Steroid hormones in the regulation of absence seizures: A putative role of the limbic system

Manipulations with the hypothalamo-pituitary-gonadal and hypothalamo-pituitary-adrenal systems

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A scientific essay in Social Sciences

Doctoral Thesis

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Chapter 1

GENERAL INTRODUCTION

1.1. Preface

Epilepsy is a neurodegenerative disorder that afflicts more than 50 million people (WHO, 2001). Seizure frequency and severity can change at various life stages that are associated with alterations in hormones (Morrell, 2002). Many people are daily exposed to stress, that transiently alters the concentration of some neuroactive steroids that can influence seizure susceptibility. In female patients with epilepsy, manifestation of complex partial seizures and generalized tonic-clonic seizures may be influenced by fluctuations in ovarian steroid hormones across reproductive cycles. Furthermore, at puberty and during adolescence, patterns of specific epilepsy syndromes change and this transition occurs due to large fluctuations in steroid hormonal milieu.

Seizure control, the primary goal of treatment, is achieved in ~ 70% of people with epilepsy using traditional antiepileptic drugs (WHO, 2001). Considering increasing awareness of the possible role of steroid hormones in seizure control, they are suggested to represent an emerging therapeutic strategy that may serve as an alternative or supplement to traditional AEDs (Reddy, 2002; Rhodes and Frye, 2005). In addition, steroid hormones can also provide more comprehensive management of other relevant issues, such as concomitant depression, cognitive impairments as well as risks for reproductive endocrine disorders associated with epilepsy (Herzog and Klein, 1998, Fawley et al, 2006).

In contrary to convulsive epilepsy, little is known about the role of steroid hormones in the pathogenesis of absence epilepsy. In addition to above mentioned issues, the relevance of this question is raised by the fact, that puberty - a period when major changes in steroid hormonal milieu occur - plays a critical role in the pathogenesis of childhood absence epilepsy. In about 60-70% of patients absence seizures remit during adolescence, while in the other 30-40% of cases it changes into a more serious form of generalized epilepsy (Loiseau et al., 1995; Panayiotopoulos, 1999).

The aim of the present thesis was to investigate the role of endogenous hormonal steroid hormones milieu in the pathogenesis of absence epilepsy and to understand whether circulating steroid hormones are involved in the ongoing seizure control.

1.2. The hypothalamo-pituitary-gonadal system and the central effects of ovarian steroid hormones

The secretion of gonadal steroids hormones is regulated by the hypothalamo-pituitary-gonadal (HPG) endocrine system, which obviously consists of the hypothalamus, the pituitary, and the gonads. A central component of this axis is a scattered population of 800-2000 neurons in the median eminence of the hypothalamus that synthesize the gonadotropin-releasing hormone (GnRH). The pulsatile release of GnRH from the hypothalamus regulates the synthesis and the release of the two gonadotropins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. In men, LH stimulates the testes to produce testosterone, which, in turn, provides negative feedback at the level of the hypothalamus and pituitary. In women, the differential production of FSH and LH determines the phase of the menstrual cycle and stimulates the production of progesterone and estrogen (Fawley et al., 2006). Progesterone has a negative feedback effect on the hypothalamus and pituitary, whereas estrogen has both positive and negative feedback effects dependent on the phase of the menstrual cycle. In addition to the feedback mechanisms of hormones, the HPG axis is regulated by the neuronal inputs from the hippocampus and amygdala (Fawley et al., 2006).

Steroid hormones influence brain functions from gestation throughout life and may affect seizure threshold by altering neuronal excitability. Many effects of steroids are known to occur via the stimulation of gene expression through well-characterized intracellular receptors. These receptors have been identified in various brain areas: amygdala, hippocampus, cortex, basal forebrain, cerebellum, locus coeruleus, midbrain raphe nuclei, glial cells, pituitary gland, hypothalamus and central grey matter (Genazzani et al, 2000). In addition to genomic effects, there are also membrane effects of steroid hormones that take place rapidly at the cell surface and influence neuronal excitability via different type of neurotransmitter receptors such as GABA, NMDA, glycine, nicotinic, cholinergic, serotonin-3 (5HT3) receptors (Stoeffel-Wagner, 2005; Melcangi and Panzica, 2006). In particular, increasing evidence suggests that most of inhibitory effects of progesterone on the cell membrane is mediated by the action of its 3a-hydroxylated metabolite (5a-pregnan-3a-ol-20-one), neurosteroid allopregnanolone (Kalkbrenner et al, 1999; van Luijtelaar et al, 2001; Rhodes and Frye, 2005). The most prominent effect of allopregnanolone is a positive allosteric modulation of the gamma-aminobutyric acid receptor A (GABA-A) (Majewska et al, 1986), which serves a major inhibitory

neurotransmitter in the mature CNS. Allopregnanolone is devoid of hormonal effects and together with other related neuroactive steroids regarded as rapid endogenous modulator of neuronal excitability in CNS with anxiolytic, antiepileptic, and sedative-hypnotic properties.

Fluctuations in plasma estrogen and progesterone (and its neuroactive metabolites) over different reproductive states have long been known to influence ictal activity in women with epilepsy. Catamenial epilepsy, or menstrual cycle-related changes in seizure disorder, may affect up to 70% of women with epilepsy (Herzog et al, 2004; Foldvary-Schaefer et al, 2004). Three distinct patterns of catamenial epilepsy have been described with increases in seizures typically occurring during the perimenstrum, around ovulation, and/or during an inadequate luteal phase (Herzog et al, 1997). In general, it is thought that estrogen enhances neuronal excitability and increases the risk of seizures, whereas progesterone diminishes neuronal excitability and has an inhibitory effect on seizures (Herzog and Klein, 1998; Rhodes at al, 2004; Rhodes and Frye, 2005). However, studies directly associating seizures with changes in hormone concentrations have shown that the expression of epileptic activity correlates at most with ratio of estrogen to progesterone plasma concentrations (Backström, 1976; Herzog, 1991). The greater levels of estrogen may be predisposing to hyperexcitability and progesterone may have anti-epileptiformic effects. These findings are reinforced by observations that seizures often suppressed during pregnancy, when the balance of progesterone to estrogen is greater (Morrell, 2002; Pimentel, 2000). In support, progesterone therapy has also been demonstrated to reduce seizure frequency in about 70% of women with epilepsy (Backström et al, 1984; Herzog, 1986; 1995).

Progesterone is also known to produce anti-seizure effects in different animal models. Recent reports indicate that administration of physiologically relevant concentrations of progesterone to ovariectomized rodents decrease seizure susceptibility in a variety of animal models of epilepsy including kainic acid and pentylenetetrazole (PTZ) induced seizures, perforant path stimulation, audiogenic seizures and kindling (for review see Herzog and Klein 1998; Rhodes et al, 2004). Consistently, kainic acid and perforant pathway induced seizure activity in rats is increased in endocrine states characterized by decline in endogenous levels of progesterone and its neuroactive derivatives (Frye and Bayon, 1998; 1999). In contrary, administration of estrogen has been reported either to have no effects, increase, or decrease seizures. For instance, estrogen administration to adult male mice or ovariectomized rats attenuates neither kainic acid nor pentylenetetrazole (PTZ)-induced seizures (Budziszewska et al, 2001; Reibel et al, 2000; Perez et al, 1988). Although estrogen administration in

rats does not alter the rate of kindling (Schultz-Krohn et al, 1986), acute estrogen treatment to adult male amygdaloid-kindled rats lead to the more severe seizure expression (Saberi et al, 2001). Estrogen administration to ovariectomized rats decreases the latency to onset of kainic-acid induced seizures, but it increases the latency to NMDA-induced seizures (Slamberova and Vathy, 2000).

It was recently demonstrated that acute i.p. administration of progesterone facilitated (and this is in contrary to convulsive seizures) the occurrence of absence seizures in both humans (Grunewald et al, 1995) and rats (Budziszewska et al, 1999; van Luijtelaar et al, 2001, Persad et al, 2004). Conversely, administration of estrogens failed to have any acute effects on absence seizures in WAG/Rij rats (van Luijtelaar et al, 2001). By contrast, cortical application of progesterone was shown to suppress spontaneous absence-like seizures evoked by application of penicillin to the cerebral cortex in cats (Landgren et al, 1978).

1.3. The hypothalamo-pituitary-adrenocortical axis and stress response

The secretion of adrenal steroid hormones is controlled by the hypothalamo-pituitary-adrenal (HPA) axis - a major control system of the neuroendocrine stress response in vertebrate organisms. The HPA axis plays a critical role in survival of all vertebrate species and helps organism to cope with physiological challenges and can be activated by a wide range of psychological experiences and physiological perturbations.

A central component of the HPA axis is a discrete set of neurosecretory neurons localized in the medial parvocellular subdivision of the hypothalamic paraventricular nucleus (PVN). These neurons integrate excitatory and inhibitory signals into appropriate secretion of corticotrophin-releasing hormone (CRH) as well as a cocktail of other peptide factors (e.g. arginine vasopressin (AVP). CRH is responsible for the synthesis and the release of adreno-cortico-trophin hormone (ACTH) from the anterior pituitary gland. The action of CRH on ACTH release is strongly potentiated by AVP that is co-produced in increasing amounts when the hypothalamic paraventricular neurons are chronically activated. ACTH enters the peripheral blood flow and reaches the adrenal cortex, where it, in turn, regulates the secretion of the glucocorticoids – the main end product of the HPA axis function. The glucocorticoids have a major negative feed-back

effect a hypothalamus and pituitary that dampens down the stress-induced activation of the HPA-axis.

Basically, the HPA axis operates in two equally important modes (or regimes) of activity. Under relatively unstressed conditions, glucocorticoids secretion undergoes a daily circadian rhythm, with peak secretion occurring at the initiation of the waking cycle in most vertebrate organisms (Keller-Wood and Dallman, 1984). Secretion during the waking phase permits circulating glucocorticoids to partially occupy glucocorticoid receptors and this is believed to be critical for optimizing functional tone of numerous systems (De Kloet et al, 1998; Reul and De Kloet, 1985). Control of this rhythmic activity is coordinated by inputs from the suprachiasmatic nucleus (Diamond et al, 1992; De Kloet et al, 1998), the critical pacemaker of numerous rhythms in the organism. The second mode of HPA action can be activated upon either real or predicted threat of any physiological challenge of homeostasis. It is initiated in the PVN, which receives inputs from the variety of cortical, limbic, and brainstem centers involved in the procession of external and internal sensory information (Herman et al, 2003).

Overall stress can alter seizure susceptibility by releasing glucocorticoid adrenal steroids hormones such as cortisol, corticosterone and deoxycorticosterone. Cortisol and corticosterone are excitatory steroids with proconvulsant or seizure facilitating properties, while deoxycorticosterone has been shown to inhibit seizures (Reddy and Rogawski, 2002). In childhood absence epilepsy i.p. corticosterone injections also induce a dose-dependent increase in the number of spike-wave discharges (SWD) (Schridde and van Luijtelaar, 2004). However, following stress there is also a release of neurosteroids facilitating GABA-ergic neurotransmission, such as progesterone, pregnenolone, allopregnanolone and 3a,5a-THDOC (Barbaccia et al, 2001; Biggio et al, 2007), which are known to decrease seizure susceptibility. Consequently, some patients with epilepsy exposed to an acute stressor show an increase, others undergo a decrease or no changes in seizure susceptibility (Haut et al, 2003; Wiener, 2003). Chronic stress is mostly associated with an increase in frequency of seizures (Minter, 1979; Temkin and Davis, 1984; Bosnjak et al, 2002).

1.4. What is an absence seizure? A short introduction to absence epilepsy

Absence seizures are characterized by spontaneously occurring bursts of bilateral synchronous spike-wave activity accompanied by the reduction in the level of consciousness (from French - "absence"), i.e. a reduced responsiveness and the inability to make voluntary movements. Episodes of this synchronized electrical activity as recorded in the electroencephalogram (EEG), the so-called spike-wave discharges (SWDs), may appear up to a few hundred times per day, and generally last 10-20 sec. Absence seizures occur during quiet behavioural states such as drowsiness and light slow wave sleep and can be provoked by sleep deprivation. In contrast to generalized convulsive or partial seizures, there is no postictal depression after a typical absence.

The pharmacological reactivity of absence seizures is also unique: they are suppressed by ethosuximide, which is ineffective in all other forms of seizures, while they are aggravated by carbamazepine, phenytoin, GABA-mimetics such as tiagabine and vigabatrin (Bouwman et al., 2003; Danober et al, 1998; Depaulis and van Luijtelaar, 2006). However, absence seizures can be also suppressed by regular antiepileptic drugs such as lamotrigine, valproate, levetiracetam and benzodiazepines (Danober et al, 1998; Depaulis and van Luijtelaar, 2006).

Typical absences are found in five different generalized syndromes such as childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy, myoclonic absence epilepsy and eyelid myoclonia with absences (Loiseau et al., 1995). Besides absence seizures, patients do not have any other neurological or neuropsychological disorders. The most common childhood absence epilepsy is characterized by the early onset of absence seizures and between the ages of 2 and 8 years and remission before puberty (Loiseau and Cohadon, 1970; Loiseau et al., 1995). Juvenile absence epilepsy and eyelid myoclonia with absences occur after the ages of 10±12 years and persist in adulthood (Porter, 1993; Loiseau et al, 1995). Absence epilepsies are thought to be associated with brain development and maturation (Danober et al, 1998).

The most dominant theory of absence epilepsy, proposed by Gloor in 1968 and nowadays widely accepted, is the classical concept of cortico-reticular epilepsy. This concept implies that SWDs represent a thalamo-cortical type of oscillation, where an abnormally excitable cortex interacts with thalamus and brain stem reticular formation (Gloor, 1968). It has also been established that absence seizures are generated in the thalamo-cortical

circuitry, which is primarily involved in the processing of incoming sensory information. The activity in this network is formed by the interplay between the reticular thalamic nucleus (the RTN), thalamic relay cells and the cortex. (Steriade, 1990; Gloor 1990; Seidenbecher et al, 1998; Avanzini et al, 2000). Hyperexcitability or, more precisely, hyperreactivity of cortico-thalamic cells, which is according to Gloor's theory a precondition for the occurrence of absence seizures, is one of the factors which can provoke the switch of thalamic relay neurons from tonic to bursting firing mode (Blumenfeld and McCormick, 2000; Destexhe, 1998) and initiate the generation of absence seizures. The firing mode of thalamic neurons is modulated by inputs from the reticular formation in the brainstem, cortical, limbic and forebrain projections.

Recently, Meeren and coauthors (2002; 2005; van Luijtelaar and Sitnikova, 2006) have shown that absence epilepsy in rodent models has a focal origin. SWDs (or absence seizures) are initiated in the distinct cortical area located in the perioral region of the somatosensory cortex and thereafter from this area epileptic activity spread over the other parts of the cortex. This theory was based on the experimental data obtained in WAG/Rij rats, a genetic model of generalized absence epilepsy (van Luijtelaar and Coenen, 1986; Coenen and van Luijtelaar, 2003). Interestingly, a similar focal zone was also found in GAERS, another genetic model of absence epilepsy in rats (Manning et al, 2004; Gurbanova et al, 2006). Preliminary results show that focal zones are also located in the prefrontal cortex in children with absence epilepsy (Westmijse et al, 2007).

1.5. WAG/Rij rats - a genetic model of absence epilepsy in rodents

Spontaneously occurring spike-wave activity mediated in a cortico-thalamic network is abundantly present in the cortical electroencephalogram of several strains of rats such as WAG/Rij and GAERS (van Luijtelaar and Coenen, 1986, Marescaux et al, 1992). WAG/Rij rats, as well as GAERS, represent fully inbred strains of albino rats considered to be well-validated and most commonly used models of absence epilepsy to study basic mechanisms underlying the pathophysiology of absence epilepsy as well as for the pharmacological tests (van Luijtelaar and Coenen, 1986; Coenen and van Luijtelaar, 2003; van Luijtelaar and Sitnikova, 2006). SWDs in rats can be easily recorded through chronically implanted either monopolar or bipolar electrodes positioned over the cortex. EEG recordings in WAG/Rij

rats showed that the spike-wave activity in rats has an inter-spike frequency of 7-11 Hz. The episodes of this activity last from 1 till 30 seconds, the mean duration is about 5 sec and may occur up to 3-30 times per hour in rats that are minimally 6 months old. Absence seizures in rats do not remit as in humans and, in contrast, they aggravate with age. The age dependency is also discussed a bit later.

The pharmacological profile of spike-wave activity is similar to those seen in patients suffering from absence epilepsy (Coenen and van Luijtelaar, 2003). In WAG/Rij rats SWDs are suppressed by the four main antiepileptic drugs, which are also effective against human absences (ethosuximide, trimethadione, valproate and benzodiazepines) and worsened by drugs which are either ineffective or aggravating absence seizures in humans (carbamazepine, phenytoin, vigabatrin, tiagabine (Depaulis and van Luijtelaar, 2006).

