the absence of epileptic activity was followed by a bar press. The authors suggested that only in 'borderline states', when motivation to act is low, the occurrence of spike-wave discharges may suppress perception of information and behavioural responsiveness (Vergnes et al 1991). The ability to press a lever upon auditory or visual stimulus presentation during spike-wave activity was also studied in the feline generalised penicillin epilepsy model (Taylor-Courval and Gloor 1984). In correspondence to the present data, interictal motor responses were found normal, whereas a total absence of the learned response was most often found after ictal presentations. The response failures were attributed to a cognitive defect or to a motor impairment associated with or without temporary amnesia (Taylor-Courval and Gloor 1984).

Keeping in with the suggestions of Taylor-Courval and Gloor (1984), our results confirm the existence of a cognitive deficit; if the total absence of ictal responses is due to a temporary, strictly ictal motor deficit such as motor initiation failure, without any cognitive defect, then the animal should be able to delay the correct response till spike-wave activity is over. In the present experiment in a majority of such presentations with a spontaneous ending of spike-wave activity no response followed.

In contrast, if the presentation of the stimulus had an abortive effect on ongoing spike-wave activity -a condition which has not been distinguished by Vergnes and coworkers (1991) nor by Taylor-Courval and Gloor (1984)-, then animals responded immediately following the spike-wave activity in about 75 % of all presentations, albeit with a lowered accuracy near chance-level. It should be noted that in the present experimental paradigm the animals not only had to respond to a stimulus as in both animal studies mentioned above, but in addition to this, the animals had to discriminate between two conditioned stimuli to obtain a foodpellet. Therefore, two earlier experiments in WAG/Rij rats are valuable to further interpretate the nature of the ictal irresponsiveness. Firstly, primary

components of the visual evoked potential could be clearly registered during spike-wave discharges (Inoue et al 1992), suggesting that during the oscillatory spike-wave mode the sensory afferent information that enters the thalamus and eventually reaches the cortex, is altered but not completely blocked. Moreover, it is argued that stimulus evaluation is possible to a certain degree, despite sensory alterations and reduced transfer ratio's in the thalamus (Coenen and Vendrik 1972; Coenen 1995). Secondly, by comparing the capacity to abort ongoing spike-wave activity of previously reinforced stimuli versus previously non-reinforced stimuli, it was affirmed that ictal stimulus evaluation is still possible. That finding suggested that ictal sensory and attention processes are diminished but not completely disturbed. However, in the present study the motor responses after spike-wave abortion -i.e. after stimulus evaluation (see Drinkenburg et al submitted)- were found to be inadequate with respect to accuracy. During spike-wave discharges animals apparently are able to 'sense' a significant stimulus, but probably due to an ictal cognitive dysfunction the perception and processing of the information of the stimulus becomes too limited to generate a correct motor response post-ictally. In studies of epileptics, an ictal anterograde or post-ictal retrograde amnesia is sometimes suggested as the responsible cognitive deficit, but further studies are needed to confirm this suggestion (Jus and Jus 1962; Mirsky, Duncan and Levavn 1995; Provinciali et al 1991; Van Luijtelaar et al 1991).

In sum, this study affirms that the genesis of an adequate motor response is restricted to non-epileptic EEG activity, suggesting an incompatibility of spike-wave activity and the initiation of motor responses. Earlier studies in WAG/Rij rats showed that sensory processing and stimulus evaluation during spike-wave discharges are still functioning to a certain extent; the present findings suggest that the ictal irresponsiveness is accompanied by a dysfunction in more advanced stages of information processing, that affects motor response programming and response accuracy after the aborted spike-wave discharges.

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„EPILEPSY REPRESENTS ONE OF THE MOST EXQUISITE EXPERIMENTS OF NATURE AND ITS STUDY MAY PROVIDE INSIGHT INTO FUNDAMENTAL FUNCTIONS OF THE BRAIN"

HERBERT JASPER, 1969

CHAPTER 10

GENERAL DISCUSSION

Chapter 10 General Discussion

10.1 Characteristics of spike-wave discharges in relation to levels of vigilance

A first objective of this thesis was to determine how spike-wave discharges relate to level of vigilance and to processing of information, as ample evidence exists that critically involved thalamic structures are controlled by ascending reticular activating systems (see chapter 1). The distribution of spike-wave paroxysms over sleep-wake states was determined in chapter 4: light slow wave sleep and drowsiness were found favourable for the occurrence of spike-wave discharges, whereas deep slow-wave sleep, REM sleep and active wakefulness were found unfavourable for the occurrence of spike-wave discharges. These findings, which in the meantime have been replicated by others (Gralewicz et al 1994), contribute to the validity of the WAG/Rij model for human absence epilepsy; in the two species the relationship between spike-wave discharges and levels of vigilance appears quite similar.

Furthermore, these findings fit nicely in recent theories about the pivotal function of the thalamus in changes of cortical EEG patterns related to shifts in sleep-wake states and to spike-wave oscillations (Snead 1995; Steriade, Jones and Llinás 1990; Steriade and Contreras 1993); from an electrophysiological perspective light non-REM sleep as well as drowsy wakefulness are considered 'unstable' states of the brain because in these sleep-wake states brief alterations in the level of neuronal synchronization are frequent (Depoortere et al 1991; Merica, Blois, and Gaillard 1989; Terzano, Parrino and Spaggiari 1988). Phasic events or sleep transients such as micro-arousals, K-complexes, vertex waves, sleep spindles, and micro-sleeps may then occur frequently (Halász 1991; Niedermeyer 1982; Wauquier and Declerck 1991). As was extensively argued in chapters 3 to 6 and recently confirmed by a study of Gralewicz and Łuczak (1995), spike-wave activity can be added to this category of phasic events, the occurrence of which is related to a transitional, unstable state of the brain. In accordance, elevation of level of vigilance turned out to be effective in reducing the number of spike-wave discharges (chapter 6), while reduction of level of vigilance by means of sleep deprivation (chapter 5) initially

increased the number of spike-wave discharges.

Moreover, these results are in line with the topic of research that was raised by Gloor (1979): spike-wave discharges are thought to represent a pathological cortical response to afferent thalamocortical volleys. Under normal conditions these volleys are involved in the elicitation of sleep spindles (Gloor 1988; Kostopoulos and Gloor 1982). The vigilance related conditions for the occurrence of spike-wave discharges -light non-REM sleep and drowsy wakefulness- concur fairly with the conditions for the occurrence of human sleep spindles (e.g. Kellaway 1985), although debate exists about the compatibility of deep non-REM sleep and different types of sleep spindles (Gaillard and Blois 1981; Nuñez et al 1992; Uchida, Atsumi and Kojima 1994). In future experiments the hypothesis that spike-wave discharges and sleep spindles share an underlying (thalamocortical) mechanism for generation can be tested in WAG/Rij rats by studying the interdependence of spike-wave discharges and sleep spindles: departing from the questions whether the number of registered sleep spindles is independent of the number of registered spike-wave discharges during each of the relevant sleep-wake states or not (chapter 4) and what is the effect of manipulating the occurrence of spike-wave discharges (chapters 5 and 6) on the occurrence of sleep spindles.