Similar to humans, the occurrence of absence seizures in WAG/Rij rats has a distinct circadian pattern closely related to the sleep-wakefulness cycle (Drinkenburg et al, 1991, Coenen and van Luijtelaar, 2003). However, in contrast to human childhood absence epilepsy, rats show a delayed developmental onset (SWDs in WAG/Rij rats became apparent after puberty at around two-three months of age) and an age-dependent increase in spike-wave discharges, concomitant to mild clinical manifestations (Coenen and van Luijtelaar, 1987; Schridde and van Luijtelaar, 2005). Interestingly, by means of the administration of a cholesterol inhibitor SWDs in rats can be elicited already before the onset of puberty, as it was shown in GAERS (Persad et al, 2002).

1.6. General outline of this thesis

The experimental focus of this thesis was aimed to the interaction between hormonal milieu (ovarian and adrenal steroid hormones) and the occurrence of absence seizures in WAG/Rij rats in a number of acute and chronic experiments. In **Chapter 2**, the first experimental chapter, we investigated the association between ovarian steroids (progesterone and estradiol) plasma concentration, as measured by ELISA kits, and the occurrence of SWDs during pregnancy, when the basal concentration of both ovarian steroid hormones are chronically increased. In the next series of experiments (**Chapter 3**), we tried to model the situation of chronic elevation of progesterone beyond the pregnancy and tested the occurrence of SWDs after daily repeated progesterone administration. To complete these

two experiments and test what would happen with SWDs in an opposite situation (chronically low level), in **Chapter 3** we also investigated the basal and stress-induced occurrence of SWDs after ovariectomy: a condition of reduced peripheral synthesis of ovarian steroid hormones. We hypothesized that if there is a relationship between hormonal milieu and the occurrence of absence seizures, the withdrawal of ovarian steroids hormones after ovariectomy should enhance the occurrence of SWDs. Considering that we found a large aggravation of SWDs in stress-sensitive condition, we characterized some of the basic parameters in the hypothalamo-pituitary-adrenal axis functioning in epileptic WAG/Rij male rats in comparison with non-epileptic control strains ACI and Wistar rats in **Chapter 4**. We measured both diurnal and stress-induced plasma corticosterone concentration and thereafter in more details investigated the effect of acute and chronic stress on the occurrence of absence seizures in male rats.

In the second part of this thesis we investigated the role excitability of the cortex and the limbic system, which are the major target areas for steroid hormones, in the pathogenesis of absence epilepsy in WAG/Rij. The cortex plays a major role in various theories on the origin of absence epilepsy and hyperexcitability of the cortex is classically regarded as a precondition of the appearance of SWDs. Our first experiment presented in **Chapter 5** was carried out to test whether the cortex of epileptic WAG/Rij rats is more excitable than the cortex of non-epileptic rats. The excitability was measured electrophysiologically by threshold values for various types of afterdischarges in 3 and 6 months old WAG/Rij, ACI and Wistar rats.

In **Chapter 6** we investigated whether pharmacological manipulations within the limbic system would affect the occurrence of absence seizures in WAG/Rij rats. Male WAG/Rij rats were implanted with permanent EEG electrodes and bilateral cannulas in the CA1-CA3 region of the dorsal hippocampus. Control rats had bilateral cannulas in the cortical area above the hippocampus. Rats received intracerebral injections of progesterone – $GABA_A$ mimetic, 45% β-cyclodextrin, saline, or tiagabine – a GABA re-uptake blocker. EEG recordings were made before and after injection.

In **Chapter 7** (General Discussion), we outlined all the major experimental findings of this thesis and proposed a new conceptual framework (the model) integrating the classical cortico-thalamo-cortical loop with the limbic system circuitry to explain the controversial effects of steroid hormones on absence seizures.

References

Avanzini, G., Panzica, F., de Curtis, M., The role of the thalamus in vigilance and epileptogenic mechanisms. Clin. Neurophysiol. 111 Suppl 2, (2000) 19-26.

Backström, T., Epileptic seizures in women related to plasma estrogen and progesterone during the menstrual cycle. Acta Neurol Scand 54, (1976) 321– 347.

Backström, T., Zetterlund, B., Blom, S., Romano, M., Effects of intravenous progesterone infusions on the epileptic discharge frequency in women with partial epilepsy. Acta Neurol Scand 69, (1984) 240–248.

Barbaccia, M.L., Serra, M., Purdy, R.H., Biggio, G., Stress and neuroactive steroids, Int Rev Neurobiol. 46, (2001) 243-272.

Biggio, G., Concas, A., Follesa, P., Sanna, E., Serra, M., Stress, ethanol, and neuroactive steroids. Pharmacol Ther. (2007).

Blumenfeld, H., McCormick, D.A., Corticothalamic inputs control the pattern of activity generated in thalamocortical networks. J. Neurosci. 20, (2000) 5153-5162.

Bouwman, B.M., van den Broek, P.L., van Luijtelaar, G., van Rijn, C.M., The effects of vigabatrin on type II spike wave discharges in rats, Neurosci. Lett. 338, (2003) 177-180.

Budziszewska, B., van Luijtelaar, G., Coenen, A., Leskiewicz, M., Lason, W., Effects of neurosteroids on spike-wave discharges in the genetic epileptic WAG/Rij rat, Epilepsy Res. 33 , (1999) 23-29.

Budziszewska, B., Leskiewicz, M., Kubera, M., Jaworska-Feil, L., Kajta, M., Lason, W., Estrone, but not 17h-estradiol, attenuates kainate-induced seizures and toxicity in male mice. Exp Clin Endocrinol Diabetes 109, (2001) 168– 173.

Bosnjak, J., Vukovic-Bobic, M., Mejaski-Bosnjak, V., Effect of war on the occurrence of epileptic seizures in children. Epilepsy Behav. 3, (2002) 502-509.

Coenen, A.M., van Luijtelaar, E.L., Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats, Behav Genet. 33, (2003) 635-655.

Danober, L., Deransart, C., Depaulis, A., Vergnes, M., Marescaux, C., Pathophysiological mechanisms of genetic absence epilepsy in the rat, Prog Neurobiol. 55 (1998), 27-57.

Destexhe, A., Spike-and-wave oscillations based on the properties of GABAB receptors. J. Neurosci. 18, (1998) 9099-9111.

De Kloet, R., Vreugdenhil, E., Oitzl, M.S., Joels, M., Brain corticosteroid receptor balance in health and disease, Endocrine Rev. 19, (1998) 269-301.

Depaulis, A., van Luijtelaar, G., Genetic models of absence epilepsy in the rat. In: Pitkänen, A., Schwartzkroin, P.A., Moshé, S.L. (Eds.), Models of Seizures and Epilepsy, Elsevier, Amsterdam, (2006) 233-248.

Diamond, D.M., Bennett, M.C., Fleshner, M., Rose, G.M., Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation, Hippocampus 2, (1992) 421–430.

Drinkenburg, W.H.I.M., Coenen, A.M.L., Vossen, J.M.H., van Luijtelaar, E.L.J.M., Spike-wave discharges and sleep-wake states in rats with absence epilepsy. Epilepsy Res. 9, (1991) 218-224.

Fawley, J.A., Pouliot, W. A., Dudek, F.E., Epilepsy and reproductive disorders: the role of the gonadotropin-releasing hormone network. Epilepsy Behav. 8, (2006) 477-482

Foldvary-Schaefer, N., Harden, C., Herzog, A., Falcone, T., Hormones and seizures. Cleve Clin J Med, 71, (2004) 11–18.

Frye, C.A., Bayon, L.E., Seizure activity is increased in endocrine states characterized by decline in endogenous levels of the neurosteroid 3a,5a-THP. Neuroendocrinology, 68, (1998) 272–280.

Frye, C.A., Bayon, L.E., Cyclic withdrawal from endogenous and exogenous progesterone increases kainic acid and perforant pathway induced seizures. Pharmacol. Biochem. Behav. 62, (1999)315-321.

Gloor, P., Generalized cortico-reticular epilepsies. Some considerations on pathophysiology of generalized bilaterally synchronous spike-and-wave discharges. Epilepsia 9, (1968) 249-263.

Gloor, P., Avoli, M., Kostopoulos, G., Thalamo-cortical relationships in generalized epilepsy with bilaterally synchronous spike-and-wave discharge. In: "Generalized Epilepsy: Neurobiological Approaches." Eds: M. Avoli, P. Gloor, R. Naquet and G. Kostopoulos. Birkhäuser Boston Inc. Boston, (1990) 190-212.

Grunewald, R.A., Aliberti, V., Panayiotopoulos, C.P., Exacerbation of typical absence seizures by progesterone, Seizure 1, (1992) 137-138.

Gurbanova, A.A., Aker, R., Berkman, K., Onat, F.Y., van Rijn, C.M., van Luijtelaar, G., Effect of systemic and intracortical administration of phenytoin in two genetic models of absence epilepsy. Br. J. Pharmacol. 148, (2006) 1076-1082.

Haut, S.R., Vouyiouklis, M., Shinnar, S., Stress and epilepsy: a patient perception survey. Epilepsy Behav. 4, (2003) 511-514.

Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C., Cullinan, W.E., Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front Neuroendocrinol, 24, (2003) 151-180.

Herzog, A.G., Intermittent progesterone therapy and frequency of complex partial seizures in women with menstrual disorders. Neurology 36, (1986) 1607–1610.

Herzog, A.G., Reproductive endocrine considerations and hormonal therapy for women with epilepsy. Epilepsia 32 Suppl 6,(1991) 27-33.

Herzog, A.G., Progesterone therapy in women with complex partial and secondary generalized seizures. Neurology 45, (1995) 1660–1662.

Herzog, A.G., Klein, P., Ransil, B.J., Three patterns of catamenial epilepsy. Epilepsia 38 (1997) 1082–1088.

Jacono, J.J., Robertson, J.M., The effects of estrogen, progesterone, and ionized calcium on seizures during the menstrual cycle of epileptic women. Epilepsia 28, (1987) 571–577.

Kalkbrenner, K.A., Standley, C.A., Kokate, T.G., Banks, M.K., Magee, T., Yamaguchi, S., Rogawski, M.A., Finasteride, a 5a-reductase inhibitor, blocks the anticonvulsant activity of progesterone in mice. J Pharmacol Exp Ther 288, (1999) 679– 684.

Inoue, M., Duysens, J., Vossen, J.M., Coenen, A.M., Thalamic multiple-unit activity underlying spike-wave discharges in anesthetized rats, Brain Res. 612, (1993) 35-40.

Keller-Wood, M., Dallman, M.F., Corticosteroid inhibition of ACTH secretion, Endocr. Rev. 5, (1984) 1–24.

Kostopoulos, G.K., Spike-and-wave discharges of absence seizures as a transformation of sleep spindles: the continuing development of a hypothesis. Clin Neurophysiol. 111 Suppl. 2, (2000) 27-38.

Loiseau, P., Cohadon, F., Considerations on epilepsia partialis continua and its relationship with somatomotor epelepsy. Encephale. 59, (1970) 362-389.

Loiseau, P., Duché, B., Pédespan, J.M., Absence Epilepsies. Epilepsia 36, (1995) 1182-1186.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor, Science 232, (1986) 1004-1007.

Manning, J.P., Richards, D.A., Leresche, N., Crunelli, V., Bowery, N.G., Cortical-area specific block of genetically determined absence seizures by ethosuximide. Neuroscience 123, (2004) 5-9.

Marescaux, C., Vergnes, M., Depaulis, A., Genetic absence epilepsy in rats from Strasbourg - A review. J. Neural Transm. 35, Suppl (1992) 37-69.

Meeren, H.K.M., Cortico-thalamic mechanisms underlying generalized spike-wave discharges of absence epilepsy. A lesional and signal analytical approach in the WAG/Rij rat. PhD thesis, NICI, Radboud University Nijmegen, 2002.

Meeren, H., van Luijtelaar, G., Lopes da Silva, F., Coenen, A., Evolving concepts on the pathophysiology of absence seizures: the cortical focus theory. Arch. Neurol. 62, (2005) 371-376.

Melcangi, R.C., Panzica, G.C., Neuroactive steroids: old players in a new game, Neurosci. 138 (2006) 733-739.

Minter, R.E., Can emotions precipitate seizures--a review of the question. J. Fam. Pract. 8, (1979) 55-59

Morrell, M.J., Epilepsy in women. Am Fam Physician 66, (2002) 1489–1494.

Panayiotopoulos, C.P., Typical absence seizures and their treatment. Arch Dis Child. 81, (1999) 351-355.

Peebles, C.T., McAuley, J.W., Moore, J.L., Malone, H.J., Reeves, A.L., Hormone replacement therapy in a postmenopausal woman with epilepsy. Ann Pharmacother 34, (2000) 1028–1031.

Peeters, B.W., van Rijn, C.M., Vossen, J.M., Coenen, A.M., Effects of GABA-ergic agents on spontaneous non-convulsive epilepsy, EEG and behaviour, in the WAG/Rij inbred strain of rats, Life Sci. 45 (1989) 1171-1176.

Perez, J., Zucchi, I., Maggi, A., Estrogen modulation of the gamma-aminobutyric acid receptor complex in the central nervous system of rat. J Pharmacol Exp Ther 244, (1988) 1005– 1010.

Persad, V., Cortez, M.A., 3rd Snead, O.C., A chronic model of atypical absence seizures: studies of developmental and gender sensitivity, Epilepsy Res. 48 (2002) 111-119.

Persad, V., Ting Wong, C.G., Cortez, M.A., Wang, Y.T., Snead III, O.C., Hormonal regulation of atypical absence seizures. Ann Neurol. 55, (2004) 353–361.

Pimentel, J., Current issues on epileptic women. Curr Pharm. 6, (2000) 865–872.

Porter, R.J., The absence epilepsies. Epilepsia. 34 Suppl. 3, (1993) 42-48.

Reddy, D.S., Rogawski, M.A., Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. J. Neurosci. 22, 9, (2002) 3795-3805.

Reddy, D.S., The clinical potentials of endogenous neurosteroids. Drugs Today (Barc) 38, (2002) 465-85.

Reibel, S., Andre, V., Chassagnon, S., Andre, G., Marescaux, C., Nehlig, A., Depaulis, A., Neuroprotective effects of chronic estradiol benzoate treatment on hippocampal cell loss induced by status epilepticus in the female rat. Neurosci Lett 281, (2000) 79– 82.

Reul, J.M., De Kloet, E.R., Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation, Endocrinology 117, (1985) 2505–2511.

Rhodes, M.E., Harney, J.P., Frye, C.A., Gonadal, adrenal, and neuroactive steroids' role in ictal activity, Brain Res. 1000 (2004) 8-18.

Rhodes, M.E., Frye, C.A., Actions at GABA(A) receptors in the hippocampus may mediate some antiseizure effects of progestins, Epilepsy Behav. 6 (2005) 320-327.

Schultz-Krohn, W.A., Thompson, J., Holmes, G.L., Effect of systemic estrogen on seizure susceptibility in the immature animal. Epilepsia 27, (1986) 538–541.

Saberi, M., Pourgholami, M.H., Jorjani, M., The acute effects of estradiol benzoate on amygdala-kindled seizures in male rats. Brain Res. 891, (2001) 1–6.

Seidenbecher, T., Staak, R., Pape, H.C., Relations between cortical and thalamic cellular activities during absence seizures in rats, Eur. J. Neurosci. 3, (1998) 1103-1112.

Schridde, U., van Luijtelaar, G., Corticosterone increases spike-wave discharges in a dose- and time-dependent manner in WAG/Rij rats, Pharmacol Biochem Behav. 78, (2004) 369-375.

Schridde, U., van Luijtelaar, G., The role of the environment on the development of spike-wave discharges in two strains of rats. Physiol Behav. 84, (2005) 379-386.

Slamberova, R., Vathy, I., Estrogen differentially alters NMDA- and kainite induced seizures in prenatally morphine- and saline-exposed adult female rats. Pharmacol Biochem Behav 67, (2000) 501– 505.

Stoffel-Wagner, B., Neurosteroid biosynthesis in the human brain and its clinical implications, Ann N Y Acad Sci. 1007, (2003) 64-78.

Temkin, N.R., Davis, G.R., Stress as a risk factor for seizures among adults with epilepsy. Epilepsia 25, (1984) 450-456.

van Luijtelaar, E.L.J.M., Coenen, A.M., Two types of electrocortical paroxysms in an inbred strain of rats, Neurosci Lett. 70, (1986) 393-397.

van Luijtelaar, E.L.J.M., Coenen, A.M.L., The WAG/Rij model for generalized absence seizures. In: J. Manelis et al (Ed.), Advances in Epileptology, Vol. 17, Raven Press, 1989, 78-83.

van Luijtelaar, G., Budziszewska, B., Jaworska-Feil, L., Ellis, J., Coenen, A., Lason, W., The ovarian hormones and absence epilepsy: a long term EEG study and pharmacological effects in a genetic absence epilepsy model, Epilepsy Res. 46 (2001) 225-239.

van Luijtelaar, G., Budziszewska, B., Tetich, M., Lason, W., Finasteride inhibits the progesterone-induced spike-wave discharges in a genetic model of absence epilepsy, Pharmacol Biochem Behav. 75 (2003) 889-894.

van Luijtelaar, G., Sitnikova, E., Global and focal aspects of absence epilepsy: The contribution of genetic models, Neurosci Biobehav Rev. 30, (2006) 983-1003.

Westmijse, I., Ossenblok, P., Gunning, B., van Luijtelaar, G., The cortical focus theory in human absence epilepsy, Neuroimage 36, 1, (2007) 204.

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Chapter 2

Chapter 2

Absence seizures during pregnancy in WAG/Rij rats

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Abstract. Spontaneously occurring spike-wave discharges and serum concentrations of ovarian steroid hormones were investigated before, during and after pregnancy in WAG/Rij rats, a rat strain with genetically determined absence seizures. Eight groups of rats were included in the assays of progesterone and estradiol: rats at diestrus, at various days of pregnancy and at lactating days. The number of spike-wave discharges in cortical EEG of W rats was decreased from 3rd up to 18th days of pregnancy and subsequently increased to control level. Thereafter a new decrease was found 2-3 days after parturition. Serum concentration of progesterone was 3-fold increased at the 3rd day of pregnancy, remained elevated till the 18th day of pregnancy and returned to control values before delivery. Over measured days estradiol was significantly elevated only at the 18th day of pregnancy. Results demonstrated that physiological conditions induced by the state of pregnancy lead to suppression of occurrence of SWDs. Changes in plasma progesterone concentration correspond to the changes in number of spike-wave discharges: an increased level of progesterone during pregnancy is accompanied by a decreased number of spike-wave discharges, while a decrease in circulating progesterone before parturition is paralleled by an increase of SWDs. Of interest, the relationship between spike-wave discharges and concentration of progesterone found during pregnancy is diametrically opposite to results obtained in acute administration studies of progesterone in non-pregnant animals.

Key words: pregnancy, progesterone, estradiol, absence seizures, WAG/Rij rats.

1. Introduction

Bilateral synchronized spike-wave discharges (SWDs) represent a hallmark of a specific form of idiopathic non-convulsive types of epilepsy, such as absence epilepsy. Spontaneously occurring spike-wave activity mediated in a cortico-thalamo-cortical network is abundantly present in the cortical electroencephalogram of several strains of rats such as WAG/Rij and GAERS. WAG/Rij strain of rats is considered as a well-validated genetic model [1,2], which offers among others a unique possibility to research occurrence of absence seizures in variety of physiological conditions. Fluctuations in plasma and brain concentrations of steroid hormones and their neuroactive metabolites over different stages of reproductive cycle might play an important role in modulation of neuronal

excitability [3,4,5] and therefore result in alterations in emotional state, sleep pattern and seizure threshold.

Several studies indicate a possible physiological role of ovarian steroid hormones in absence epilepsy [6,7,8]. In a single case human study absences were aggravated after administration of progesterone [6]. In animal model systemic acute administration of progesterone, but not estradiol increased the number and total duration of SWDs in WAG/Rij rats and injection of an antagonist of the intracellular progesterone receptors RU 38486 had no effect on SWDs and did not block the stimulatory effect of progesterone [7]. The latter data suggest that the effects of progesterone were likely due to membrane transmitter receptors. Investigation of spontaneously occurring SWDs during the estrous cycle, again in WAG/Rij rats, has revealed an increase in the number of SWDs at proestrus day at the time of day in which the level of progesterone is enhanced [8]. These data suggest not only a clear role of progesterone in generalised absence epilepsy but they also demonstrate that in contrast to what has been found in convulsive types of epilepsy [9,10], progesterone may exacerbate epileptic activity of the SWD type. The precise mechanisms of this involvement are still unclear. Earlier it was suggested that the increase of SWDs after acute administration of progesterone in WAG/Rij rats was due to the GABA-mimetic action of its active metabolite allopregnanolone [7]. This was recently confirmed in an experiment in which the increase induced by progesterone was antagonized by finasteride, a 5alpha-reductase inhibitor, which itself did not have an effect on SWDs [11]. These data suggested that some of the central effects of progesterone on SWDs are exerted through the GABA-ergic system. A major metabolite of progesterone, allopregnanolone, is an allosteric modulator of $GABA_A$ receptor which may facilitate GABA mediated response by an increase in both Cl^- channel opening time and frequency [12]. Several studies have indicated that long-term treatment with neuroactive steroids including genomic component may lead also to various changes in density, plasticity and sensitivity to modulation induced by benzodiazepines and neurosteroids itself [4,5,13,14,15]. All these changes are region specific and can be different for the low and high affinity binding sites [1,13,14,15].

Variety of changes which could be induced by genomic effects progesterone make it difficult to predict their ultimate impact on characteristics of thalamo-cortical circuits to generate SWDs. Pregnancy is a dominant physiological state, which is associated with striking changes in hormonal milieu and offer a unique model to study the chronic effects of

large (but physiological) changes in progesterone in an experimental way [16]. Present study was designed to investigate the possible functional relation between changes in plasma concentrations of progesterone and estradiol and level of occurrence of SWDs during pregnancy and after delivery.

2. Materials and methods

Adult female WAG/Rij rats with body weight of 190 to 210 g were used as experimental subjects. They were housed five per cage under artificial 12-h light, 12-h dark cycle (light on from 8.00 to 20.00) at a constant room temperature. All manipulations with animals were according to the locally approved codes of practice for the care and use of animals for scientific purposes.

Stage of estrous cycle (diestrus, proestrus, or estrus) was determined from daily vaginal smears taken between 9.00 and 12.00 for 2 weeks. Only rats exhibiting at least three regular cycles with duration of 4-5 days were included in the study. For the induction of pregnancy, females were caged with males on the evening of proestrus. Mating was verified by observation of spermatozoa in the vaginal smear taken next morning, which was designated as day 0 of pregnancy. Dams were housed individually from the third week of pregnancy onwards. The duration of pregnancy in WAG/Rij rats was found to be 21 days.

Eight groups of rats were included in progesterone and estradiol assay: diestrus rats, rats at day 3, 7, 15, 18, 20 of pregnancy and lactating rats, 1 and 3 days after delivery. Samples of blood from the v. jugularis were taken between 12.00-14.00 Sodium citrate (0.11 M) in proportion 1:9 was used as an anticoagulant. Blood was centrifuged at 1000g for 15 min, after which the plasma was frozen until assayed for steroid hormones. The concentration of estradiol and progesterone in all samples was simultaneously determined with ELISA kits to determine the concentrations (“Diagnostics”, USA).

For EEG study, 10 animals were provided, under Nembutal anesthesia (40 mg/kg i.p.), with a standard EEG-electrode set with coordinates: AP +2.0, L 3.0 for active, AP –6.0, L 4.0 –for the indifferent electrode and the earth electrode was placed in the cortex of the cerebellum. Following surgery, rats were allowed to recover for at least 1 week. All rats were first familiarized with the recording and then were adapted to experimental cage during the first hour before each recording. During this study, the cortical EEGs were recorded between 17.00 and 20.00 at two days before (this was

considered as base-line), during various days of pregnancy and at 3rd postpartum day. The dams were not separated from their pups during the EEG-session. The EEGs were amplified, filtered between 1 and 100 Hz, and stored for off-line analyses. The hourly number and total duration of SWDs per hour were counted according to standard criteria.

Although it is acknowledged that there is a delay 5-7 hours between EEG and blood samplings for steroid assays and that both variables show a circadian pattern, it can be remarked that there is a high positive correlation between the hourly number of SWDs during all hours of the day [17]. Moreover, unpublished data showed that differences between strains in concentrations of HPA-axis hormones were stable across a 12 hr period.

The analyses of variance (ANOVA)-statistic for independent (progesterone and estradiol plasma levels) and repeated (number of SWDs) measurements were used, if necessary followed by Newman-Keuls post-hoc comparisons between days, a significant level of $p<0.05$ was chosen for all tests.

3. Results

The data for the level of SWDs along over the course of pregnancy and after delivery are presented in Fig.1. Obtained results demonstrate considerable changes for the number of SWDs in WAG/Rij rats during pregnancy. The ANOVA for repeated measurements has revealed significant day effects for hourly number of SWDs ($F(8,40)= 55.75$, $p<0.000$). A significant constitutive suppression of SWDs generation was found from 7rd day onward until 18th day of pregnancy. However level of SWDs activity was progressively increasing up to base line values for 2-3 days before the delivery while was found to be decreased anew on 2-3 postpartum day.

Marked changes in circulating steroids concentration during pregnancy were found as presented in Table I. Significant day effects for progesterone ($F (7,20) = 164.29$, $p<0.000$) were found. The serum concentration of progesterone was significantly increased from the 3rd day onwards and remained elevated till the 18th day of pregnancy compared with Diestrus day.

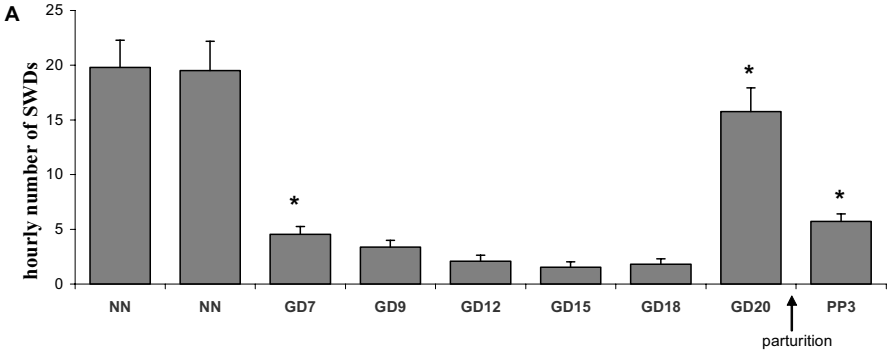


Figure 1. The number (mean and s.e.m.) of spike-wave discharges (SWDs) per hour before, during and after pregnancy in WAG/Rij rats. NN –base line days, GD-gestation day, PP-postpartum. * - significant ($p<0.05$) increase/decrease compared to previous day.

A progressive and significant increase in serum progesterone level was found on 7th and 18th day of pregnancy compared to previous measured days (3rd and 15th days correspondingly). Thereafter and immediately before delivery, on 20th day, plasma concentration of progesterone returned to control values, remained unchanged on 1 day after delivery, and then it was found to increase again on 3rd postpartum day. The time-course of changes in the occurrence of SWDs during and after pregnancy and circulating progesterone concentration were in parallel over this period of time. This may suggest a functional relationship between SWDs and progesterone. Also a day effect for estradiol was found ($F(7,20) = 4.02$, $p<0.01$).

Table I. Plasma progesterone and estradiol concentrations (mean and s.e.m.) on Diestrus day, 3rd, 7rd, 15th, 18th, 20th day during pregnancy and on 1st and 3rd postpartum (PP) days. E- Estradiol, P-Progesterone.

	Diestrus1 n=6	**3 day** n=3	**7 day** n=5	**15 day** n=3	**18 day** n=3	**20 day** n=3	**1 PP day** n=3	**3 PP day** n=4
E nm/l	46 ± 7	50 ± 8	54 ± 10	59 ± 2	76 ± 9	41 ± 13	49 ± 15	31 ± 25
P nm/l	42 ± 14	139 ± 3	166 ± 4	159 ± 7	180 ± 5	46 ± 15	36 ± 6	83 ± 2

There were no considerable changes in plasma estradiol concentration during pregnancy with a single exception. Only at day 18 of pregnancy a significant elevation in estradiol concentration compared to diestrus day and all other measured days of pregnancy and postpartum days was found.

4. Discussion

This study was aimed to establish the level of SWDs occurrence during pregnancy and after parturition and to investigate a possible functional relation between changes in spike-wave activity and changes in endogenous plasma steroids concentrations over these periods. Obtained results clearly demonstrate that the level of occurrence of SWDs is constitutively decreased in WAG/Rij rats during pregnancy. Under normal conditions the daily number of SWDs in 6 months old rats is more or less stable. This is the first report demonstrating a marked decrease in basal levels in occurrence of SWDs in a special physiological condition such as pregnancy. As expected, major changes during pregnancy including a rapid increase after onset of pregnancy, a maximum at day 18, a rapid decrease and a smaller postpartum increase were found for serum progesterone level that were consistent with those observed in the literature [4,18,19]. Plasma estradiol level was significantly enhanced only at day 18^{th} of pregnancy, its increase at that specific time was also mentioned by Weizman et al [18] while strain related differences in the time-course of estradiol plasma level during pregnancy were found in the last few days before parturition [19]. The increased level of progesterone, starting at day 3 up to day 18 of pregnancy, was accompanied by a decrease in the number of spontaneously occurring SWDs. The sudden decrease in the concentration of progesterone at three days before parturition was also accompanied by a change in SWDs, but now by an increase. Based on the data obtained in the present experiment it can be assumed that during pregnancy in WAG/Rij rats an increase in concentration of progesterone is paralleled by a decrease in number of SWDs and a decrease in concentration by an increase in SWDs. Therefore it seems the relationship between progesterone and SWDs during pregnancy is opposite to what has been found in a few acute dose-effect studies of progesterone in male and female WAG/Rij rats and during the estrus cycle in female WAG/Rij rats [7,8,11.

In contrast to the short-term effect in the acute dose-response studies, the long-term steroids effects that are studied here involve delayed genomic effects mediated by citosolic receptors, which are wide-spread in the brain, mainly in cortex and structures of the limbic system [20,21]. These citosolic receptors are involved in the regulation of GABA-ergic system functioning. In fact, constitutive changes in plasma and brain concentrations of progesterone and its active metabolites during pregnancy were found to be in association with changes in density, sensitivity and plasticity of

$GABA_A$ receptor complex [4, 18, 22]. Another mechanism for regulation of $GABA_A$ receptor sensitivity to modulation is based on balance between endogenous phosphate and protein kinase C activity [23]. The shift of this balance induced by oxytocin before parturition led to insensitivity of $GABA_A$ receptors to allopregnanolone in supraoptic nucleus [23]. On the other hand, an up regulation of $GABA_A$ receptors in cortical neurons and a down regulation of these receptors in the ventral lateral thalamus was found after repeated administration of progesterone in the female hamster [15]. These areas form the key-players in thalamo-cortical oscillations of the SWD type [25, 26]. Considering the diminished GABA-ergic intracortical inhibitory mechanisms in WAG/Rij rats in normal condition [27], a positive modulation of GABA receptors in cortical neurons could prevent the hyperexcitability of the cortex, which is postulated as a major factor controlling the occurrence of SWD [28, 29]. It is therefore assumed that increased functioning of GABA in the cortex and simultaneously down regulation of $GABA_A$ receptors at the lateral basal complex of the thalamus might cause opposite effects during pregnancy as under normal circumstances when a positive relation between progesterone and SWD activity was found.

In addition, neuroactive steroid hormones are able to modulate not only the GABA-ergic system but also the glutamatergic (NMDA), cholinergic and opioid system [30], which were shown to be involved in absence epilepsy [31, 32, 33] and therefore these influences can not be ruled out to play a role. Furthermore, pregnancy and lactation are physiological conditions associated with striking alterations in the hormone milieu and metabolism, also largely varying between different strains of rats [19]. It may be that various hormones have indeed direct or indirect effects on pathogenesis of absence epilepsy. To our knowledge, for only a few hormones, such as prolactin, estradiol and hypothalamo-pituitary-adrenal-(HPA-) axis related hormones, effects on absence seizures were actually examined. Under physiological conditions and after systemic administration no effects of estradiol or its antagonist on SWDs were established in WAG/Rij rats [7, 8]. The effects of prolactin on absence seizures were not previously described, known is however that prolactin-releasing peptide suppressed absence seizures in GAERS [34]. However, since prolactin plasma level is substantially decreased during pregnancy [19], it is not likely that its effects contribute extensively to the decrease of occurrence of SWDs during pregnancy, although its contribution cannot fully be excluded. Fluctuations in plasma corticosterone levels and other HPA-axis hormones as found during pregnancy [19] might also be implicated in the regulation of the occurrence of SWDs since concentrations of ACTH and corticosterone

were found to be correlated with number of SWDs in normal circumstances [35]. Finally, effects of HPA-axis related hormones on SWDs were established in a genetic mouse model of absence epilepsy [36].

It can be concluded that a constitutive decrease in the number of SWDs can be found during the state of pregnancy. Therefore it seems that during pregnancy the fetus of genetically epileptic rats is protected. During pregnancy levels of progesterone and other hormones are chronically and seriously changed. A different relation between progesterone and SWDs was established here than in three earlier studies. It is thought that opposite effects on GABA-receptors in cortex and thalamus, which may emerge only after repeated injections and not after acute ones, might explain the negative relation between levels of progesterone and SWDs. Whether this is sufficient reason for opposite effects on SWD presence between acute injection and chronic endogenous elevation in progesterone level during pregnancy is not immediately clear.