10.2 Characteristics of spike-wave discharges in relation to information processing

A main question in our research had to do with the possibilities for and the extent of information processing during spike-wave paroxysms. Research of information processing in rats is traditionally done in such a way that variables, that are thought to influence response behaviour are controlled as much as possible: learning and memory tests require alertness and engagement in the task to be effective. Because in a state of active wakefulness spike-wave discharges were shown to occur rarely (chapters 4 to 6), studying ictal information processing during alert wakefulness is awkward. In WAG/Rij rats (chapter 7) it was shown that, although production of conditional motor responses in a bar press task indeed initially led to a reduction in spike-wave activity, in the course of the task a level of reduced alertness was present during which both spike-wave discharges as well as bar press responses could be reliably registered. As continuation of the task led to

a further decrease, a period was established optimal for testing response behaviour during spike-wave discharges.

Based on the existence of this period it is assumed that although before entering a spike-wave activity the organism is mostly in a somewhat drowsy or sleepy state, the level of alertness is not so low as to exclude information processing. Attempts to further test this assumption in pilot studies by presenting external stimuli in the immediate temporal proximity of the expected onset of spike-wave discharges failed because stimulus presentations led to a reinstatement or continuation of awake (alert) EEG. Therefore, it cannot be completely excluded that spike-wave activity is accompanied with a sudden, temporary diminishment of alertness or motivation to perform operant behaviour; the results -immediately after the disappearance of the spike-wave activity operant performance diminishments were no longer present- as described in chapter 7 , however, corroborate the notion that ample alertness and engagement in the task is present during the period following as well as preceding the spike-wave paroxysms. An alternative approach to study the interaction between motivational states and epileptogenesis is to refrain from using appetitive reinforcement and overt behaviour as an operant: within an operant conditioning paradigm it might be possible to make reinforcement conditional upon the generation of spike-wave activity itself. In such an experiment the rat is electrophysiologically (e.g. intra-cranial stimulation) 'rewarded' if he is able to generate spike-wave discharges (anecdotal evidence exists that children with absence epilepsy are sometimes able to wilfully generate an absence seizure). If the genesis of spike-wave discharges is restricted to lowered levels of vigilance, which are characterized by the incompatibility with motivational effort, then the rat will not be able to generate spike-wave discharges (i.e. receive rewards). If, in contrast, the rat is able to wilfully obtain rewarding stimulation, then it must be concluded that a lowered level of vigilance is not a prerequisite for the genesis of spike-wave discharges. Such an experimental approach may help to disentangle the relationships between vigilance, (peri-)ictal cognition, and constraints on information processing.

In chapter 8 it was shown that during spike-wave activity the rats were still able to evaluate the meaning of externally presented stimuli in a large majority of all

presentations. Given that stimulus discrimination was still possible during spike-wave activity, it is suggested that the ictal irresponsiveness as reported in chapter 7 and in the literature (e.g. Provinciali et al 1991), is most likely caused by paroxysm-induced disturbances in response programming or in motor execution. It was tried in a subsequent experiment (chapter 9) to establish whether response programming and motor execution indeed were disturbed; it appeared that during spike-wave activity a motor response was never given, indicating incompatibleness of spike-wave activity and response initiation. Analyses of the response behaviour directly after the spike-wave activity had ended revealed that, notwithstanding that post-ictal motor execution functions adequately (see chapter 7), quality of responding was impaired. It should be noted, however, that the post-ictal response behaviour consists of delayed responses, the accuracy of which may be not directly comparable to zero-delay inter-ictal response accuracy. Based on the outcomes of the present studies it is possible to construct an additional control group with appropriate inter-ictal stimulus-response delays to obtain mnemonically comparable inter-ictal response behaviour. Furthermore, if there is a mnemonic problem -encoding, consolidation or rehearsal of stimulus information might be inadequate or the contextual retrieval might be hampered by differences between the ictal and the inter-ictal condition of the brain (Binnie et al 1987; Jus and Jus 1961; Provinciali 1991)-, this problem can be addressed by presenting the stimuli at different points of time in the course of spike-wave discharges and analysing whether time of presentation is related to the quality of response execution. In case of retrograde amnesia, for example, a clear-cut forgetting-curve should be producible based on differences in the duration of post-stimulus spike-wave activity. An additional complicating factor, which such studies should account for, can be the intra-spike-wave paroxysm dynamics, as both in rats (see also chapter 3) and humans there are indications for the existence of paroxysm-related processes that may influence information processing differentially in the course of a spike-wave activity (e.g. Shimazono et al 1953). Moreover, the cognitive correlates of spike-wave discharges are no all-or-none phenomena (Andermann 1995; Chatrian et al 1970); in absence epilepsy some neural circuits continue to perform their functions, though with varying degree of alterations in the face of unrelenting spike-wave activity. Because in patients execution of simple motor

response patterns per se is not severely disturbed during spike-wave paroxysms (e.g. Sengoku et al 1990; Yeager and Guerrant 1957;), future experiments in WAG/Rij rats not only have to further pinpoint the disturbed stages in the chain from sensory information processing to motor execution, but should also take into consideration the complexity of the required response organization, the mnemonic demands of the task, and the dynamic nature of the discharges.

10.3 Spike-wave discharges, vigilance, and information processing: a converging approach on the base of the transfer ratio concept.

The considerations mentioned above with respect to physiology and cognition during spike-wave discharges should ideally be fitted into a model, which accounts for the subsequent disturbances of information processing as well as for the underlying physiological substrates. Since Broadbent's (1958) attempt to integrate multiple processes into a model of information processing, several interesting models have been developed, trying to encompass human information processing aspects such as filtering, capacity, and different types of attention and memory (Shiffrin and Schneider 1977; Shallice 1982; Cowan 1988). However, these models rely heavily on data from human neuropsychological studies, which often refer specifically to elements of human perception and cognition such as several types of attention or verbal comprehension (e.g. Aalders and Eling 1991; Aldenkamp and Vermeulen 1995), and this restricts the use of these models for interpretation of results of animal studies.

An alternative approach is to formulate the results of studies of cognition during spike-wave paroxysms from a neurophysiological perspective. In 1988 Gloor stated: *„It seems therefore more useful to describe the cardinal feature of the behavioral deficit during an absence attack as a variable degree of interference with corticothalamic function as it pertains to its perceptual, cognitive, voluntary motor, and mnemonic aspects rather than to call it a 'loss of consciousness'."* p.199. Following Gloor's line of reasoning the results presented in this thesis can be interpreted as follows: spike-wave paroxysms appear to be transient-like phenomena, that predominantly occur on transitions from wakefulness to sleep and vice versa. At wake-sleep transitions neurons of various thalamic nuclei quit the

relay or tonic firing mode -the substrate of EEG beta waves- to enter the bursting or oscillatory mode; a mode of activation that is characterized by pause-burst like discharges of many cells. It is during this bursting mode that spindles and eventually spike-wave discharges become manifest (for a review see: Coenen 1995). When thalamic networks are in the rhythmic bursting mode they respond to afferent stimulation by producing a stereotypical oscillation, which is characterized by the properties of the neurons involved, but not by the properties of the afferent signal (Steriade, McCormick and Sejnowski 1993). Additional neurophysiological evidence exists for a reduction in thalamic transfer of sensory information to the cortex during this mode of activation. Based on intracellular recordings of feline thalamic relay cells Coenen and Vendrik (1972) postulated the concept of the transfer ratio. During wakefulness the excitatory postsynaptic potentials produced by action potentials of retinal ganglion cells readily pass the lowered thalamic threshold characteristic of the tonic firing mode and result in outgoing action potentials. During this tonic firing mode the transfer ratio has a value of 1.0. The authors further noted that given identical input, the output reduces during drowsiness and light non-REM sleep; when the bursting mode is entered the transfer ratio lowers to 0.7 and this ratio further drops to about 0.3-0.4 when the slow (delta) waves of deep non-REM sleep appear. Nevertheless, upon awakening the transfer ratio immediately climbs up to 1.0 (Coenen 1995; Coenen and Vendrik 1972). Likewise, upon electrical stimulation of the activating reticular system the transfer ratio is also immediately increased, accompanied by an arousal response (Singer 1973).