References

1. van Luijtelaar, E.L.; Coenen, A.M. Two types of electrocortical paroxysms in an inbred strain of rats. Neurosci. Lett. 70: 393-397, 1986.
2. Coenen, A.M.; van Luijtelaar, E.L. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. Behav Genet. 33: 635-655, 2003.
3. Majewska, M.D.; Harrison, N.L.; Schwartz, R.D.; Barker, J.L.; Paul, S.M. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232: 1004-1007, 1986.
4. Concas, A.; Follesa, P.; Barbaccia, M.L.; Purdy, R.H.; Biggio, G. Physiological modulation of $GABA_A$ receptor plasticity by progesterone metabolites. Eur. J. Pharmacol. 30: 225-235, 1999.
5. Biggio, G.; Barbaccia, M.L.; Follesa, P.; Serra, M.; Purdy, R.H.; Concas, A. Neurosteroids and $GABA_A$ Receptor Plasticity. In: Martin, D.; Olsen, R.W.; eds. GABA in the Nervous System. Lippincott-Williams and Wilkins, Philadelphia, 1999.
6. Grunewald, R.A.; Aliberti, V.; Panayiotopoulos, C.P. Exacerbation of typical absence seizures by progesterone. Seizure 1: 137-138, 1992.
7. Budziszewska, B.; Van Luijtelaar, G.; Coenen, A.; Leskiewicz.; M.; Lason, W. Effects of neurosteroids on spike-wave discharges in the genetic epileptic WAG/Rij rat. Epilepsy Res. 33: 23-29, 1999.
8. van Luijtelaar, G.; Budziszewska, B.; Jaworska-Feil, L.; Ellis J.; Coenen, A.; Lason, W. The ovarian hormones and absence epilepsy: a long term EEG study and pharmacological effects in a genetic absence epilepsy model. Epilepsy Res. 46: 225-239, 2001.

9. Frye, C.A.; Scalise, T.J. Anti-seizure effects of progesterone and 3alpha,5alpha-THP in kainic acid and perforant pathway models of epilepsy. Psychoneuroendocrinol 25: 407-420, 2000.
10. Kokate, T.G.; Svensson, B.E.; Rogawski, M.A. Anticonvulsant activity of neurosteroids: correlation with gamma-aminobutyric acid-evoked chloride current potentiation. J. Pharmacol. Exp. Ther. 270:1223-1229, 1994.
11. van Luijtelaar, G.; Budziszewska, B.; Tetich, M.; Lason, W. Finasteride inhibits the progesterone-induced spike-wave discharges in a genetic model of absence epilepsy. Pharmacol Biochem Behav. 75: 889-894, 2003.
12. Martin, I.L. and Dunn, S.M.J. GABA receptors. Review written on request by Tocris, www.tocris.com.
13. Yu, R.; Ticku, M.J. Chronic neurosteroid treatment produces functional heterologous uncoupling at the γ-aminibutyric acid type A benzodiazepine receptor complex in mammalian cortical neurons. Molecular Pharmacol. 47: 603-610, 1995.
14. Gavish, M.; Weizman, A.; Moussa, B.H.; Okun, Y.; Okun, F. Regulation of central and peripheral benzodiazepine receptors in progesterone-treated rats. Brain Res. 409: 386-390, 1987.
15. Canonaco, M.; O'connor, L.H.; Pfaff, D.W.; McEwen, B.S. Longer term progesterone treatment induces changes of $GABA_A$ receptor levels in forebrain sites in the female hamster: quantative autoradiography study. Experimental Brain Research 77: 407-411, 1989.
16. Chepurnov, S.A.; Chepurnova, N.E.; Tolmacheva, E.A.; Kochetkov, I.A.; van Luijtelaar, E.L.; Coenen, A.M.; Pregnancy in WAG/Rij rats-changes in the levels of progesterone, estradiol and generalized absence epilepsy. Fiziol. Zh. Im. I. M. Sechenova 88: 741-750, 2002.
17. Van Luijtelaar, E.L.; Coenen, A.M. Circadian rhythmicity in absence epilepsy in rats. Epilepsy Res. 2: 331-336,1988.
18. Weizman, R.; Dagan, E.; Snyder, S.H.; Gavish, M. Impact of pregnancy and lactation on $GABA_A$ receptor and central-type and peripheral-type benzodiazepine receptors. Brain Res. 752: 307-314, 1997.
19. Garland, H.O.; Atherton, J.C.; Baylis, C.; Morgan, M.R.; Milne, C.M.J. Hormone profiles for progesterone, oestradiol, prolactin, plasma renin activity, aldosterone and corticosterone during pregnancy and pseudopregnancy in two strains of rat: correlation with renal studies. Endocrinol. 113: 435-444, 1987.
20. McEwen, B.S.; Davis, P.; Gerlach, J. Progestin receptors in the brain and pituitary gland. In: C.W. Bardin, J. Mauvais-Jarvis, E. Mil-Grom. Progesterone and progestins. NewYork: Raven Press, 1983: pp. 59-76.
21. Pfaff, D.W.; Keiner, M.; Estradiol-concentrating cells in the rat amygdala as part of a limbic-hypothalamic hormone-sensitive system. In: The neurophysiology of the amygdala. (Ed: B.E. Elefheriou). New York, Plenum Publishing, 1973: pp.775-792.
22. Follesa, P.; Floris, S.; Tuligi, G.; Mostallino, M.C.; Concas, A.; Biggio, G. Molecular and functional adaptation of the GABA(A) receptor complex during pregnancy and after delivery in the rat brain. Eur. J. Neurosci. 10: 2905-2929, 1998.
23. Koksma, J.J.; van Kesteren, R.E.; Rosahl, T.W.; Zwart, R.; Smit, A.B.; Luddens, H.; Brussaard, A.B. Oxytocin regulates neurosteroid modulation of GABA(A) receptors in supraoptic nucleus around parturition. J Neurosci. 23: 788-797, 2003.
24. Brussaard, A.B.; Koksma, J.J. Short-term modulation of $GABA_A$ receptor function in the adult female rat. Progress in Brain Res. 139: 31-42, 2002.

25. Avanzini, G.; de Curtis, M.; Marescaux, C.; Panzica, F.; Spreafico, R.; Vergnes, M.J. Role of the thalamic reticular nucleus in the generation of rhythmic thalamo-cortical activities subserving spike and waves. Neural Transm Suppl. 35: 85-95, 1992.
26. Meeren, H.K.; Pijn, J.P.; Van Luijtelaar,E.L.; Coenen, A.M.; Lopes da Silva, F.H. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. J. Neurosci. 22: 1480-1495, 2002.
27. Luhmann, H.N.; Mittmann, T.; van Luijtelaar, G.; Heinemann, U. Impairment of intracortical GABA-ergic inhibition in a rat model of absence epilepsy. Epilepsy Res. 22: 43-51, 1995.
28. Gloor, P.; Pellegrini,A.; Kostopoulos, G.K. Effects of changes in cortical excitability upon the epileptic bursts in generalized penicillin epilepsy of the cat. Electroencephalogr. Clin. Neurophysiol. 46: 274-289, 1979.
29. Kostopoulos, G. Spike and wave discharges of absence seizures as a transformation of sleep spindles: the continuing development of a hypothesis. Clin. Neurophysiol. 111(S2): 27-38, 2000.
30. Melcangi, R.C.; Panzica, C. Steroids in the nervous system: Pandora's box? Trends Neurosci. 24: 311-312, 2001.
31. Coenen, A.M.L.; Drinkenburg, W.H.I.M.; Inoue, M.; van Luijtelaar, E.L.J.M. Genetic models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. Epilepsy Res. 12: 75-86, 1992.
32. Lason, W.; Przewlocka, B.; Coenen, A.; Przewlocki, R.; van Luijtelaar, G. Effects of mu and delta opioid receptor agonists and antagonists on absence epilepsy in WAG/Rij rats. Neuropharmacol. 33:161-166, 1994.
33. Berdiev, R.K.; Chepurnov, S.A.; Chepurnova, N.E.;. van Luijtelaar, E.L.J.M, Coenen, A.M.L. Effects of neuropeptide galanin on spike-wave discharges in WAG/Rij rats. In: Kuznetsova, G.D.; Coenen, A.M.L.; Chepurnov S.A.; van Luijtelaar, E.L.J.M.; eds. The WAG/Rij model of absence epilepsy: the Nijmegen-Moscow research. Nijmegen: Nijmegen University Press, 2000: pp 71-78,
34. Lin, S.H.; Arai, A.C.; Espana, R.A.; Berridge, C.W.; Leslie, F.M.; Huguenard, J.R.; Vergnes, M.; Civelli, O. Prolactin-releasing peptide (PrRP) promotes awakening and suppresses absence seizures. Neuroscience 114: 229-238, 2002.
35. Tolmacheva, E.A.; Oitzl, M. S.; Chepurnov, S.A.; van Luijtelaar, E.L.J.M. Activity of the hypothalamus-pituitary-adrenal axis in female absence epileptic rats. Epilepsia 44, S8:107, 2003.
36. Capasso, A.; Sorrentino, L.; Di Giannuario, A.; Palazzesi, S.; Pieretti, S.; Loizzo, A. Dexamethasone and hormones related to the hypothalamic-pituitary-adrenal axis modulate inherited neocortical spindling in DBA/2J mice. Neuropsychobiology 29: 143-151,1994.

Chapter 3

Ovarian steroid hormones in the regulation of basal and stress induced absence seizures

Elena A. Tolmacheva and Gilles van Luijtelaar

Abstract. Ovarian hormones play an important role in the regulation of absence seizures in humans as well as in animal models. The present study examined whether chronic progesterone exposure induces tolerance and whether reductions in gonadal steroids (via ovariectomy) alters basal and stress induced absence seizures in a genetic model for absence epilepsy. In Experiment 1, female WAG/Rij rats equipped with EEG electrodes received progesterone (P) (20 mg/kg) or cyclodextrin (CD, solvent) i.p. injections once a day for three days while a third group received CD injections on days one and two and P on day three. The EEG was recorded on the day preceding the injections and at each day after injections. In Experiment 2, female WAG/Rij rats equipped with EEG electrodes, were ovariectomized (OVX) or sham operated. EEG recordings were made before and at the 4th, 8th, 10th, 20th, and 35th day after surgery. Rats were then exposed to three series of 10 foot-shocks (FS, 1.5 mA, 1 s). The EEG was recorded 1h before and 2h after each FS series.
Tolerance developed after a single P injection and the effect of P on SWDs was facilitated by two preceding control injections. No differences were found between OVX and sham operated females in the occurrence of SWDs either in resting conditions or after acute FS exposure. However, OVX females showed a more prominent day-to-day aggravation in SWDs after repeated FS administration. The data suggest an important interaction between hormones of the hypothalamo-pituitary-adrenal and hypothalamo-pituitary-gonadal axes in seizure control. On the one hand, stress interferes with and facilitates the acute effects of progesterone on the occurrence of SWDs and, on the other hand, rats with an intact hypothalamo-pituitary-gonadal axis can better regulate the stress response and develop tolerance to the stressor.

Key words: absence seizures, WAG/Rij, progesterone, ovariectomy, repeated stress, anticipation.

1. Introduction

Absence epilepsy is a generalized non-convulsive form of epilepsy, which is characterized by spontaneously occurring bursts of bilateral synchronous spike-wave activity accompanied by a decrease of consciousness. Although mechanisms underlying the generation of absence seizures are extensively described and represented in different models [1],

the pathogenesis of this disorder including the impact of endocrine factors is far less understood.

Endogenous fluctuations in plasma and brain concentrations of ovarian steroid hormones and their neuroactive metabolites across stages of the reproductive cycle are thought to play an important role in modulation of neuronal excitability and may result in alterations in emotional state, sleep patterns, and seizure threshold [2, 3]. It has been demonstrated that acute administration of progesterone aggravates the number of absence seizures both in humans [4] and in rats [5, 6, 7] and that this is a non-genomic effect mediated by the neuroactive derivative allopregnanolone known to facilitate $GABA_A$ receptor inhibitory function [6]. No changes in SWDs were found after acute administration of estrogen [5]. However, in contrast, we found that chronic elevation of progesterone during pregnancy was accompanied by a decrease in the number of SWDs in WAG/Rij rats [8]. Rats of the WAG/Rij strain are considered to be one of the well-validated models of absence epilepsy and the seizures originate in the cortico-thalamo-cortical network [10, 11]. It is suggested that a number of adaptive changes in the system, such as tolerance, as well as possible genomic effects of ovarian steroids, which can take place during pregnancy, may account for this contradiction and explain the decrease in the occurrence of absence seizures [8].

The present study was aimed to examine this hypothesis and further investigate the role of the endogenous steroid hormonal milieu in the pathogenesis of absence seizures. Therefore, we first questioned (Experiment 1) whether repeated progesterone exposure could induce tolerance. Next (Experiment 2a), we tested whether chronic diminution of gonadal steroids by ovariectomy would alter the occurrence of SWDs in basal conditions, as during pregnancy.

It is also known that ovarian hormones are involved in the modulation of the activity of the hypothalamo-pituitary-adrenal (HPA) axis (a major system of the neuroendocrine stress response in vertebrate organisms) and alter stress reactivity in adult female rats [12, 13, 14]. The increased level of progesterone and its neuroactive derivatives following acute stress [14, 15] implies their direct involvement in neuronal adaptations and is thought to counteract neuronal excitation elicited by stressful stimuli [15, 16]. A mutual effect between stress (or HPA axis functioning) and convulsive seizures is well-known. Consistently, we have recently demonstrated that both stress and anticipation of stressful stimuli aggravate

the incidence of absence seizures in WAG/Rij rats [17]. Based upon these data, we also investigated (Experiment 2b) whether chronic diminution of gonadal steroids reduces adaptation to stress in OVX females by enhancing the number of SWDs after repeated foot shock stress.

2. Materials and methods

2.1. Animals and housing

The present study was performed with female WAG/Rij rats, 4-5 months of age, obtained from the breeding colony at the Department of Biological Psychology, Radboud University Nijmegen. All rats were group-housed prior to surgery and individually following surgery in a temperature-controlled room (21±1°C), on a 12/12-h reversed light cycle (lights off at 8 a.m.). Food and water were available *ad libitum*. Rats were handled for one week prior to the experiment and placed in the recording cages one day before the first recording session to habituate to the experimental conditions. All manipulations were approved by the Institutional Ethical Committee of the Radboud University of Nijmegen.

2.2. Surgery

Surgery to implant a standard tripolar EEG-electrode set (MS333/1-A, Plastic One, Roanoke, VI, USA) was performed under isoflurane inhalation anesthesia. Electrodes were placed using the following coordinates: AP = + 2.0, L = + 3.0 and AP = -6.0, L = + 4.0 as active electrodes, the ground electrode was placed in the cortex of the cerebellum. The assembly of the three electrodes was attached to the skull surface using dental cement and jewelers screws. Rats were allowed to recover for at least 2 weeks following surgery.

2.3. Ovariectomy (OVX)

OVX or sham operation was performed under isoflurane inhalation anesthesia, two weeks after the implantation of the EEG electrodes. The lower back was shaved at the midline and a single rostral– caudal incision was made. Fascia was separated from the skin to expose the lateral peritoneum above the ovary on one side. Next, a small incision of the

peritoneum was made and the ovary was then cut away from the uterus and the uterus was settled back into the abdominal cavity. The peritoneal incision was sutured, and the entire procedure was repeated on the other side to remove the second ovary. The midline skin incision on the back was closed with wound clips and animals were placed in their home cage under a heat lamp until recovery a few hours later. In the sham operated group the operation procedure was repeated excluding the removal of the ovaries.

2.4. Drugs

Progesterone (Sigma) (20 mg/ml) was dissolved in 20% 2-hydroxypropyl-γ-cyclodextrin (CD) immediately prior to administration. CD is most often used as solvent for steroid hormones such as progesterone. Drugs were i.p. injected in a volume 1 ml/kg.

2.5. Foot-shock (FS) administration

Rats were individually placed in a Perspex box (25x25x40), which has an electrified grid on the floor, through which shock could be delivered. Scrambled electrical shocks (1.5 mA, 1 sec) were administered with random (range 1 - 10 sec) inter-shock intervals.

2.6. Experimental design

Experiment 1. Rats were randomly assigned to one of three groups (see also Table 1). The first and second groups (Groups P, n=11 and CD (n=9) were administered progesterone (20 mg/kg) or CD, respectively, once a day for three days. The third group (Group CD-P, n=7) received CD on Day 1 and 2 and progesterone on Day 3. The experimental deign is also presented in Table I. The EEG was recorded on Day 0 (base-line level) and than on Day 1-3 immediately after injections, for 2 hours between 12.00 and 14.00.

Experiment 2a. EEG recordings were conducted one day before and at the 4th, 6th, 8th, 10th, 12th, 20th, 23rd, and 35th day after surgery in both OVX (n=7) and sham-operated (n=7) females. The recordings lasted four hours and were carried out between 10.00 and 14.00 in the home cages.

Experiment 2b. Next, the same rats (OVX and sham operated) were placed individually into Perspex boxes each experimental day and returned after the experimental procedure to their home cages. Animals were habituated to the boxes on the first day. Three hour base-line EEG recordings were made on the second day. On the next three days, rats were placed into the Perspex boxes again and after one hour they received a series of 10 FS; the EEG was recorded 1h before and 2h after the FS. On the 6th day only EEG recordings were made in the Perspex boxes. All experiments were carried out between the 3rd and 6th hour after light offset (between 11.00 and 14.00).

2.7. EEG analysis

The EEG's were amplified and filtered between 1 and 100 Hz, digitized at 200 Hz and stored for off-line analyses. The EEG data were processed by a program, which searched in the EEG for the presence of high-voltage activity with a minimal duration of 1 s. The selected periods of aberrant EEG activity were visually inspected on the basis of published criteria, whether these periods contained SWDs [9]. Both duration and number of SWDs were analyzed in 15 or 60 min (Exp 2a).