The transfer ratio is a challenging concept, that, albeit on a speculative base, elegantly concurs with the presently reported ictal disturbances in information processing. During spike-wave activity the organism is mostly able to discriminate between two differentially reinforced stimuli; a sufficiently strong arousing reaction consequently results in the abortion of ongoing spike-wave activity (see chapter 8). At stimulus onset a reduced transfer ratio may be assumed as the thalamus is at that moment thought to be in the bursting mode. The transfer ratio model suggests that in the latter mode, depending on the outcome of an evaluation of the reduced stimulus information by the cortex, the thalamic 'sensory channels' either stay closed or are opened -switch to tonic firing mode- by the

ascending activating system. Immediately after this activation -i.e. after the spike-wave abortion- the animal is again capable to respond adequately to presented stimuli (see chapter 7) as the thalamic tonic firing mode is again reached and thus a transfer ratio of about 1.0 is quickly restored. In contrast, if the presence of a stimulus is restricted to ictal activity or for yet unknown reasons not followed by an arousal reaction, then the organism is not able to initiate a (correct) motor response (see chapter 9). This can be considered a consequence of the reduced transfer of stimulus information to the cortical areas involved in sensorimotor planning and due to the bursting mode of thalamic neurons.

The transfer ratio model, of which many underlying assumptions have yet to be tested, offers a useful framework to study ictal information processing, although with some limitations regarding the putative multiple functions of the thalamus. Besides the gateway function, McCormick, Bal and Von Krosigk (1993) pointed at a second function of the thalamus, when they stated: *„the thalamus should not be viewed only as the 'gateway' to the cerebral cortex, but also as the entry point of sensory and motor information into a ongoing thalamocortical loop, the state of which is controlled by ascending and descending modulatory transmitter systems in accordance with behavioural demands so as to allow the forebrain to perform the sensory processing which is appropriate for the actual or perceived cognitive tasks"* p. 370. With this restriction and the neurophysiological characteristics of spike-wave discharges in mind the presented studies into ictal information processing can be viewed as a first attempt to behaviourally quantify the neurophysiological concept of transfer ratio.

10.4 The epileptic nature controversy

When discussing a series of experiments, it is tempting to close with an evaluation of the findings in relation to an old controversy (e.g. Friedmann 1906): 'are spike-wave discharges really of epileptic nature'? A crucial role in the definition of epilepsy -recurrent pathological neuronal discharges- seems to be reserved for the adjective 'pathological'. As already noted in chapter 1, the epileptic nature of rodent spike-wave discharges has been doubted by some scientists, who stress the dissimilarities with the human absence spike-wave discharges and argue that no known animal model mimics the conditions of human

spike-wave discharges completely. Rodent spike-wave discharges are suggested to be equals of alternative nonpathological rhythms, such as feline sensorimotor rhythm or human μ-rhythm (Semba, Szechtman and Komisaruk 1980). However, in chapters 4, 5 and 6 it was shown that spike-wave discharges have a distinct relationship with level of vigilance and can therefore be readily set apart. Furthermore, in chapter 3 spike-wave discharges were successfully differentiated from several other transients on the base of the spectral content of the cortical EEG; it turned out that spike-wave discharges, classified according to the criteria firstly elaborated by Van Luijtelaar and Coenen in 1986 (peak frequency about 8 Hertz, minimal duration of 1 second, amplitude twice as large as background, and an asymmetrical morphology), possess distinctively more power in the domain of the high frequencies than any other transient investigated. This EEG characteristic is responsible for the 'sharp' morphology of the discharges -spikes in particular- and is generally accepted as a major indication of pathological, highly synchronized neuronal activity and, accordingly, of epileptic activity.

Finally, the pathological nature of the spike-wave discharges was questioned by Kaplan (1985), who suggested that spike-wave discharges can be considered as epileptic phenomena if evidence is brought forward that indicates that rodents are „*unconscious*" while spike-wave activity was registered. Such evidence, however, is difficult to provide; besides the conceptual awkwardness of the term consciousness (Trevarthen 1979), it is legitimate to question whether unconsciousness is a valid prerequisite to establish the epileptic nature of spike-wave discharges (Myslobodsky 1988). Though impairment of responsiveness is the cardinal clinical symptom in patients with absence epilepsy, numerous studies have shown that during spike-wave discharges a patient not necessarily loses contact with reality completely (e.g. Browne et al 1974; Goode, Penry and Dreifuss 1970; Mirsky and Van Buren 1965), nor that a patient is completely incapable of perceiving and retaining information during spike-wave activity (Gloor 1986; Goldie and Green 1961; see also § 10.2). During spike-wave activity WAG/Rij rats (chapters 8 and 9) were mostly able to perform an evaluation of the presented stimulus („so they were conscious?"), whereas an adequate motor response was never given during the spike-wave activity („so they were unconscious?").

10.5 Epilogue

Regardless whether spike-wave discharges of WAG/Rij rats are of pathological nature or not, spike-wave discharges as a model offer valuable opportunities for studies on the functioning of the brain; from an experimental point of view one may look at spike-wave discharges for example as a temporary, reversible 'lesion' of certain thalamo-cortical circuits, as a temporary cortical spreading depression, as a short lasting disturbance in equilibrium of excitatory and inhibitory neural activity, as a reversible disturbance of excitatory and inhibitory neurochemical processes, as a temporary disturbance in information processing, as a genetic model for each of the phenomena mentioned above, or ultimately as a model for human absence epilepsy (Coenen et al 1992).

Having emphasized the merits and heuristic potentials of spike-wave discharges in WAG/Rij rats, this thesis ends, citing Myslobodsky and Mirsky (1988): „*(...) with a plea against premature oversimplification of what is a complex and still largely enigmatic phenomenon*" p.386.

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„A CASE OF SIMPLE ABSENCE SEIZURES IS NOT A FORMIDABLE CONDITION. ASIDE FROM SLEEP ITSELF, IT REPRESENTS PERHAPS THE ONLY SAFE ROUND-TRIP EXCURSION INTO UNCONSCIOUSNESS."

MICHAEL MYSLOBODSKY AND ALLAN MIRSKY, 1988

SUMMARY

Summary

In **chapter one** the object of study is introduced and the rationale and outline of this thesis presented. Ever since antiquity, sudden changes in behaviour have been related to epilepsy. Its neurophysiological correlate, however, had not been identified until at the start of the present century electroencephalography was developed. Recurrent discharges with a characteristical electroencephalographic (EEG) spike-wave-like morphology form the hallmark of a non-convulsive type of epilepsy: absence epilepsy. The clinical concomitant of spike-wave discharges is behavioural immobility, accompanied by unresponsiveness to external stimulation. Studies of behavioural tasks in absence epileptics showed that during spike-wave discharges information processing is disturbed, but these studies cannot yet explain within a consistent theoretical framework how the brain functionally changes during spike-wave activity.

Spike-wave activity has also been recorded from the brains of animals, such as cats and rodents. Research on animal models can help to build a theoretical framework, because these models offer ample possibilities to study mechanisms underlying epileptogenesis and epileptic brain functioning. Animal models allow systematic intra-cerebral manipulations and are not hampered by anti-epileptic medication as is inevitably the case in clinical studies of absence epileptics.

Up to now only a few animal studies have investigated information processing during spike-wave discharges. In contrast, much attention has been focused on the biochemical, neuro-anatomical and neurophysiological systems involved in the genesis of spike-wave discharges in animal models. Cortical spike-wave discharges represent oscillations in the thalamo-cortical circuits. The thalamus is an extremely complex nuclear complex within an extensive network of afferents and efferents. Its activity is partly under control of the ascending reticular activating system, a system that is held responsible for controlling waking and sleeping. As a consequence, the level of vigilance is expected to be of influence on the occurrence of spike-wave discharges and is thought to interact with epileptic information processing.

Therefore, in this thesis experiments are presented in which the interrelationships between sleep-wake control, level of vigilance and spike-wave discharges were studied. Based on the findings on these relationships, in this thesis experiments are also reported which systematically addressed disturbances in information processing during spike-wave activity. All studies were done in one strain of rats, the WAG/Rij rat, which reliably mimics several aspects of human childhood epilepsy. Since Van Luijtelaar and Coenen's first publication in 1986 on the epileptic properties of this strain, various characteristics of spike-wave discharges in the WAG/Rij model have been described and several hypotheses concerning many aspects of these discharges have been tested. Spike-wave paroxysms are not exclusive for the WAG/Rij strain: other animal 'spike-wave' models have produced relevant results.

In **chapter two** an overview is given of the research on genetic animal rat models of absence epilepsy up to 1991. Electrophysiological studies indicated that abnormal discharges in the cortical EEG are generalized and that the hippocampus is not involved. The thalamic reticular nucleus, together with other parts of the thalamus, apparently acts as a pacemaker for the paroxysms. Cognitive studies showed that the number of spike-wave discharges is modulated by mental or physical activity (elaborated in chapter 6) and that the occurrence of spike-wave discharges disrupts time-estimation in WAG/Rij rats. Finally, the aberrant shape of visual evoked potentials obtained during spike-wave activity suggests that sensory processing during spike-wave discharges is not absent, but is different from that occurring during normal states of vigilance.

In **chapter three** similarities and differences between spike-wave discharges and other aberrant electroencephalographic transients were determined by means of spectral analysis of the cortical EEG. The EEG of WAG/Rij rats contains, besides normal sleep spindles, high voltage spiky phenomena, epileptic spike-wave discharges, and deviant intermediate stage. Spectral analysis of these transient phenomena showed that some features (e.g. peak frequency) are alike, but that they differ in other spectral characteristics (e.g. the first harmonic of the peak frequency and in the domain of the high frequencies). The results provide arguments for the view that spike-wave discharges can be considered as unique aberrant phenomena, presumably related to but dissimilar to the high voltage spiky

phenomena and intermediate stage. Next to this, the intra-phenomenal dynamics of spike-wave discharges were studied. The peak frequency was found to decrease monotonously from about 10 Hz at the beginning of the spike-wave discharge to about 8 Hz at the end. Other frequency bins showed maximal values of amplitude in the middle part of the spike-wave discharge, followed by a decrease in value. A correlation of these time-variant EEG dynamics in spike-wave discharges with the cognitive disturbances during absence seizures in man is suggested.

In **chapter four** conditions of the brain in relation to sleep-wake states and to the transitions therein which are favourable for the occurrence of spike-wave discharges were determined. Description of these conditions accentuates the vigilance-related prerequisites of the brain to generate spike-wave discharges. It was found that spike-wave discharges predominantly occur during light slow wave sleep and passive wakefulness. REM sleep, active wakefulness, and deep slow wave sleep are less susceptible to the occurrence of spike-wave discharges. Furthermore, spike-wave discharges tended to prevail in transitional states. It is suggested that degree of stability of the level of vigilance plays a crucial role in the genesis of absence seizures.

To manipulate the level of vigilance in **chapter five** a provocation technique was used, that is derived from clinical practice: total sleep deprivation. The hypothesis was tested that changes in sleep-wake state distribution influence epileptogenesis. This was done by analysing spike-wave activity and the occurrence of sleep-wake states (wakefulness, light non-REM sleep, deep non-REM sleep, and REM sleep) before, during and after a period of twelve hours of nearly total sleep deprivation. A substantial increase in the number of spike-wave discharges was found during the first four hours of the deprivation period; in the following deprivation hours epileptic activity returned to baseline values. Immediately after termination of deprivation, a decrease in the number of spike-wave discharges ran parallell to a rebound of REM sleep and of deep non-REM sleep. This initial increase as well as the epileptogenic effects during the course of the sleep deprivation and during the recovery period after sleep deprivation can be interpreted in terms of changes in sleep-wake states. Although the epilepsy provoking mechanisms are not yet fully understood, an explanation is suggested based on changes of transitions between sleep-wake states and

shifts in level of synchronization.

To gain further insight in the relationship between vigilance and spike-wave discharges a series of experiments is reported in **chapter six** in which the interaction between the occurrence of spike-wave discharges and several other aspects of alertness was studied. In the first experiment of this chapter continuous recordings were made for a period of 48 hours and a clear circadian rhythm was established for the number of spike-wave discharges. A maximum appeared during the middle of the dark period, whereas a minimum was detected directly after the onset of the light period; the time period during which deep slow wave sleep predominates. The relationship of spike-wave discharges with states of vigilance was elaborated in a second study, which is also described in chapter four in more detail. It is concluded that discharges preferably occur when the level of vigilance of the brain is close to the level noticed at the transitions from sleep to wakefulness. In the last three experiments, the level of alertness was enhanced by various procedures such as photic stimulation, a learning task, and selective deprivation of REM sleep. In all cases, an increase of alertness appears to decrease the number of spike-wave discharges, indicating that modulation of the level of vigilance influences the occurrence of spike-wave discharges in rats of the WAG/Rij strain.

In **chapter seven** the findings concerning the relationship between level of vigilance and occurrence of spike-wave discharges were linked to a paradigm that enables behavioural quantification of information processing. It was tested whether ictal information processing and spike-wave activity are mutually exclusive. This was done for the reason that during conditions favourable for the occurrence of spike-wave activity, level of vigilance is supposed to be so low that reliable testing of information processing may be expected to be no longer possible. WAG/Rij rats were trained on an appetitively motivated visual detection task. The animals had to press a lever upon presentation of a light stimulus, which was presented for a period of ten seconds on a variable-interval schedule. Responses made within ten seconds after the onset of the stimulus were rewarded with a food-pellet. During a three hours lasting test session of 180 trials both cortical EEG activity and operant performance were registered and analysed in blocks of 15 minutes. The occurrence of spike-wave discharges was largely restricted to the middle

part of the test session, when animals responded on 75 till 90 percent of all stimulus presentations. Spike-wave discharges occurred less frequently during the periods with near maximal and low percentages of responses, which are mainly found at the beginning and at the end of the test session, respectively. These results suggest that within a test session a period exists which allows behaviourally testing of ictal information processing; both the chance of occurrence of a spike-wave paroxysm and the chance of generation of an operant response is high. Additionally it was observed that responses were never initiated during ongoing spike-wave discharges, while immediately after the disappearance of the spike-wave activity response performance was again normal.