2.8. Statistical Analysis

In Experiment 1, a two-way ANOVA (with day as a within-subject factor and groups as a between-subject factor) followed by *post-hoc* one-way ANOVAs and pair wise comparisons were used to analyze the hourly number of SWDs.

In Experiment 2a, a two factor ANOVA with operation as a between-subjects factor and day as a within-subjects factor followed by orthogonal trend analysis and a *post-hoc* comparison were used to examine changes in SWDs (number and mean duration) that occurred over time before and after operation in OVX and sham-operated animals.

In Experiment 2b, a three factor ANOVA for repeated measures with OVX as a between-subjects factor and time (four 15 min episodes) and day as within-subjects factors (five days for pre-FS data, 4 days for post-FS data) followed by orthogonal trend analysis and *post-hoc* pair-wise comparisons were used to test the effects of repeated FS on SWDs in OVX and sham-operated rats. The SWDs were separately analyzed in the base line (pooled data) and in the first and the second hour after FS. The level of statistical significance was set at 5 % (two tailed tests) for all variables.

3. Results

3.1. Experiment 1: the effects of repeated progesterone injections.

As depicted in Figure 1, there was a significant main effect of day (F_{day}=18.81, df=3,72, p<0.001) and a significant interaction between day and group ($F_{day*group}$=6.39, df=6,72, p<0.001) in the hourly number of SWDs. This interaction was further analysed with a one-way ANOVA (with group as the between-subject factor) for each day separately. There were no differences between groups on the base-line day. However, there was a significant main effect of group on the number of SWDs in the first hour post injection of Day 1 (F_{group}= 3.27, df=2,26, p=0.05) and Day 3 (F_{group}=4.50, df=2,26, p<0.01). Group P had more SWDs than Group CD on Day 1 and Group CD-P had more SWDs than both groups P and CD on Day 3. No difference between the groups was found on Day 2.

The fluctuations between days on the hourly number of SWDs were subsequently analysed. The ANOVA with day as a within-subject factor for each group of rats separately showed a significant main effect of day for Group P (F_{day}=9.78, df=3,30, p<0.001); the number of SWDs was higher after the first (Day 1) and the third progesterone injection (Day 3) than on the base-line day (Day 0). The number of SWDs after the second injection of progesterone (Day 2) was significantly lower than after the first injection (Day 1) (t=2.9, df 10, p<0.05), no significant difference was found between the effects of the second and the third injections. This fact, as well as the lack of difference between the groups on this day, may indicate the rapid onset of tolerance to progesterone after the first injection.

A significant effect of day was also found in Group CD-P (F_{day} =11.06, df=3,18, p<0.001): there were more SWDs after progesterone on Day 3 than after CD on Day 0, 1 and 2. There were no day effects on SWDs in rats of Group CD, supporting that tolerance had developed to progesterone and not to the injection procedure per se. A comparison between the effects of the first progesterone injections (Group CD-P and Group P) showed that progesterone had larger effects when preceded by two control injections of CD, than when it was injected to naïve animals (t=2.05, df 16, p<0.05) (Figure 1).

The ANOVA for the number of SWDs during the second hour after injection revealed a significant effect of day (F_{day}= 4.56, df=3,72, p<0.01). The number of SWDs was larger on days 1 and 3 than on days 0 and 2.

Table 1. Experimental design (*Experiment 1)*: the first group was given three injections of progesterone (P), the second group - three injections of cyclodextrin (CD), and the third group - two injections of CD followed by an injection of P. The dose of P was 20 mg/kg, all injections were i.p.

Group P (n=11)	Base-line	Prog	Prog	Prog
Group CD-P (n=7)	Base-line	CD	CD	Prog
Group CD (n=9)	Base-line	CD	CD	CD

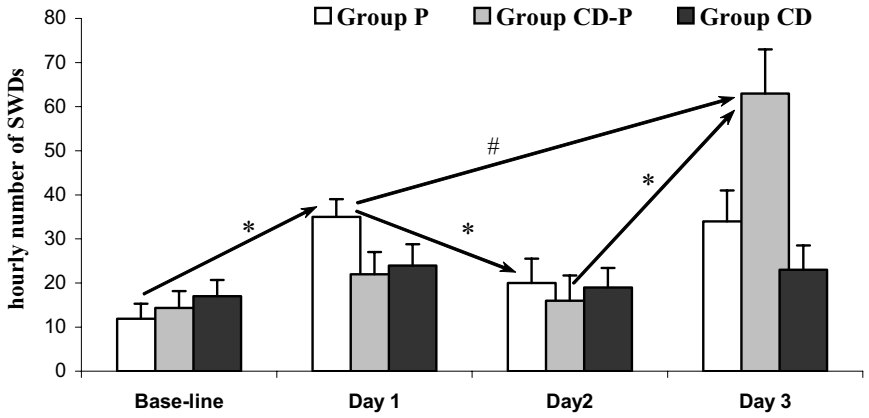


Figure 1. The number of SWDs (mean±S.E.M.) in the base-line and experimental days (Day1–Day3) in the three groups during the first hour after injection of progesteron (P) and cyclodextrin (CD). Asterisk (*) shows significant difference between days ($p < 0.05$) in Group P and CD-P according to *post hoc* test; symbol (#) shows significant difference in the effect of P in naive rats *vs.* CD pretreated rats ($p < 0.05$) according to *post hoc t*-test.

3.2. Experiment 2a: the effects of ovariectomy on basal SWDs.

The two-way ANOVA revealed only a significant main effect of day (F_{day}=5.55, df=8,80, p<0.01) for the duration of SWDs. Orthogonal trend analysis showed significant linear (F_{lin}=11.64,df=1.10, p<0.01) and cubic (F_{cub}=5.68, df=1,10, p<0.05) trends. They described the time course of changes in the mean duration of SWDs: the operation caused a slight

increase followed by a decrease and again a slow increase. No significant effects were found for the number of SWDs.

3.3. Experiment 2b: the effects of ovariectomy on stress-induced level of SWDs.

SWDs in the base-line preceding FS exposure

The ANOVA revealed a significant main effect of day (F_{day}=15.13 df=4,32, p<0.001) on number of SWDs in the base-line period (before FS administration). The day effect was characterized by a significant linear trend (F_{lin}=62.77, df=1,8 , p<0.001) characterizing an increase in SWDs in the base-line preceding the exposure to stress from day to day. This increase was more prominent in the OVX rats than in the sham-operated rats as suggested by a significant interaction between day and operation (F_{lin}= 13.61, df=1,8, p<0.01). In both groups the increase was characterized by a significant linear orthogonal trend (OVX: F_{lin}=127.95, df=1,6, p<0.000; sham: F_{lin}=18.63, df=1,6, p<0.01), *post-hoc* comparisons revealed an increase in the number of SWDs in the base-line hour over the four experimental days: Day 2 > Day 1 and Day 4 > Day 3 for the OVX rats and no significant increase from day to day in the sham operated rats. However, sham operated rats had a significantly larger number of SWDs on Day 3 and 4 compared to Day 0 (base-line). The difference between the OVX and sham group was significant at Days 3 and 4 (Figure 2).

SWDs in the first hour after FS

As shown in Figure 3, there was a main effect of day (F_{day}=10.55, df=3,36, p<0.001) for the number of SWDs. Changes over days were characterized by significant linear and quadratic orthogonal trends (F_{lin}= 13.72, df=1,12, p< 0.01; F_{quad}= 7.02, df=1,12, p< 0.05), suggesting that there was the significant increase in the number of SWDs discuss. There was also a significant effect of time (F_{time}=44.23, df=3.36, p<0.001) characterized by a significant linear trend (F_{lin}= 85.74, df=1,12, p<0.001) indicating a linear increase in the number of SWDs over the four 15 min episodes. There was also a significant effect of operation ($F_{operation}$=11.27, df=1,12, p<0.01): OVX rats had more SWDs than sham-operated animals. The significant interaction between day and time ($F_{day*time}$=9.50, df=9,108, p<0.001) was characterized by significant linear and quadratic orthogonal trends (F_{lin}=74.06, df=1,12, p<0.001; F_{quad}=11.19, df=1,12, p<0.01)

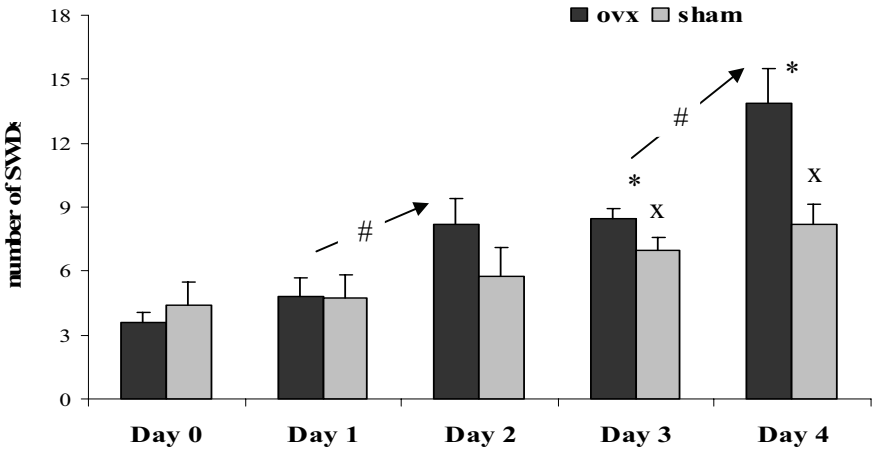
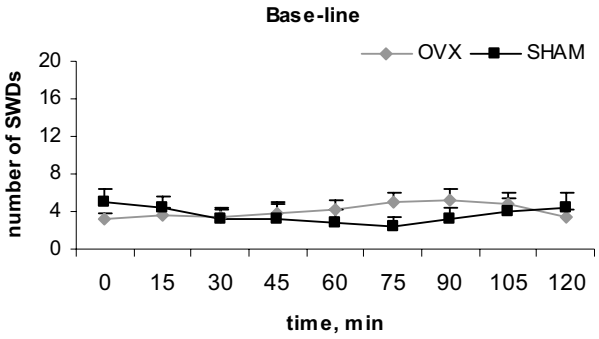


Figure 2. The hourly number (mean and S.E.M.) of SWDs in base-line (Day0) and before the first (Day 1), second (Day 2), third FS exposure (Day 3)and anticipated fourth (Day 4) FS exposure. Asterisk (*) shows significant difference between the groups ($p < 0.05$ according to *post hoc t*-test); symbol (#) shows significant increase from Day 1 to Day 2 in the group of OVX females ($p < 0.05$ according to *post hoc t*-test); symbol (X) shows significant difference compared to Days 0, 2, 3, and 4 ($p < 0.05$ according to *post hoc t*-test).

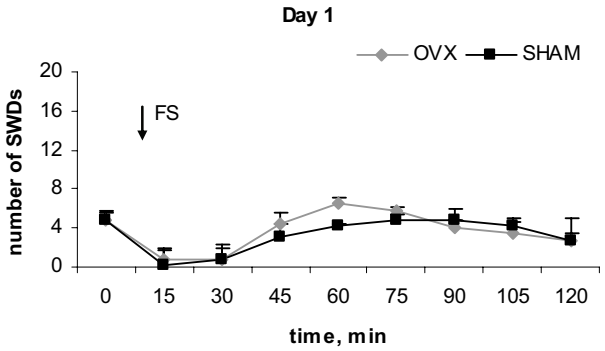
indicating that the trends over the four 15 min episodes were changing over the four experimental days. Initially, at the first day, when rats did not receive FS, the number of SWDs remained stable. The next day, when animals received the first series of FS, SWDs were suppressed during the first 30 min after FS administration and, thereafter, the number gradually increased. After the second and third FS series on the two following days respectively, SWDs were suppressed only for the first 15 min episodes and the subsequent aggravation started in the second 15 min episode and it was larger from day to day after each subsequent series of FS.

A significant interaction between time and operation ($F_{time*operation}$=3.54, df=3,36, p<0.05) with a linear orthogonal trend (F_{lin}= 6.34, df=1,12, p<0.05) and a significant linear trend in the day*time*operation interaction (F_{lin}=10.12, df=1,12, p<0.01) indicated that OVX animals had both a significantly larger aggravation in the number of SWDs after FS (except the first 15 min suppression in SWDs, which was equal for both sham and OVX animals); and a more prominent increase of this aggravation over days after each FS series compared to the sham-operated rats.

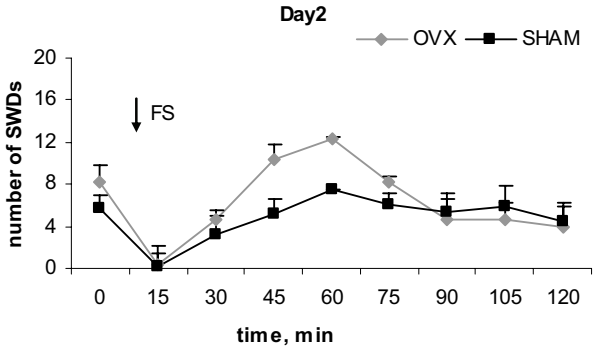
A


Base-line
OVX
SHAM
number of SWDs
0
4
8
12
16
20
0
15
30
45
60
75
90
105
120
time, min

B


Day 1
OVX
SHAM
FS
number of SWDs
0
4
8
12
16
20
0
15
30
45
60
75
90
105
120
time, min

C


Day2
OVX
SHAM
FS
number of SWDs
0
4
8
12
16
20
0
15
30
45
60
75
90
105
120
time, min

D

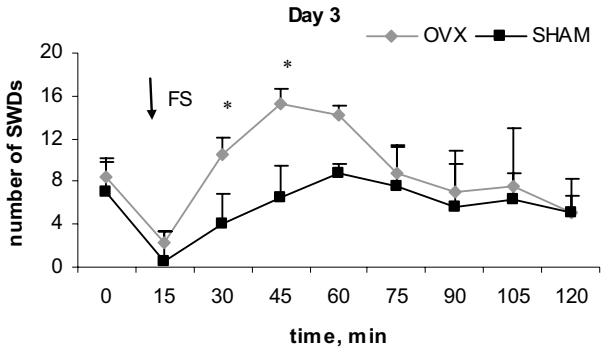


Figure 3. The number of SWDs in the base-line (A, 3 h) and before (1 h) and after (2 h) the administration of 10 FS repeated for 3 subsequent days (B–D). Asterisk (*) shows a significant difference between the groups at Day 3 ($p < 0.05$) according to *post hoc test.*

Subsequent *post-hoc* t-tests revealed no significant difference between OVX and sham-operated rats in either basal conditions or after the first FS session. At the second session, OVX rats showed a tendency (marginally significant – $p<0.08$) to have a larger number of SWDs between 30-60 min after FS administration. At the third session the difference became significant: OVX rats demonstrated a higher number of SWDs between 15-30 and 30-45 min ($t=2.38, df=12, p<0.05$; $t=2.61, df=12, p<0.05$) after FS administration compared to sham-operated animals.

SWDs in the second hour after FS

There was a significant main effect of day ($F_{day}=7.18, df=3,36, p<0.001$) and a significant main effect of time ($F_{time}=4.68, df=3,36, p<0.01$) in SWDs in the second hour after FS. An orthogonal trend analysis revealed a significant linear trend ($F_{lin}=20.31, df=1,12, p<0.001$) for the main effect of day, indicating a linear increase in the number of SWDs over days, and a significant linear orthogonal trend ($F_{lin}=9.55, df=1,12, p<0.01$) for the main effect of time, indicating a decrease in the number of SWDs.

SWDs before and after FS

The analysis of the last 15 min episodes of the base line and the first 15 min episodes after FS, in which a clear decrease was found in the number of SWDs, revealed a significant effect of day ($F_{day}=6.72, df=2,24, p<0.01$)

and time (F_{time}= 162.87, df=1,12, $p<0.001$) and a significant interaction between day and time ($F_{day*time}$=3.59, df=2,24, $p<0.05$). There was neither a significant effect of operation, nor significant interaction with operation, indicating that the initial suppression of SWDs after FS was the same in both the OVX and sham-operated females.

The analysis of the base line preceding the administration of FS and the period of aggravation in SWDs after FS exposure revealed a marginally significant ($p<0.06$) higher number of SWDs in OVX females between 45-60 min after the second FS session and a significantly higher number of SWDs between 30-45 and 45-60 min after the third FS session (t=2.5, t=4.5, df 6, $p<0.05$). No significant aggravation (compared to the base-line level) in the number of SWDs after FS was found for sham-operated WAG/Rij females. All this illustrates a higher aggravation in SWDs in response to stress in OVX animals than in controls.

4. Discussion

Several relevant outcomes concerning the role of ovarian steroids in the regulation of absence seizures were found. First, tolerance to the central effects of progesterone developed rapidly. Next, the effect of progesterone injection was facilitated by two preceding injections with CD, suggesting that anticipation to an injection aggravated the action of progesterone. Finally, and most relevant, chronic diminution of gonadal steroids by ovariectomy did not alter basal absence seizure activity, but increased the aggravation in the occurrence of SWDs after repeated stress exposure. Taken together, these data suggest that ovarian hormones are involved in the regulation of the occurrence of SWDs in a situation of stress anticipation and therefore may play a critical role in the pathogenesis of absence epilepsy.