In **chapter eight** a new testparadigm is introduced, based on the results of the study in chapter 7: information processing during spike-wave activity was tested by quantifying a physiological variable (i.e. ongoing EEG activity). A conditioning paradigm in a successive discrimination task was used to find out whether evaluation of the meaning of a stimulus is still possible during spike-wave activity. In an appetitively motivated learning task animals were trained to discriminate between two auditory stimuli with equal duration and frequency, but with different intensities. One group of animals learned that the low intensity stimulus was always followed by a food reward, whereas the high intensity stimulus was never reinforced. In a second group reinforcement was given in a counterbalanced way. In the test phase, both stimuli were presented in pseudorandom order during spike-wave discharges and the reactivity of the ongoing EEG activity was analyzed. It was found that presentation of the reinforced stimulus has significantly more effect on ongoing EEG activity than the non-reinforced stimulus, regardless of the intensity of the stimuli. This shows that during generalized spike-wave discharges the brain is still capable of evaluating the meaning of an ictally presented stimulus and that sensory and attentional processes are not completely disturbed. The outcomes are discussed within the context of brain structures involved in the genesis of spike-wave discharges.

In **chapter nine** a more demanding discrimination experiment is presented, in which additionally motor response organisation and execution during spike-wave paroxysms was tested. In that study a successive discrimination task was used in which, additional to a discrimination, an instrumental response (lever press) was required to obtain a food

reward. The animals were trained to discriminate between two auditory stimuli with equal intensity and duration, but with different frequencies. A food reward was delivered if the animals pressed the right lever upon presentation of a low frequency tone or the left lever upon presentation of a high frequency tone. In the test phase, both reinforced stimuli were presented during spike-wave activity as well as during non-epileptic EEG activity; operant performance was analysed by means of the numbers of correct and incorrect lever presses and omissions (no response). It was found that lever presses were never generated during ongoing spike-wave activity, which affirms the incompatibility of spike-wave discharges and response initiation. Next to impairments in motor initiation, that are held responsible for the ictal irresponsiveness, a cognitive deficit is suggested. Following paroxysms which were not aborted by the presentation of the stimulus, motor responses were mostly omitted, whereas after spike-wave activity that was aborted by the presentation of the stimulus, animals responded in a vast majority of stimulus presentations. The accuracy of the latter type of responses, however, was near chance level. While the study of chapter eight showed that during spike-wave discharges sensory processing and stimulus evaluation function to a certain degree, the present findings lend support for the existence of a dysfunction in more advanced stages of information processing, such as a sensory-motor integration failure or perhaps, amnesia.

Finally, in **chapter ten** the results of the various experiments are linked and the 'state of the art' concerning spike-wave paroxysms, level of vigilance, and cognition is discussed. Suggestions are made for future experiments in WAG/Rij rats, including studies on sleep spindles, on motivational states and epileptogenesis, and on the disturbances in information processing related to spike-wave discharges. Finally, it is argued that the findings with respect to the interrelationships of level of vigilance, information processing and epileptogenesis of this thesis can be most appropriately interpreted from a neurophysiological perspective, instead of in terms such as 'perception', 'attention', or 'consciousness'. The concept of thalamic transfer ratio is used to integrate present results and existing neurophysiological data into testable working hypotheses for future research.

SAMENVATTING

Samenvatting

In **hoofdstuk 1** wordt het onderzoeksonderwerp geïntroduceerd en worden rationale en opbouw van deze dissertatie uitgelegd. Al reeds in de klassieke oudheid werden plotselinge veranderingen in het gedrag in verband gebracht met epilepsie. De neurofysiologische processen die met deze gedragsveranderingen samenhangen, konden echter pas bestudeerd worden nadat in het begin van deze eeuw de electroencefalografie was ontwikkeld. Herhaald optredende elektriche ontladingen van zenuwcellen met een karakteristieke, electroencefalografische (EEG) piek-golf vorm zijn het kenmerk van een niet-convulsief type epilepsie: absence epilepsie. Het klinische beeld dat deze piek-golf ontladingen vergezelt, is gedragsmatige immobiliteit en een verminderd reageren op van buiten af aangeboden stimuli. Studies naar het presteren van absence patiënten op gedragstaken leerden dat tijdens piek-golf ontladingen aangeboden informatie niet goed verwerkt wordt. Er bestaat echter omtrent de functionele veranderingen van de hersenen tijdens piek-golf ontladingen nog geen consistente theorie waarbinnen de resultaten van deze studies verklaard kunnen worden.

Piek-golf ontladingen kunnen ook afgeleid worden van de hersenen van dieren, vooral van katten en knaagdieren. Onderzoek aan diermodellen is nuttig bij het ontwikkelen van het noodzakelijke theoretische kader, omdat dergelijke modellen bijzondere mogelijkheden bieden om de mechanismen te bestuderen die ten grondslag liggen aan het ontstaan van epileptische hersenactiviteit en de functionele consequenties daarvan.

Tot nu toe heeft slechts een klein aantal dierstudies informatieverwerking tijdens piek-golf ontladingen bestudeerd. De bij piek-golf ontladingen betrokken biochemische, neuroanatomische en neurofysiologische processen zijn daarentegen veelvuldig in diermodellen onderzocht. Corticale piek-golf ontladingen weerspiegelen oscillaties in thalamo-corticale circuits. De thalamus is een buitengewoon ingewikkeld complex van groepen neuronen binnen een uitgebreid netwerk van ingaande en uitgaande zenuwvezels. De mate van activiteit van de thalamus staat deels onder controle van het reticulair activerende systeem (ARAS) waaraan men de controle over slapen en waken ofwel

vigilantie toeschrijft. Als gevolg van de nauwe neurofysiologische relatie tussen de thalamus en het ARAS, kan verwacht worden dat het vigilantie niveau (de mate van alertheid ofwel de bewustzijnstoestand) gerelateerd is aan het optreden van piek-golf ontladingen, en dat het vigilantie-niveau mogelijk van invloed is op het verwerken van informatie tijdens de epileptische activiteit.

In het voor u liggende proefschrift worden daarom experimenten gepresenteerd waarin de relatie tussen slaap-waak stadia, vigilantie niveau en piek-golf ontladingen wordt bestudeerd. In deze dissertatie worden vervolgens experimenten beschreven die, voortbouwend op de bevindingen van eerstgenoemde experimenten, systematisch de verstoringen in informatieverwerking tijdens piek-golf ontladingen bestudeerden. Alle onderzoeken werden uitgevoerd bij ratten van één bepaalde stam, de WAG/Rij stam, die qua verscheidene eigenschappen overeenkomt met absence epilepsie bij de mens. Sedert de eerste publikatie over de epileptische eigenschappen van deze stam door Van Luijtelaar en Coenen in 1986, zijn verschillende karakteristieken beschreven van piek-golf ontladingen zoals die bij WAG/Rij ratten optreden en zijn enige hypothesen omtrent deze ontladingen getoetst. Het optreden van piek-golf paroxysmen is niet exclusief voor de WAG/Rij rat en ook andere diermodellen hebben belangrijke resultaten opgeleverd.

In **hoofdstuk 2** wordt een overzicht gegeven van onderzoek aan genetische diermodellen voor absence epilepsie tot aan 1991. Electrofysiologische studies toonden aan dat de piek-golf ontladingen in het corticale EEG gegeneraliseerd over de cortex optreden, terwijl de hippocampus niet bij deze activiteit betrokken is. De nucleus reticularis van de thalamus functioneert waarschijnlijk tezamen met andere delen van de thalamus als een pace-maker voor de paroxysmen. Cognitieve studies leerden dat het aantal piek-golf ontladingen gemoduleerd wordt door mentale en fysieke activiteit (zie voor uitvoeriger beschrijving onderzoek hoofdstuk 6) en dat bij WAG/Rij ratten door het optreden van de piek-golf ontladingen het vermogen om correct tijdschattingen uit te voeren is verstoord. Tenslotte wordt op grond van de afwijkende vorm van de tijdens piek-golf activiteit opgewekte potentialen gesuggereerd dat de sensoriek tijdens piek-golf ontladingen niet volledig verstoord is, maar wel verschilt van de sensoriek zoals die bestaat tijdens normale vigilantie-niveau's.