The present data support previous findings on effects of progesterone on SWDs and also extend them in several ways. As previously reported, acute progesterone exposure aggravated the number of SWDs for 1-1.5 hours after injection [5, 6]. A clear reduction in the SWD promoting effect of progesterone on SWDs already after the second injection indicates a rapid development of tolerance to progesterone or its derivatives. Consistently, Zhu and coauthors showed that tolerance to allopregnanolone developed already after 60-90 of continuous exposure [16]. Based on our data we

suggest that this acutely developed tolerance may present for at least 2 days after the first exposure.

Although there are several recently published data indicating that neuroactive steroids may have a tolerance liability similar to that of benzodiazepines [18, 19, 20], the mechanisms of tolerance development under chronic or repeated exposure to neuroactive steroids are not yet well understood. However, it has been shown that neurosteroids not only interact with $GABA_A$ receptors but also down-regulate the expression of genes that encode the subunits of this receptor complex, reducing the sensitivity of the receptor to allosteric modulation [15, 20].

The present results also demonstrated a significantly larger increase of SWDs after progesterone injection preceded by two cyclodextrin injections compared to injection of progesterone to naïve rats. A similar effect was reported by Czlonkowska and coauthors [19]: they observed that allopregnanolone, given repeatedly, produced a less salient anticonvulsant effect than a single dose of allopregnanolone following repeated cyclodextrin injections. Recent data from an *in vitro* experiment suggest that the stimulation of GABA-mediated chloride ion uptake by allopregnanolone, mediating the aggravation of SWDs after progesterone injection [6], may be enhanced in the presence of glucocorticoid metabolites [21]. Based upon these data, we suggest that mild stress associated with an injection procedure may interfere with and enhances the effect of progesterone on SWDs. However, neither the mechanisms, nor the physiological significance of the interaction between different neuroactive steroids, such as gonadal and stress related steroids, are well understood and we will come back to this question in the discussion of experiment 2b.

The outcomes of the second experiment (2a) indicated that the removal of the peripheral steroids hormones did not influence the basal level of SWDs for 35 days after surgery. Although there was a slight increase in the SWDs in both OVX and sham-operated animals over time, this was likely due to effects of surgery (or the anesthesia) and ageing process, which is known to increase both the occurrence and duration of SWDs [10].

The lack of any changes in absence seizures after ovariectomy was rather unexpected, since progesterone has an imperative impact on the GABA-ergic transmission, which is ultimately involved in the generation of thalamo-cortical oscillations of the absence type (GABA-mimetics enhance SWD after systemic administration [10, 22]. Moreover, the outcomes of an acute pharmacological study in which progesterone was injected as well as fluctuations in SWD in the course of the ovarian period show pronounced effects of progesterone on SWDs [5]. However, similar results were also found in the pilocarpine convulsive seizure model, in which chronic

diminution of ovarian hormones did not change the incidence of status epilepticus, although a more rapid progression to the status development was found [23]. To account for the discrepancy, it can be suggested that chronic depletion of ovarian steroid may trigger counteractive mechanisms such as increased density and/or sensitivity of $GABA_A$ receptors and increased local synthesis of neurosteroids (or/and other endogenous neuromodulators) within the brain to adjust to the dramatic loss of steroids and restore the impaired balance. Virtually, a number of adaptive changes in specific binding associated with $GABA_A$ receptors as well as in density of dopamine receptors was indeed observed following ovariectomy in different brain structures such as striatum and prefrontal cortex [24, 25]. The lack of any changes in the basal occurrence of SWDs in OVX females raises doubts whether and in which way ovarian steroids play a role in the regulation of SWDs under resting conditions [5, 6, 7, 8].

Interestingly, however, the difference in the incidence of SWDs between OVX and sham operated animals became apparent in the second experiment (2b), in which OVX females showed a more prominent increase in the number of SWDs after daily repeated FS administration. Therefore, although the incidence of either SWDs or convulsive seizures under resting conditions was not disturbed after ovariectomy, OVX females became less resistant to repeated stress exposure.

Stress and, in particularly, repeated stress induces structural changes in neuronal networks, in particular in hippocampus, prefrontal cortex and amygdala [26, 27]. An aggravation in SWDs from session to session was found in both groups of animals and this is in agreement with our recent findings in male rats [17]. Moreover, an increase in SWDs following FS was mediated by an increase in the base-line period preceding FS. The increase in SWDs FS was suggested to reflect an anticipatory response [17], which is known to be generated in limbic structures by memory to a fearful context. This anticipatory response is capable to activate the HPA axis under conditions, in which physical challenges can be predicted [28]. Consistently, it was recently shown that OVX rats display an increased numbers of c-Fos – positive nuclei response in the number of limbic structures such as hypothalamus, dentate gyrus, medial prefrontal cortex and central and medial amygdala after re-exposure to the stressor in the same environment [29]. Considering that the hippocampus as well as some other limbic structures, such as nucleus accumbens and prefrontal cortex are also involved in the regulation of SWDs [22, 30, 31], we suggest that more

salient aggravation of SWDs in the base-line before and after repeated FS exposure in OVX females might be attributed to an increased activation of limbic structures and a lack of ovarian steroids.

Interestingly, an abrupt withdrawal of ovarian hormones via ovariectomy is thought to produce a depression like state that is reversed by the administration of estradiol or progesterone, mimicking the effects of antidepressants [23, 32]. Based on this, we suggest that the aggravated anticipation response in OVX females might also be linked to a larger predisposition of these animals to develop depressive-like symptoms and this needs to be further investigated.

In addition, the dynamic changes in the number of SWDs after exposure to stress might reflect the changes in the excitability induced by different types of neurosteroids released after stress [14, 21]. On the other hand, these changes could be linked to brainstem catecholamine-ergic systems which are activated by stress and are may mediate the alterations in the incidence of SWDs. [11, 31]. Therefore additional experiments will also be required to establish the mechanisms underlying the dynamic of SWDs both preceding and following repeated stress exposure.

In conclusion, the present data demonstrate the quick development of tolerance to progesterone and an interaction between hormones of the hypothalamo-pituitary-gonadal and hypothalamo-pituitary-adrenal axis. The outcomes suggest that rats with an intact hypothalamo-pituitary-gonadal axis are better able to regulate the stress response and develop tolerance to the stressor.

References

1. Pitkanen, A., Schwartzkroin, P.A., Moshe, S.L. (Eds), Models of Seizures and Epilepsy, Elsevier Academic Press, San Diego, (2006).
2. Rhodes, M.E., Harney, J.P., Frye, C.A., Gonadal, adrenal, and neuroactive steroids' role in ictal activity, Brain Res. 1000 (2004) 8-18.
3. Melcangi, R.C., Panzica, G.C., Neuroactive steroids: old players in a new game, Neurosci. 138 (2006) 733-739.
4. Grunewald, R.A., Aliberti, V., Panayiotopoulos, C.P, Exacerbation of typical absence seizures by progesterone, Seizure 1 (1992) 137-138.
5. van Luijtelaar, G., Budziszewska, B., Jaworska-Feil, L., Ellis, J., Coenen, A., Lason, W., The ovarian hormones and absence epilepsy: a long term EEG study and pharmacological effects in a genetic absence epilepsy model, Epilepsy Res. 46 (2001) 225-239.
6. van Luijtelaar, G., Budziszewska, B., Tetich, M., Lason, W., Finasteride inhibits the progesterone-induced spike-wave discharges in a genetic model of absence epilepsy, Pharmacol. Biochem. Behav. 75 (2003) 889-894.
7. Persad, V., Ting Wong, C.G., Cortez, M.A., Wang, Y.T., Snead 3rd, O.C., Hormonal regulation of atypical absence seizures, Seizure 1 (1992) 137-138.
8. Tolmacheva, E.A., Chepurnov, S.A., Chepurnova, N.E., Kochetkov, Y.A., van Luijtelaar, G., Absence seizures during pregnancy in WAG/Rij rats, Physiol. Behav. 81 (2004) 623-627.
9. van Luijtelaar, E.L., Coenen, A.M., Two types of electrocortical paroxysms in an inbred strain of rats, Neurosci. Lett. 70 (1986) 393-397.
10. Coenen, A.M., van Luijtelaar, E.L., Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats, Behav. Genet. 33(6) (2003) 635-655.
11. van Luijtelaar, G., Sitnikova, E., Global and focal aspects of absence epilepsy: The contribution of genetic models, Neurosci. Biobehav. Rev. 30 (2006) 983-1003.
12. Carey, M.P., Deterd, C.H., de Koning, J., Helmerhorst, F., de Kloet, E.R., The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat, J Endocrinol. 144 (1995) 311-321.
13. Young, E.A., Altemus, M., Puberty, ovarian steroids, and stress, Ann N Y Acad Sci. 1021 (2004) 124-133.
14. Romeo, R.D., Bellani, R., McEwen, B.S., Stress-induced progesterone secretion and progesterone receptor immunoreactivity in the paraventricular nucleus are modulated by pubertal development in male rats. Stress, 8 (2005) 265-271.
15. Barbaccia, M.L., Serra, M., Purdy, R.H., Biggio, G., Stress and neuroactive steroids, Int Rev Neurobiol. 46 (2001) 243-272.
16. Saalmann, Y.B., Morgan, I.G., Calford, M.B., Neurosteroids involved in regulating inhibition in the inferior colliculus, J Neurophysiol. 96 (2006) 3064-3073.
17. Tolmacheva, E.A., Oitzl, M.S., Van Luijtelaar, G., Basal and stress induced activity of the hypothalamus-pituitary-adrenal (HPA) axis in genetic epileptic male rats, submitted.
18. Zhu, D., Birzniece, V., Backstrom, T., Wahlstrom, G., Dynamic aspects of acute tolerance to allopregnanolone evaluated using anaesthesia threshold in male rats, Br. J. Anaesth. 93 (2004) 560-567.

19. Czlonkowska, A.I., Krzascik, P., Sienkiewicz-Jarosz, H., Siemiatkowski, M., Szyndler, J., Maciejak, P., Bidzinski, A., Plaznik, A., Tolerance to the anticonvulsant activity of midazolam and allopregnanolone in a model of picrotoxin seizures, Eur. J. Pharmacol. 425 (2001) 121-127.
20. Birzniece, V., Turkmen, S., Lindblad, C., Zhu, D., Johansson, I.M., Backstrom, T., Wahlstrom, G., GABA(A) receptor changes in acute allopregnanolone tolerance, Eur. J. Pharmacol. 535 (2006) 125-134.
21. Stromberg, J., Backstrom, T., Lundgren, P., Rapid non-genomic effect of glucocorticoid metabolites and neurosteroids on the gamma-aminobutyric acid-A receptor, Eur J Neurosci. 21 (2005) 2083-2088.
22. Danober, L., Deransart, C., Depaulis, A., Vergnes, M., Marescaux, C., Pathophysiological mechanisms of genetic absence epilepsy in the rat, Prog Neurobiol. 55 (1998), 27-57.
23. Scharfman, H.E., Goodman, J.H., Rigoulot, M.A., Berger, R.E., Walling, S.G., Mercurio, T.C., Stormes, K., Maclusky, N.J., Seizure susceptibility in intact and ovariectomized female rats treated with the convulsant pilocarpine, Exp Neurol. 196 (2005) 73-86.
24. Wilson, M.A., Biscardi, R., Effects of gender and gonadectomy on responses to chronic benzodiazepine receptor agonist exposure in rats, Eur J Pharmacol. 215(1992) 99-107.
25. Bosse, R., Di Paolo, T., Dopamine and GABAA receptor imbalance after ovariectomy in rats: model of menopause. J Psychiatry Neurosci. 20 (1995) 364-71.
26. McEwen, B.S., Effects of adverse experiences for brain structure and function, Biol Psychiatry. 48 (2000) 713-714.
27. Fuchs, E., Flugge, G., Czeh, B., Remodeling of neuronal networks by stress, Front Biosci. 11 (2006) 2746-2758.
28. Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C., Cullinan, W.E., Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front Neuroendocrinol. 24 (2003) 151-158.
29. Gerrits, M., Bakker, P.L., Koch, T., Ter Horst, G.J., Stress-induced sensitization of the limbic system in ovariectomized rats is partly restored by cyclic 17beta-estradiol administration, Eur J Neurosci. 23 (2006) 1747-1756.
30. Tolmacheva, E.A., van Luijtelaar, G., Absence seizures are reduced by the enhancement of GABA-ergic inhibition in the hippocampus in WAG/Rij rats,2007.
31. Midzyanovskaya, I.S., Kuznetsova, G.D., van Luijtelaar, E.L., van Rijn, C.M., Tuomisto, L., Macdonald, E., The brain 5HTergic response to an acute sound stress in rats with generalized (absence and audiogenic) epilepsy, Brain Res Bull. 69 (2006) 631-638.
32. Stoffel, E.C., Craft, R.M., Ovarian hormone withdrawal-induced "depression" in female rats, Physiol Behav. 83 (2004) 505-513.

Chapter 4

Cortical and limbic excitability in rats with absence epilepsy

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Abstract. The classical cortico-reticular theory on absence epilepsy suggests that a hyperexcitable cortex is a precondition for the occurrence of absence seizures. In the present experiment seizure thresholds and characteristics of cortical epileptic afterdischarges (AD) were determined in a comparative cortical stimulation study in young and old adult genetically epileptic WAG/Rij, congenic ACI and Wistar rats. Fifteen-second series of 8 Hz stimulation of the sensory-motor cortex were applied in 80- and 180-day-old rats with implanted electrodes.

Strain differences were found for the threshold for movements directly induced by stimulation, low frequency spike-and-wave AD, maximal clonic intensity of seizures accompanying direct stimulation, and frequency characteristics of low frequency AD. None of these results agreed with a higher cortical excitability exclusively in WAG/Rij rats. However, WAG/Rij rats had the longest duration of the low frequency AD, and the lowest threshold for the transition to the limbic type of AD. The decrease of this threshold correlated with the increase of the incidence and total duration of spontaneous SWDs in WAG/Rij rats.

It is concluded that the elevated excitability of the limbic system or pathways mediating the spread of the epileptic activity into this system can be attributed to the development of genetic epileptic phenotype in WAG/Rij rats.

Key words: excitability, cortex, sensory-motor cortex, rats WAG/Rij, absence epilepsy, afterdischarges.

1. Introduction

The pathophysiology of idiopathic generalized absence epilepsy is not fully understood. Gloor's concept of cortico-reticular epilepsy, nowadays widely accepted, postulates that an abnormally excitable cortex interacts with thalamus and brain stem reticular formation (Gloor and Fariello, 1988; Kostopoulos, 2000). Gloor and coworkers showed that systemic administration of high doses of penicillin induced spike-wave discharges (SWDs) in cats and suggested that a pharmacologically induced change in cortical excitability was the underlying factor. They reasoned that spindle volleys coming from the thalamus were transformed into SWDs at the level of the cortex, when this was made hyperexcitable by penicillin (Gloor and

Fariello, 1988). However it is not clear whether the cortex is hyperexcitable permanently (i.e. also interictally) or only during the appearance of SWDs.

The inbred WAG/Rij albino rat strain is considered to be an appropriate genetic animal model for absence epilepsy (van Luijtelaar and Coenen, 1986; Crunelli and Laresche, 2002; Coenen and van Luijtelaar, 2003). This type of epilepsy, a generalized non-convulsive form, is associated with spontaneously occurring bursts of bilateral synchronous SWDs which can be recorded in the electroencephalogram (EEG). SWDs in WAG/Rij rats consist of trains of spike and waves with a duration of 1 to 30 seconds and a frequency between 7 and 10 Hz. At 2-3 months of age, SWDs start to become present in the EEG and the number of SWDs increases with age. In 6-month-old rats 16 to 18 SWDs emerge per hour, adding up to about 400 SWDs per day. Mild behavioral concomitants can be seen during the presence of a SWD, such as episodes of vibrissae twitching; otherwise the animals are immobile (van Luijtelaar and Coenen, 1986). Increased cortical excitability in WAG/Rij rats is conceivable since Meeren et al (2002) found that cortex of WAG/Rij rats contains zones in which the epileptoformic activity may be triggered before being fully synchronized in or by the cortico-thalamic network.

Local rhythmic electrical stimulation of the sensorimotor cortex in freely moving rats was used to investigate whether genetic epileptic rats are endowed with a more excitable cortex interictally. This stimulation paradigm allows the study of four events with different mechanisms:

1. movements elicited by individual stimuli, due to a direct activation of the motor system
2. afterdischarges (AD) characterized by low frequency (around 3 Hz) spike-and-wave EEG rhythm, generated by a thalamocortical system
3. clonic seizures of head and forelimbs muscles accompanying AD, indicating a spread of epileptic activity into the motor system
4. transition into AD similar to those elicited by stimulation of limbic structures (Dyer et al., 1979) accompanied by behavioral arrest or automatisms – e.g. elements of orienting reaction in a well known cage and wet dog shakes. These phenomenon's presumably represent that epileptic activity has spread into limbic structures. A detailed description of this stimulation paradigm is at disposal because this test was routinely used to study the ontogeny of cortical epileptic AD (Mareš et al. 2002) and the effects of anticonvulsant (Kubová et al., 1996, 1999; Haugvicová et al., 2002) as well as convulsant drugs (Koryntová et al. 2002, Živanović et al. 2003).