In **hoofdstuk 3** werden met behulp van spectraal analyse van het corticale EEG overeenkomsten en verschillen bepaald tussen piek-golf ontladingen en overige afwijkende EEG transiënten. Het EEG van WAG/Rij ratten vertoont namelijk naast normale slaapspoeltjes (spindels) hooggevolteerde piekvormige fenomenen, piek-golf ontladingen, en afwijkende 'intermediate stage'. Spectraal-analyse van deze transitionele fenomenen toonde aan dat enkele spectrale eigenschappen, zoals de frequentie van herhaling van de pieken, voor alle geteste fenomenen hetzelfde zijn, terwijl deze fenomenen op andere eigenschappen onderling verschillen (bijvoorbeeld de eerste harmonische van de piek-frequentie en in het gebied van de hogere frequenties). De resultaten ondersteunen de gedachte dat piek-golf ontladingen gezien moeten worden als unieke, afwijkende fenomenen die weliswaar gerelateerd, maar niet identiek zijn aan de hooggevolteerde piekvormige fenomenen en intermediate stage. Tevens werd de intra-fenomenale dynamiek van piek-golf ontladingen onderzocht. Het bleek dat de piek-frequentie monotoon verminderde van ongeveer 10 Hertz (Hz) aan het begin van de piek-golf ontladingen tot ongeveer 8 Hz aan het einde van de piek-golf ontladingen. Een samenhang tussen dergelijke dynamische intra-piek-golf veranderingen en verstoringen van het cognitief functioneren tijdens absence aanvallen bij de mens wordt gesuggereerd.

In **hoofdstuk 4** werd voor de slaap-waak toestanden en voor hun onderlinge overgangen bepaald in hoeverre ze gunstig zijn voor het optreden van piek-golf ontladingen. Door de 'gevoeligheid' voor het optreden van piek-golf ontladingen per bewustzijnstoestanden vast te stellen, worden de met het vigilantie-niveau samenhangende voorwaardelijke omstandigheden voor het optreden van piek-golf ontladingen in de hersenen nader beschreven. Het bleek dat piek-golf ontladingen hoofdzakelijk optreden tijdens lichte niet-REM slaap en tijdens passief wakker zijn. REM-slaap, actief wakker zijn, en diepe niet-REM slaap werden minder gevoelig bevonden voor het optreden van piek-golf ontladingen. Daarnaast bleek dat piek-golf ontladingen tenderen meer voor te komen gedurende transities tussen bewustzijnstoestanden. Een cruciale rol bij het ontstaan van piek-golf ontladingen wordt gesuggereerd voor de mate van stabiliteit in het vigilantieniveau.

Om het vigilantieniveau te kunnen manipuleren werd in **hoofdstuk 5** gebruik gemaakt

van een provocatietechniek, die afkomstig is uit de klinische praktijk: totale slaapdeprivatie. Getoetst werd de hypothese dat veranderingen in de verdeling van slaap-waak toestanden het ontstaan van de epileptische activiteit beïnvloedt. Hiertoe werd zowel het optreden van piek-golf ontladingen als ook het optreden van slaap-waak stadia (wakker, lichte niet-REM slaap, diepe niet-REM slaap, en REM slaap) vóór, tijdens, en na een periode van twaalf uren met vrijwel totale slaapdeprivatie geanalyseerd. Tijdens de eerste vier uren van de slaapdeprivatie-periode viel een significante toename in het aantal piek-golf ontladingen te constateren, terwijl tijdens de daarop volgende uren het aantal piek-golf ontladingen terugkwam op base-line niveau. Onmiddellijk na het einde van de slaapdeprivatie-periode werd parallel aan een rebound van REM slaap en van diepe niet-REM slaap een afname in het aantal piek-golf ontladingen gevonden. De aanvankelijke toename in epileptische activiteit, als ook de effecten op de epileptogenese gedurende het vervolg van de slaapdeprivatie-periode en gedurende de rebound slaap periode worden geïnterpreteerd in termen van veranderingen in het optreden van slaap-waak stadia. Hoewel de mechanismen die verantwoordelijk zijn voor het optreden van de epileptische activiteit nog niet geheel begrepen zijn, wordt een verklaring bediscussieerd, waarin veranderingen in de aard van de transities tussen slaap-waak stadia en fluctuaties in het synchronisatie-niveau van de betrokken neuronen centraal staat.

Om verder inzicht te krijgen in de relatie tussen het vigilantie-niveau en piek-golf ontladingen wordt in **hoofdstuk 6** van een reeks experimenten verslag gedaan waarin de interactie tussen het optreden van piek-golf ontladingen en verscheidene aspecten van alertheid bestudeerd werd. In het eerste experiment van hoofdstuk 6 werd van WAG/Rij ratten gedurende een periode van 48 uren continu EEG geregistreerd en werd slaap-waak classificatie uitgevoerd; piek-golf ontladingen bleken op te treden volgens een duidelijk circadiaan ritme. Een maximum in aantal werd gevonden tijdens de middelste uren van de donker-periode, terwijl een minimum in aantal te vinden was tijdens de eerste uren van de licht-periode; de periode van het etmaal waar de meeste diepe niet-REM slaap voorkomt. In de laatste drie experimenten van dit hoofdstuk werd het niveau van alertheid experimenteel verhoogd met behulp van verschillende activatie-technieken, zoals licht-stimulatie, een gedragstaak, en selectieve REM slaap deprivatie. In alle gebruikte

technieken werd een toename in alertheid verkregen die gepaard ging met een afname in het aantal piek-golf ontladingen. Deze resultaten tonen aan dat bij ratten van de WAG/Rij stam het optreden van piek-golf ontladingen is te beïnvloeden door het moduleren van het vigilantie-niveau.

In **hoofdstuk 7** worden de bevinden met betrekking tot de relatie tussen het optreden van piek-golf ontladingen en het vigilantie-niveau gekoppeld aan een onderzoeksparadigma waarmee informatieverwerking op een gedragsmatige wijze gekwantificeerd kan worden. In dit paradigma wordt onderzocht of onderzoek naar ictale informatieverwerking en het optreden van piek-golf ontladingen moeten worden beschouwd als elkaar uitsluitende zaken; de achterliggende gedachte bij dit experiment is dat tijdens condities die gunstig zijn voor het optreden van piek-golf ontladingen het vigilantie niveau verondersteld kan worden zo laag te zijn dat het op een betrouwbare, gedragsmatige wijze testen van informatieverwerking niet meer mogelijk is. WAG/Rij ratten werden getraind op een voedsel-gemotiveerde visuele detectie taak. De dieren moesten op een pedaal drukken wanneer er een licht-stimulus verscheen. Deze licht-stimulus, die een duur van tien seconden had, werd op een variabel interval schema gepresenteerd. Drukte het dier binnen tien seconden na het aangaan van de lichtstimulus, dan werd hij beloond met een voerkorreltje. Tijdens een drie uren durende test-sessie van in totaal 180 trials werden zowel het corticale EEG als het operante presteren geregistreerd, waarna de registratie geanalyseerd werd in blokken van 15 minuten. Het optreden van piek-golf ontladingen was beperkt tot het middelste deel van de test-sessie, wanneer de dieren op 75 tot 90 procent van alle stimulus-aanbiedingen reageerden met een pedaaldruk. Piek-golf ontladingen traden minder frequent op tijdens perioden waarin op vrijwel alle stimulus aanbiedingen gereageerd werd, gedurende het begin van de test-sessie, en tijdens perioden waarin op heel weinig stimulus aanbiedingen gereageerd werd, gedurende het einde van de test-sessie. Deze bevindingen tonen aan dat er binnen een test-sessie een periode voorkomt, waarin ictale informatieverwerking betrouwbaar gedragsmatig getest kan worden; zowel de kans op optreden van piek-golf ontladingen als ook de kans op het geven van een operante respons (pedaaldruk) is dan hoog. Bovendien werd waargenomen dat er tijdens pick-golf ontladingen nooit pedaaldrukken werden gegeven, terwijl onmiddellijk na het verdwijnen

van de epileptische activiteit responderen weer normaal was.

In **hoofdstuk 8** wordt een nieuw testparadigma geïntroduceerd dat is gebaseerd op de bevindingen van het experiment in hoofdstuk 7. Informatieverwerking tijdens piek-golf ontladingen werd getest door een fysiologische variabele, het momentane EEG, te kwantificeren. Er werd gebruik gemaakt van een conditionerings-paradigma binnen een successieve discriminatie taak om te onderzoeken of evaluatie van de betekenis van een aangeboden stimulus nog mogelijk is tijdens piek-golf ontladingen. Dieren werden getraind in een voedsel-gemotiveerde leertaak om twee auditieve stimuli te onderscheiden, die een gelijke duur en toonhoogte hadden, maar die verschilden in intensiteit. Eén groep dieren leerde dat presentatie van de stimulus met de hoge intensiteit altijd gevolgd werd door de presentatie van een voerkorreltje, en dat na presentatie van de toon met de lage intensiteit nooit een voerkorreltje werd aangeboden. Aan een andere groep dieren werd de bekrachtiging precies omgekeerd gegeven: na de harde toon volgde nooit een bekrachtiging, terwijl na de zachte toon altijd een bekrachtiging volgde. In de test-fase werden beide stimuli in een pseudo-random volgorde aangeboden tijdens piek-golf ontladingen en werd de reactiviteit van het momentane (epileptische) EEG geanalyseerd. Het bleek dat het aanbieden van de stimulus die voorheen bekrachtigd was geweest significant meer effect op het momentane EEG-beeld had dan aanbiedingen van de voorheen niet-bekrachtigde stimulus, onafhankelijk van de intensiteit van de stimuli. Dit resultaat toont aan dat tijdens piek-golf ontladingen het brein nog steeds in staat is om de betekenis van ictaal gepresenteerde stimuli te evalueren en dat sensorische en aandachtsprocessen niet volledig verstoord zijn. De discussie van de resultaten van dit onderzoek vindt plaats naar aanleiding van de betrokkenheid van verschillende hersenstructuren bij zowel informatieverwerking als bij de genese van piek-golf ontladingen.

In **hoofdstuk 9** wordt vervolgens van het dier tijdens piek-golf ontladingen niet alleen een discriminatie verlangd om een voerkorrel te krijgen, maar tevens een motorische respons (drukken op één van twee pedalen). De dieren werden getraind om twee auditieve stimuli met een gelijke intensiteit en duur maar met verschillende toonhoogten te onderscheiden. Een voedselbeloning werd gegeven als de dieren het rechts-geplaatste

pedaal indrukten na presentatie van een toon met een lage frequentie of het links-geplaatste pedaal na presentatie van een toon met een hoge frequentie. In de test-fase werden beide bekrachtigde stimuli successief gepresenteerd zowel tijdens piek-golf ontladingen (ictaal) als buiten piek-golf ontladingen (inter-ictaal); operant gedrag werd geanalyseerd aan de hand van het aantal correcte, incorrecte, en afwezige pedaaldrukken. Tijdens piek-golf ontladingen werd geen enkele keer op een pedaal gedrukt, hetgeen bevestigd dat piek-golf ontladingen en respons initiatie incompatibel zijn. Behalve een stoornis in motorische initiatie welke verantwoordelijk geacht wordt voor het uitblijven van responsen tijdens piek-golf ontladingen, wordt tevens een cognitieve stoornis gesuggereerd. Na afloop van paroxysmen welke niet afgebroken werden door het aanbieden van de stimulus, bleef een pedaaldruk meestal uit. Daarentegen werd in een meerderheid van de stimulus-aanbiedingen die leidden tot het afbreken van de piek-golf ontladingen, na het afbreken alsnog een motorische respons gegeven; de accuratesse van dit type responsen lag echter rond kansniveau. Terwijl het onderzoek in hoofdstuk 8 aantoonde dat tijdens piek-golf ontladingen sensoriek en stimulus evaluatie tot op zekere hoogte adequaat functioneren, levert dit onderzoek evidentie voor het bestaan van een stoornis in de meer geavanceerde stadia van informatieverwerking, zoals een falende sensori-motor integratie of, misschien, een amnesie.

Ter afsluiting worden in **hoofdstuk 10** de resultaten van de verschillende studies geïntegreerd en wordt de 'state of the art' met betrekking tot piek-golf paroxysmen, vigilantie-niveau, en cognitie besproken. Suggesties voor toekomstig onderzoek aan WAG/Rij ratten gaan in de richting van het bestuderen van slaap spindels, van de relatie tussen motivationele toestanden en epileptogenese, en van de stoornissen in informatieverwerking zoals tot nu toe vastgesteld tijdens piek-golf ontladingen. Tot slot wordt betoogd dat de bevindingen van deze dissertatie met betrekking tot de relaties tussen vigilantie-niveau, informatieverwerking, en epileptogenese zich het best laten interpreteren vanuit een neurofysiologische perspectief, in plaats van vanuit modellen die gebruik maken van termen als 'perceptie', 'aandacht', of 'bewustzijn'. Het concept van de thalamische transfer ratio wordt gebruik om de huidige resultaten met bestaande neurofysiologische data samen te voegen tot toetsbare werkhypothesen voor toekomstig onderzoek.

„QUIS EST NOSTRUM LIBERALITER EDUCATUS, CUI NON EDUCATORES CUM GRATA RECORDATIONE IN MENTE VERSENTUR ?"