In order to investigate whether increased interictal cortical excitability may underlie the pathogenesis of absence epilepsy in WAG/Rij rats, we used this stimulation paradigm and determined threshold values and characteristics of AD. Two groups of WAG/Rij rats were used, 2-3 month old which have only few SWD, 6 month old with many SWDs, age matched control rats from an inbred strain with no or a minimal number of SWD's (ACI, Inoue et al., 1990; de Bruin et al., 2001) and from an outbred control strain (Wistar). This design allowed us to compare effects of age, genotype (epileptic vs non-epileptic and inbred vs outbred) in order to figure out whether differences between groups can be attributed to the development of genetic absence seizures.

2. Materials and Methods

2.1. Animals

Experiments were performed in 2-3 (80 days) and 6 months male WAG/Rij (n=12, n=11), ACI (n=12, n=11) and Wistar rats (n=8, n=8). The animals were housed under standard conditions (temperature 22±1^{o}C, 12/12 light/dark cycle with light onset at 6 a.m.). WAG/Rij rats were purchased from Charles River Co, ACI from Harlan Winkelmann GmbH, Borchen, and Wistar rats from the breeding colony of the Institute of Physiology Academy of Sciences of the Czech Republic. All experiments were approved by the Animal Care and Use Committee of the Institute of Physiology and declared to be in agreement with Czech Animal Protection Law (fully compatible with European Community Council directives 86/609/EEC).

2.2. Electrode implantation

Rats were surgically prepared under pentobarbital anesthesia (Nembutal® Abbott, 40 mg/kg i.p.). Silver ball electrodes were placed epidurally; two stimulation electrodes over right sensorimotor cortex (AP = -1 and +1; L = 2.5 mm in relation to bregma), recording electrodes over left sensorimotor cortex (AP = 0; L = 2.5 mm), left parietal cortex (AP = 3; L = 3 mm) and over occipital, visual cortical areas of both hemispheres (AP = 6; L = 4 mm). A reference electrode was inserted into the nasal bone, ground electrode into the occipital bone. All electrodes were connected to a plug

and the whole assembly was fixed to the skull with fast curing dental acrylic cement. Experiments started after a one-week recovery period.

2.3. EEG recording and cortical stimulation

Animals were put into Plexiglas cages and connected to the input of a paperless ECoG (sampling rate of 200 Hz) to perform video-ECoG monitoring in a semi sound proof room with regulated air temperature. Base-line recordings (duration 2 hours) of spontaneous ECoG started after two-hour adaptation to experimental conditions, it allowed us to establish the number and duration of spontaneously occurring SWD.

Next the animals were transferred to another room with the stimulation facilities. For recording of AD, the ECoG activity was digitized at a higher rate of 500 Hz. A constant current stimulator was used. Current intensity had to be confirmed before the stimulation started and was registered by the computer. It was possible to mark directly on the recording so that motor as well as behavioral phenomena were registered. Series of 15-s stimulation (biphasic rectangular pulses of 1-ms duration and 8-Hz frequency) were applied. Stimulation series were repeated after at least a 10 min interval, the intensity of stimuli was always increased for the next series in the following steps: 0.2; 0.4; 0.6; 0.8; 1.0; 1.2; 1.4; 1.6; 1.8; 2.0; 2.2; 2.4; 2.6; 2.8; 3.0; 3.5; 4.0; 4.5; 5.0; 6.0; 8.0; 10.0; 12.0; 14.0; 15.0 mA up to the transition of the AD into the limbic type. At the moment this second type of AD was present, stimulation intensities were not further increased. Electrocorticographic activity was always recorded 20 s before the start of stimulation and at least two minutes after the end of stimulation. The same protocol was used in earlier studies on the ontogenetic development (Mareš et al., 2002) and on the influence of GABA receptor antagonists (Źivanović et al. 2003) on the AD thresholds in Wistar rats.

Concerning the events induced by electrical stimulation, four different phenomena were evaluated: movements of head or contralateral paws (usually forepaw) directly elicited by stimulation, epileptic AD characterized by low frequency spike-and-wave rhythm (Fig.1), clonic seizures of head and forepaw muscles accompanying spike-and-wave AD and epileptic AD consisting of huge delta waves and fast low amplitude spikes (Fig.1) that were accompanied by behavioral automatisms. Means and variability measures of threshold current intensities for these four phenomena were calculated in all six groups. In addition, the duration of spike-wave AD as well as severity of movements directly elicited by

stimulation and clonic seizures accompanying epileptic AD were measured in the stimulation series with the threshold and two times threshold intensities for spike-and-wave AD elicitation. The severity of seizures was expressed by means of the slightly modified Racine's five-point scale (Racine, 1972) as described by Kubová et al. (1996). Frequency of low frequency spike-and-wave AD was measured in the first and the last 3-s sections of all spike-and-wave AD longer than 6 s. Animals were used only once since pilot experiments demonstrated marked changes of thresholds at the second exposure (Haugvicová et al, 2002).

SWDs were identified in WAG/Rij rats in the two-hour period of ECoG recording according to well known criteria (van Luijtelaar and Coenen, 1986) and expressed as number of SWDs per hour. Data from both age groups were put together to have animals with low and high number of SWDs for calculation of possible correlations.

2.4. Statistics

Two-way ANOVA with age (2 levels) and strain (3 levels) as between groups factors with subsequent pairwise comparisons by LSD tests were used for statistical evaluation of the thresholds, a three way-ANOVA for the frequency of the low-frequency AD (begin and end of the AD was used as within groups factor, strain and age as between groups factors). Spearman rank correlations between individual threshold current intensities for elicitation of spike-and-wave AD and hourly number and mean duration of spontaneously occurring SWDs in WAG/Rij rats were conducted. The incidence of fast activity during AD was statistically evaluated by means of Fisher exact test. The level of statistical significance was set at 5 % (two tailed tests) for all variables.

3. Results

All rats included in the present study exhibited the first three phenomena that were evaluated: movements during stimulation, low frequency spike-and-wave AD (Fig.1) and clonic seizures accompanying this type of AD. Transition into the second, limbic type of AD (Fig.1) was recorded under our experimental conditions (15 mA as the highest intensity of stimulation current) in 12-13 rats in each strain (i.e. in 80-100% of animals) without any significant strain or age difference in the number of animals showing this transition.

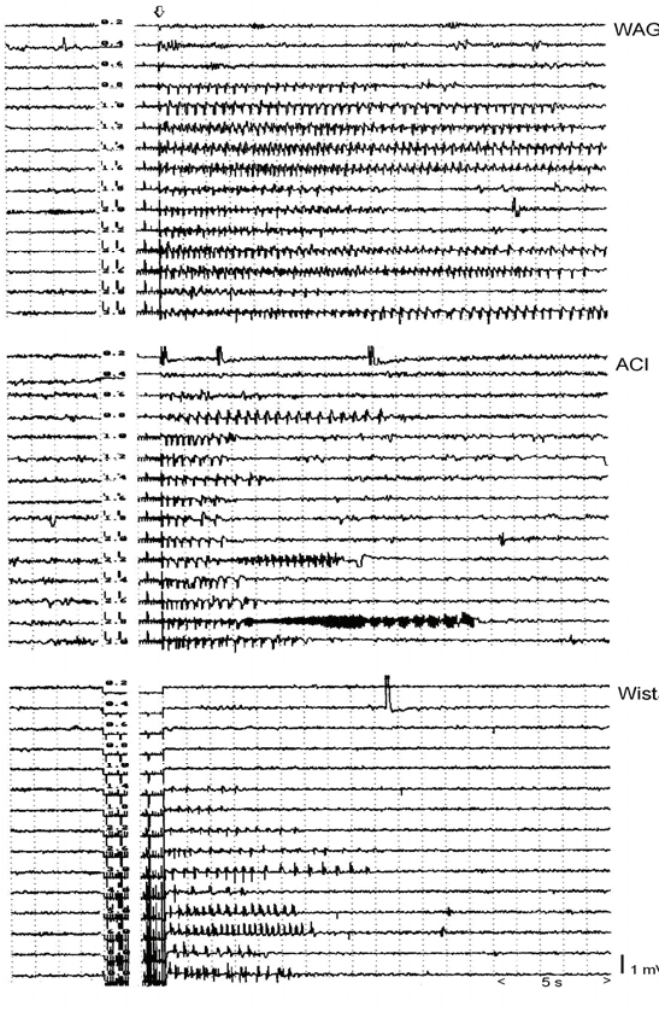

Figure 1. Recordings of EEG effects of stimulations with increasing intensities from 3-month-old rats. From top to bottom: WAG/Rij rat; ACI rat; Wistar rat. Individual sections from top to bottom: current intensities from 0.2 to 3.0 mA in WAG/Rij and ACI rats, from 0.2 to 10.0 mA in Wistar rats. Left part of each curve: last 4 s before the stimulation, right part: the last second of stimulation and 19 s poststimulation. Time mark 5 s, amplitude callibration 1 mV. Slow frequency spike and wave afterdischarge first appears at current intensity of 0.8 mA in the traces of WAG/Rij rat, at 0.6 mA in ACI, and at 1.4 mA in Wistar rat. Note also the longer duration of the slow frequency spike and wave afterdischarges in WAG/Rij rats compared to ACI and Wistar rat. Finally, fast activity characteristic for ACI rats can be seen in the traces of current intensities of 2.2 and 2.8 mA.

3.1. Thresholds for movements elicited by stimulation

The ANOVA showed significant strain effects for the thresholds for elicitation of stimulus-bound movements, reflecting direct activation of the motor cortex ($F_{2,56}$=28.1, $p<0.001$). The results are presented in Figure 2. There were neither age effects nor an interaction between age and strain. Post-hoc tests showed that Wistar rats demonstrated a lower excitability of the motor cortex in comparison with WAG/Rij as well as ACI rats ($p<0.01$).

3.2. Threshold of low frequency spike-and-wave AD

A significant strain effect was found for the threshold intensity necessary for elicitation of low frequency spike-and-wave AD ($F_{2,56}$ =33.1, $p<0.001$), see also Figure 2.

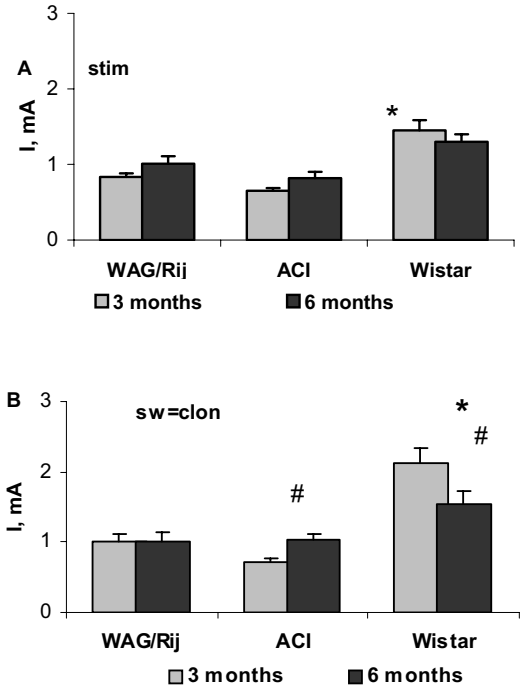

Figure 2. Thresholds current intensities (mean+S.E.M.) necessary for (A) elicitation stimulus-bound movements (stim) and (B) low frequency spike and wave afterdischarges (sw)/clonic seizures (clon) in WAG/Rij, ACI and Wistar rats at 3 (gray columns) and 6 months (black columns). In all cases the thresholds current intensities for sw and clon were exactly the same. Ordinate: current intensity (I) in mA. * - $p<0.001$ significant difference in comparison with both other strains, # - $p<0.05$ significant age difference.

This threshold was in all subjects equal to that for elicitation of clonic movements of head and forelimbs muscles accompanying this type of AD. Both ACI and WAG/Rij rats had significantly lower thresholds for elicitation of these phenomena in comparison with Wistar rats ($p<0.01$). There was no age effect but a significant interaction between age and strain was found ($F_{2,56}=6.4$, $p<0.01$), pointing to difference in age-related shifts among the tested strains. Both thresholds (for low-frequency spike-and-wave AD) and elicitation of clonic movements of heads and shoulders tended to increase with age in both inbred strains, whereas they declined significantly ($p<0.01$) with age in Wistar rats. This demonstrates that the differences between strains were attenuated with age.

3.3. Frequency of low frequency spike-and-wave AD

Frequency of the low frequency spike-and-wave AD (data are presented in Fig. 3) was always higher at the beginning of the AD than at the end ($F_{1,54}=217.7$, $p<.0001$). There was no age effect, however the strain difference was significant ($F_{2,54}=7.44$, $p<.001$). Post hoc analyses for a strain effect demonstrated that the frequency (overall) was lower for Wistar than for ACI and WAG/Rij rats. The interaction between strain and frequency (beginning-end) just failed to reach significance ($0.05>p>.10$), however it prompted us to analyse the parameters in more detail. Separate analyses were performed for frequency of this AD at the beginning, at the end and the difference score. For the frequency at the beginning only a strain effect ($F_{2,54}=5.54$, $p<.01$) was found; post-hoc tests showed that the frequency is higher for ACI and WAG/Rij rats compared to Wistar rats. For the frequency at the end of the AD again a strain effect ($F_{2,54}=4.84$, $p<.05$) was found; post hoc's for the strain effect showed that now the frequency is higher for ACI compared to Wistar and WAG/Rij rats. The ANOVA on the difference pre-post again revealed only a strain effect ($F_{2,57}=3,15$, $p=.05$), post hoc tests show that the change in frequency is larger for WAG/Rij than for Wistar rats, but not for ACI. In addition, ACI rats often exhibited a change in the ECoG pattern of the AD. Section of fast spikes (Fig.1) suddenly appeared in the course of AD in 70% of 3-month-old and in 35.7% of 6-month-old animals. Frequency of these spikes varied in the range from 9 to 12 Hz. Similar phenomenon was registered in only one out of 10 WAG/Rij rats 3 months old and never in the remaining three (6 months WAG/Rij, 3 and 6 months Wistar) groups of animals; the distribution of these probabilities is significant.

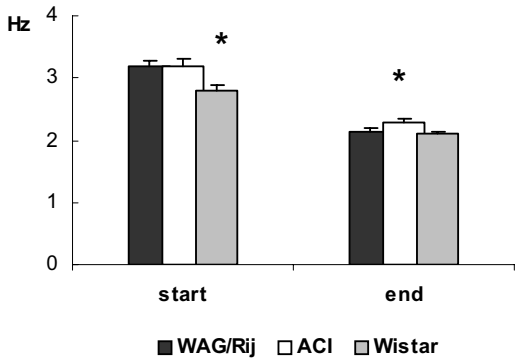

Figure 3. The frequency of spikes (mean+S.E.M.) in the first three (start) and the last three seconds (end) of spike and wave afterdischarges. Details as in Fig.3, only ordinates – frequency of spike-and-wave complexes in Hz.

3.4. Duration of low frequency spike-and-wave AD

A strain effect ($F_{2,56}$ =28.43, p<0.000) was found for duration of low frequency spike-and-wave AD; the data are presented in Figure 4. WAG/Rij rats demonstrated a significantly longer duration of spike-and-wave AD compared to both ACI and Wistar rats (p<0.01 in both cases). There were no other effects.

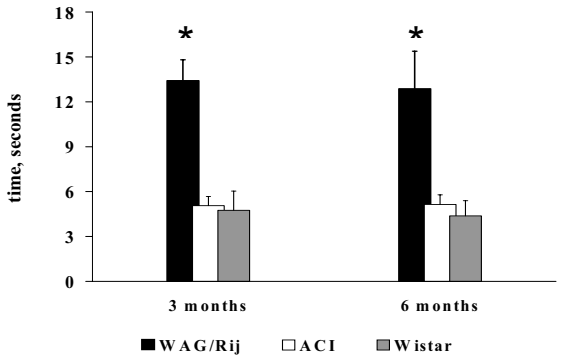

Figure 4. Mean duration of spike-wave afterdischarges (mean+S.E.M.) calculated from individual data for the threshold and twofold threshold current intensities. Details as in Fig.3, only ordinate – duration of afterdischarges in seconds

3.5. Maximal intensity of clonic seizures

Maximal intensity of clonic seizures accompanying AD (Fig.5) exhibited a strain effect ($F_{2,47}$ =16.01, $p<0.001$). ACI rats demonstrated significantly ($p<0.001$) more severe clonic seizures than WAG/Rij as well as Wistar rats. Sections of fast spikes in ACI rats were accompanied by fast clonic movements of forelimbs synchronous with individual spikes.

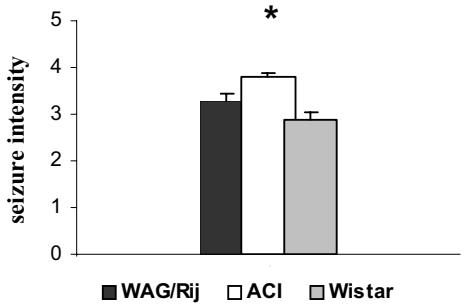

Figure 5. Maximal severity of seizures (MEAN+S.E.M.) accompanying stimulation and spike-and-wave afterdischarges in WAG/Rij, ACI and Wistar rats at 3 and 6 months (Racine's five-point scale modified by Kubová et al. (1996)). * - significant difference in comparison with both other strains at $p<0.05$.