CICERO, PRO PLANCIO 81

Dankwoord

Dankwoord

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Curriculum Vitae

De auteur van dit proefschrift, Wilhelmus Helena Ignatius Maria (Pim) Drinkenburg, werd geboren op 28 oktober 1962 in Nijmegen. Na de lagere school 'Sint Petrus Canisius' succesvol doorvoetbald te hebben, volgde hij voorbereidend wetenschappelijk onderwijs (Gymnasium β) aan het Stedelijk Gymnasium te Nijmegen. In 1982 begon hij de studie Psychologie aan de Katholieke Universiteit Nijmegen (KUN). Na de propaedeuse in 1983, werd deze studie in 1988 met goed gevolg afgesloten met als afstudeerrichting Vergelijkende en Fysiologische Psychologie bij prof. dr. J.M.H. Vossen. Zijn werkzaamheden binnen deze vakgroep werden gecontinueerd toen hij in november 1988 in dienst trad als onderzoeksmedewerker voor het project 'Nachtelijk Vliegtuiglawaai en Hinder' in opdracht van het raadgevend ingenieursbureau Couberg-Huygen. Aansluitend hieraan trad hij in mei 1989 in dienst van de Nederlandse organisatie voor Wetenschappelijk Onderzoek (NWO) als onderzoeker-in-opleiding op het vierjarige project 'Karakteristieken van Absence Epilepsie', toegewezen aan dr. E.L.J.M. van Luijtelaar en prof. dr. A.M.L. Coenen. Het certificaat 'artikel 9 functionaris' naar aanleiding van de Wet op de Dierproeven ontving hij in maart 1991. In de periode januari - april van 1993 verbleef hij als gastonderzoeker aan de Eötvös Loránd Universiteit bij prof. dr. G.A. Juhász op de vakgroep Vergelijkende Fysiologie te Budapest, Hongarije. Tegelijkertijd was hij als gastonderzoeker verbonden aan de Postgraduate Medical School van de Haynal Imre University of Health Sciences bij prof. dr. P. Halász op de vakgroep Neurologie te Budapest, Hongarije. Gedurende dat jaar werkte hij tevens als projectmedewerker binnen een samenwerkingsstudie van Organon International b.v. (dr. J.S. Andrews) en de KUN (prof. dr. J.M.H. Vossen) naar de effecten van cholinomimetica op leergedrag. In november 1993 kreeg hij een tweejarige aanstelling als Universitair Docent bij de vakgroep Vergelijkende en Fysiologische Psychologie van de KUN. Dat voor een promovendus een dergelijke UD-aanstelling vruchtbaar kan zijn, mag blijken uit het gegeven dat in deze periode zowel dit proefschrift als twee zonen (Stijn en Mees) het levenslicht zagen; hoewel op zijn minst dit laatste meer een verdienste van Moniek Roosendaal geweest is met wie de auteur sedert oktober 1990 gehuwd is.

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STELLINGEN

BEHORENDE BIJ HET PROEFSCHRIFT

INFORMATION PROCESSING IN AN ANIMAL MODEL OF ABSENCE EPILEPSY: CHARACTERISTICS OF SPIKE-WAVE DISCHARGES IN WAG/RIJ RATS.

I TIJDENS KORTDURENDE SLAAPDEPRIVATIE NEEMT HET AANTAL PIEK-GOLF ONTLADINGEN TOE. BIJ EEN LANGER VOORTDUREN VAN DE SLAAPDEPRIVATIE, VERDWIJNT DEZE TOENAME VAN HET AANTAL EPILEPTISCHE ONTLADINGEN.

- RODIN EA, LUBY ED, GOTTLIEB JS. *ELECTROENCEPH CLIN NEUROPHYSIOL* 14:544-551;1962;
- DIT PROEFSCHRIFT.

II HET COMBINEREN VAN MILDE SENSORISCHE STIMULATIE TER VOORKOMING VAN INSLAPEN EN HET BESTUDEREN VAN DE ANTI-EPILEPTISCHE WERKING VAN FARMACA MOET OP GROND VAN DE EPILEPTOGENE WERKING VAN KORTDURENDE SLAAPDEPRIVATIE ALS METHODOLOGISCH ONGEWENST GEZIEN WORDEN.

- N.A.V. DEPAULIS ET AL *NEUROPHARMACOLOGY* 27.683-689 1988.

III TIJDENS HET OPTREDEN VAN PIEK-GOLF ONTLADINGEN KAN DE BETEKENIS VAN EXTERNE STIMULI NOG GEËVALUEERD WORDEN. HET BLIJKT EVENWEL ONMOGELIJK OM OP DERGELIJKE STIMULI ADEQUAAT TE REAGEREN MET EEN MOTORISCHE RESPONS.

- DIT PROEFSCHRIFT

IV HET DUIDEN VAN GEDRAGSSTOORNISSEN TIJDENS PIEK-GOLF ONTLADINGEN IN TERMEN VAN FUNCTIONELE MODELLEN VOOR NEURALE CIRCUITS EN MECHANISMEN, ZOALS HET TRANSFER RATIO CONCEPT, VERDIENT VOORALSNOG DE VOORKEUR BOVEN DUIDINGEN IN TERMEN VAN MODELLEN VOOR INFORMATIEVERWERKING.

- DIT PROEFSCHRIFT

V PROEFDIER-ONDERZOEKERS DIE SPREKEN OVER 'BEWUSTZIJN' BIJ DIEREN MAKEN ZICH SCHULDIG AAN DEZELFDE ESSENTIËLE FOUT ALS FILOSOFEN DIE SPREKEN OVER 'BEWUSTZIJN' BIJ COMPUTERS: EEN GEBREK AAN CONSTRUCT VALIDITEIT.

- N.A.V. STELLING 5 PROEFSCHRIFT DR. FRANÇOISE WEMELSFELDER

VI HET VERWERVEN VAN BUITENLANDSE ONDERZOEKSERVARING DRAAGT BIJ TOT EEN NOG BREDERE VISIE EN HET RELATIVEREN VAN EIGEN ONDERZOEK EN DE WAARDEBEPALING ERVAN. HETZELFDE GELDT EIGENLIJK VOOR ALLE PERSOONLIJKE ERVARINGEN IN DEN VREEMDE, HET ALLEDAAGSE LEVEN WORDT DUIDELIJKER.

- DR. GILLES VAN LUUTELAAR. E-MAIL BERICHT 15 MAART 1993 VERZONDEN VAN NIJMEGEN NAAR BOEDAPEST.

VII DAT MENIGE WETENSCHAPPELIJKE PROMOTIE IN DE JAREN '90 HET EINDE IN PLAATS VAN HET BEGIN VAN EEN WETENSCHAPPELIJKE CARRIÈRE AANGEEFT, TAST NIET ALLEEN DE WAARDE VAN DE UNIVERSITAIRE PROMOTIE, MAAR OOK HET BESTAANSRECHT VAN DE UNIVERSITEIT ALS WETENSCHAPPELIJK OPLEIDINGSINSTITUUT AAN.

VIII HET STREVEN VAN FABRIKANTEN OM KINDERLUIERS TE PRODUCEREN DIE STEEDS LANGER, DROGER, EN MINDER MERKBAAR TE DRAGEN ZIJN, MOET STOELEN OP OMZETVRIENDELIJKE GEMAKZUCHT VAN OPVOEDERS IN PLAATS VAN OP LEERTHEORETISCH VERANTWOORDE ZINDELIJKHEIDSTRAINING.

IX DE SOORTTYPISCHE VRAAG NAAR DE ZIN VAN HET BESTAAN IS NAUW VERWANT AAN HET ANTWOORD OP DE VRAAG WAAROM EEN HOND NOOIT HET REQUIEM VAN MOZART MEEKWISPELT.

X DAT VANDAAG DE DAG DE AFKORTING 'AUTO' IN PLAATS VAN HET OORSPRONKELIJKE 'AUTOMOBIEL' ALGEMEEN GEBRUIKT WORDT, DOET RECHT AAN DE TOENEMENDE BEPERKINGEN IN GEBRUIKSMOGELIJKHEDEN; GEZIEN HET GROTE AANTAL ONGELUKKEN IN HET WEGVERKEER ZOU DE VOORKEUR ECHTER MOETEN UITGAAN NAAR DE BENAMING 'WAGEN'.

W.H.I.M. DRINKENBURG,

NIJMEGEN, 30 OKTOBER 1995.

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