3.5. Threshold for transition into limbic type of AD

Both an age effect ($F_{2,56}$ =4.75, $p<0.05$) and a strain effect ($F_{2,56}$ =13.4, $p<0.001$) were found for the fourth phenomenon – the threshold for the transition to the limbic type of AD, indicating spread of epileptic activity into the limbic system (see also Fig. 2).

The interaction between age and strain was not significant. WAG/Rij rats showed a significantly ($p<0.01$) lower threshold for this phenomenon compared to each of the other strains. No difference was found between ACI and Wistar rats. Older rats had significantly ($p<0.05$) more excitable limbic system than younger rats. The fact that, no interaction between age and strain was found, suggests that the differences between strains remain stable.

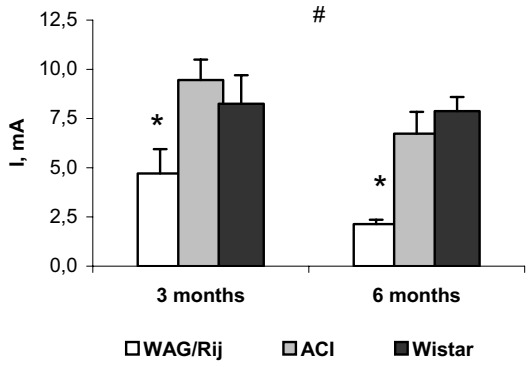

Fig. 6. Thresholds current intensities (mean+S.E.M.) necessary for elicitation limbic type of afterdischarges in WAG/Rij (black columns), ACI (white columns) and Wistar (gray columns) rats 3 (A) and 6 (B) months old. Ordinate: current intensity in mA. * - $p<0.001$ significant difference in comparison with both other strains, # - $p<0.05$ significant difference between age groups.

3.6 Relation to spontaneously occurring spike-wave discharges

The number of spike-and-wave episodes counted in WAG/Rij rats during the base-line recording varied between 9 and 63, the total duration between 27 and 264 s. The correlation between individual threshold current intensities for transition into limbic seizures and hourly number and mean duration of spontaneously occurring SWDs in WAG/Rij rats showed a significant inverse correlation between the threshold and both mean and total duration of SWDs ($R=-0,57$, $R= -0,53$, $p<0.05$).

4. Discussion

The present study was designed to investigate whether genetically epileptic rats are endowed with an increased cortical excitability interictally. The outcomes of cortical stimulation study demonstrated no difference in excitability of sensorimotor cortex between epileptic WAG/Rij and congenic ACI rats. All the first three phenomena, reflecting (i) direct activation of motor cortex (stimulus-bound movements), (ii) spread of epileptic activity into the motor system (clonic movements following after

stimulation period) and (iii) involvement of cortico-thalamic system (low frequency AD) had the same thresholds in WAG/Rij rats as in ACI rats. However, comparison between Wistar rats on the one side and the two inbred strains on the other showed a difference in excitability. Both inbred strains had from 1.5 till 2-fold lower thresholds for these three phenomena than age-matched Wistar rats. Consistent with this, Wistar rats demonstrated a lower intensity of seizures and stimulus-bound movements compared to ACI and WAG/Rij rats. Similar results were found in an *in vitro* study, in which neocortical slices of WAG/Rij rats in comparison to Wistar rats exhibited significant reduction in the efficiency of GABAergic inhibition concomitant with hyperexcitability (Luhmann et al., 1995). Another report showed that WAG/Rij rats have a lower seizure threshold than Wistar rats in a model of convulsive epileptic seizures (pentylenetetrazol) (Kliqueva et al., 2001). However, in contrast to what has been found before, our study showed that both inbred rats are characterized by increased interictal cortical excitability of the sensorimotor cortex as well as motor system compared to outbred Wistar rats. Since ACI rarely show SWDs, our data imply that precondition of higher interictal cortical excitability is necessary but not sufficient condition for the development of absence epileptic phenotype.

Our experiment tested the cortical excitability only during the interictal period and was not aimed to test cortical excitability during or immediately before episodes of SWDs. However, it might be that only a temporal ictal (or periictal) change in cortical excitability is necessary for the appearance of SWDs in WAG/Rij rats. Moreover, there might be only local changes in excitability (Meeren et al., 2002; van de Bovenkamp-Janssen et al, 2004), which could not be found in the stimulation paradigm used in present experiment. These possibilities remain to be further explored.

Oscillation frequency within the AD is a parameter that describes thalamo-cortical interaction. First of all, we found that the frequency of the low-frequency oscillations was always higher at the beginning compared to the end of the AD. Interestingly, the decrease in frequency over the course of spontaneous SWDs was also found in WAG/Rij rats and in children with absence epilepsy and might be a reflection of a mechanism which is involved in arresting of an AD or train of SWDs. At the beginning, the frequency of the AD was higher in WAG/Rij and ACI rats than in Wistar rats, whereas at the end, ACI rats have a higher frequency both in comparison to Wistar and to WAG/Rij rats. The decrease in frequency was larger in WAG/Rij than in Wistar rat and did not differ between WAG/Rij and ACI rats. In addition, old WAG/Rij rats did not differ from young

WAG/Rij rats. These outcomes do not give any evidence to assume that frequency characteristics of this type of AD are associated with the presence of SWDs in WAG/Rij rats. On the other hand, of interest, ACI rats were found to be peculiar: they were characterized by a higher frequency at the end of the AD and not in the beginning. Moreover, ACI rats frequently demonstrated sudden shift from low 3 - 4 Hz to much higher till 9 - 12 Hz frequency of oscillations within AD. As concerns the other two strains, a similar shift was recorded in only a single WAG/Rij rat. We observed exceptionally this fast activity in Wistar rats in other experiments (unpublished observations). These facts give a reason to suggest that different reactivity rather than threshold characteristic of nervous cells may account for increased excitability in agouti (ACI) vs albino (Wistar and WAG/Rij) rats. Since majority of Wistar rats also develop spontaneous SWDs during aging (van Luijtelaar et al., 1995), we suggest that this difference in reactivity of cortico-thalamic cells in response to the same stimuli may also underlie the development of SWDs.

A parameter that revealed a difference between epileptic WAG/Rij and other two strains, was the duration of low frequency spike-and- wave AD. Both young and old WAG/Rij rats had considerably longer duration of low frequency spike-and-wave AD in comparison to age-matched ACI and Wistar rats. This result clearly indicates impaired inhibition of generalized thalamo-cortical oscillations in WAG/Rij rats. Interestingly, the lack of either age effect and age-strain interaction for this parameter suggests that this seizure arresting mechanism is already altered before the presence of SWD in WAG/Rij rats. To date, little is known about seizures arresting mechanisms. Adenosine is one of the neurotransmitters that affect duration of different epileptic phenomena. In our laboratory, it was shown that caffeine, an antagonist of adenosine receptors, prolongs epileptic AD more efficiently than agents compromising GABA-ergic inhibition (Koryntova et al. 2002). On the other hand, adenosinergic system is altered in genetic epileptic rats from Strasbourg (GAERS). Reticular (nRT) and anterior ventral (AV) thalamic nuclei as well as basal ganglia of GAERS have a lower density of A1 receptors (15% decrease) compared to control animals (Ekonomou et al, 1998). An adenosinergic inhibition is considered to exert an anti-oscillatory effect on thalamic nuclei by suppressing (via A1 receptors) excitatory as well as inhibitory neurotransmitter release (Kostopoulos, 2000). However, an injection of adenosine induces a dose-dependent increase in the appearance of SWDs in WAG/Rij rats (Ilbay et al., 2001). Taken together, these results may indicate diverse, region specific role of adenosine in the control of SWDs and in the duration of AD.

The last, but not the least finding concerned the fourth phenomenon, it indicates the excitability of the limbic system. Unexpectedly, WAG/Rij rats exhibited the lowest threshold for the spread of epileptic activity into the limbic structures in comparison with two other strains; moreover, in all strains this threshold decreased with age. The low threshold of this transition in WAG/Rij rats may be due to either an augmented excitability of structures mediating spread of epileptic activity into the limbic system (probably thalamic nuclei with limbic projections) or an increased excitability of limbic structures in WAG/Rij rats. Little evidence is available concerning the role of limbic system in absence epilepsy. The inverse correlation between individual threshold current intensities of this phenomena and individual total and mean duration of spontaneously occurring SWDs found in WAG/Rij rats suggests that excitability of the limbic system is apparently related to the occurrence of spontaneous SWDs in WAG/Rij rats. It is currently difficult to account for the role of limbic system in genetic absence epilepsy. However, there are some data in GAERS that also point to changes in the limbic system. In comparison to non-epileptic control rats, at postnatal day 21 (before the occurrence of SWDs), GAERS have higher brain metabolic activation in limbic regions, but not in the thalamo-cortical loop (Nehlig et al, 1998). There is no longer any difference between brain regions in adult GAERS expressing SWDs. It was suggested that metabolic changes are present throughout the brain and translated into SWDs in the cortico-thalamic loop. Lason et al (1992) found also elevated levels of a-neoendorphin in the hippocampus of 6 months old WAG/Rij rats in comparison with younger rats of the same strain and age matched ACI rats. Finally, the rostral pole of the nucleus reticular thalamus (nRT), which is known to play a role in the generation of SWD, does belong to the limbic system. The nRT is a key structure in the generation of sleep spindles and spike-wave discharges (Avanzini et al., 1992). Moreover, lesions of the lateral thalamus including the rostral pole of the nRT abolished SWD in GAERS and WAG/Rij rats (Avanzini et al., 1992; van Luijtelaar et al., 2001; Meeren, 2002). The rostral pole is connected with various motor and limbic centres, the middle and caudal parts of the nRT are connected with the thalamocortical relay cells (Lubke, 1993). Most of the projections to and from the nRT are ipsilateral. However, commissural connections from the rostral pole of the nRT to selected nuclei of the contralateral thalamus have been reported in the rat (Chen et al, 1992; Raos and Bentivoglio, 1993; Battaglia et al, 1994). Through these bilateral connections the nRT may influence the activity of wide territories of the

cerebral cortex of both hemispheres and these areas might be involved in the synchronization of spontaneously occurring SWD as well as the limbic seizures. A final argument for the role of the thalamic relay nuclei and the nRT in limbic seizures was demonstrated by injections of carbachol in the ventral basal complex of the thalamus and in the nRT. It induces behavioural and electrocortical limbic seizures in rats (Mraovitch and Calando, 1995). The strain difference suggests that the lowered seizure threshold for limbic seizures precipitates the presence of SWD in the EEG. Therefore, the mechanisms involved in the limbic seizure threshold might be causative for the development of SWD from 2-3 months onwards. In addition, the age-dependent decrease of the threshold for limbic seizures may reflect general characteristic of the aging process of the limbic part of the brain. The role of the limbic in the development of absence seizures deserve to be further investigated.

In addition, it should be taken into account that repeated electrically induced seizures lead to a step-wise increase in the corticosterone response (Young et al., 1990). Neurons of the limbic system containing large quantities mineralocorticoid receptors are one of the first potential target affected by corticosterone (Joels and de Kloet, 1992; Reddy and Rogawski, 2002). Considering the duration of present experiment, which last up to 4-5 hours, effects of corticosterone on excitability of limbic system may interfere with effects of cortical stimulation. Consequently, presumable differences in the pattern and amount of corticosterone released over the course of repeated stimulation may also account for strain differences found in the present experiment.

In conclusion, WAG/Rij rats of six months have lower thresholds than Wistar rats but not to ACI rats for most tests. WAG/Rij rats have a lower threshold for the spread into the limbic system and prolonged duration of low frequency of spike-and-wave AD. This outcome is in disagreement with Gloor's theory, that absence seizures are due to a hyperexcitable cortex. The threshold of limbic type of AD is susceptible to aging processes and goes in parallel with an age-dependent increase in the occurrence and duration of SWDs. In addition, the correlation between the number and duration of SWDs with the limbic seizure threshold in WAG/Rij rats further emphasizes a role of the limbic system in generalized absence epilepsy.

References

Avanzini, G., de Curtis, M., Marescaux, C., Panzica, F., Spreafico, R., Vergnes, M., 1992. Role of the thalamic reticular nucleus in the generation of rhythmic thalamo-cortical activities subserving spike and waves. J. Neural. Transm. Suppl. 35, 85-95.

Battaglia, G., Lizier, C., Colacitti, C., Princivalle, A., Spreafico, R.A., 1994. Reticulo-reticular commissural pathway in the rat thalamus. J. Comp. Neurol. 347, 127-138.

Chen, S., Raos, V., Bentivoglio, M., 1992. Connections of the thalamic reticular nucleus with the contralateral thalamus in the rat. Neurosci. Lett. 147, 85-88.

Coenen, A.M., Drinkenburg, W.H., Inoue, M., van Luijtelaar, E.L., 1992.Genetic models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. Epilepsy Res. 12, 75-86.

Coenen, A.M., van Luijtelaar, E.L., 2003. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. Behav. Genet. 33, 635-655.

Crunelli, V., Leresche, N., 2002. Childhood absence epilepsy: genes, channels, neurons and networks. Nat. Rev. Neurosci. 3, 371-382.

De Bruin, N.M., van Luijtelaar, E.L., Cools, A.R., Ellenbroek, B.A., 2001. Auditory information processing in rat genotypes with different dopaminergic properties. Psychopharmacology 156, 352-359.

Dyer, R.S., Swartzwelder, H.S., Eccles, C.U., Annau, Z., 1979. Hippocampal afterdischarges and their post-ictal sequelae in rats: a potential tool for assessment of CNS neurotoxicity. Neurobehav. Toxicol. 1, 5-19.

Ekonomou, A., Angelatou, F., Vergnes, M., Kostopoulos, G., 1998. Lower density of A1 adenosine receptors in nucleus reticularis thalami in rats with genetic absence epilepsy. Neuroreport 9, 2135-2140.

Gloor, P., Fariello, R.G., 1988. Generalized epilepsy: some of its cellular mechanisms differ from those of focal epilepsy. Trends Neurosci. 11, 63-68.

Haugvicová, R., Bílková, E., Kubová, H., Mareš, P., 2002. Effects of classical antiepileptics on thresholds for phenomena induced by cortical stimulation in rats. J. Pharmacol. 54, 1011-1015.

Ilbay, G., Sahin, D., Karson, A., Ates, N., 2001. Effects of adenosine administration on spike-wave discharge frequency in genetically epileptic rats. Clin. Exp. Pharmacol. Physiol. 28, 643-646.

Inoue, M., Peeters, B.W., van Luijtelaar, E.L., Vossen, J.M., Coenen, A.M., 1990. Spontaneous occurrence of spike-wave discharges in five inbred strains of rats. Physiol. Behav. 48, 199-201.

Joels, M., de Kloet, E.R., 1992. Control of neuronal excitability by corticosteroid hormones. Trends Neurosci. 15, 25-30.

Kliouеva, I.A., van Luijtelaar, E.L., Chepurnova, N.E., Chepurnov, S.A., 2001. PTZ-induced seizures in rats: effects of age and strain. Physiol. Behav. 72, 421-426.

Kostopoulos, G.K., 2000. Spike-and-wave discharges of absence seizures as a transformation of sleep spindles: the continuing development of a hypothesis. Clin Neurophysiol. 111, Suppl. 2, 27-38.

Koryntová, H., Kubová, H., Tutka, P., Mareš, P., 2002. Changes of cortical epileptic afterdischarges under the influence of convulsant drugs. Brain. Res. Bull. 58, 49-54.

Kubová, H., Lanštiaková, M., Mocková, M., Mareš, P., Vorlíček J., 1996. Pharmacology of cortical epileptic afterdischarges in rats. Epilepsia 37, 336-341.

Kubová, H., Mocková, M., Mareš, P., 1999. Midazolam suppresses spike-and-wave rhythm accompanying three different models of epileptic seizures. Physiol. Res. 48, 491-500.

Lason W, Przewlocka B, Van Luijtelaar EL, Coenen AM, Przewlocki R., 1992. Endogenous opioid peptides in brain and pituitary of rats with absence epilepsy. Neuropeptides 21:147-152.

Lubke, J., 1993. Morphology of neurons in the thalamic reticular nucleus (TRN) of mammals as revealed by intracellular injections into fixed brain slices. J. Comp. Neurol. 329, 458-471.

Luhmann, H.J., Mittmann, T., van Luijtelaar, G., Heinemann, U., 1995. Impairment of intracortical GABAergic inhibition in a rat model of absence epilepsy. Epilepsy Res. 22, 43-51.

Mareš, P., Haugvicová, R., Kubová, H., 2002. Unequal development of thresholds for various phenomena induced by cortical stimulation in rats. Epilepsy Res 49, 35-43.

Meeren, H.K.M., 2002. Cortico-thalamic mechanisms underlying generalized spike-wave discharges of absence epilepsy. A lesional and signal analytical approach in the WAG/Rij rat. PhD Thesis NICI Nijmegen University, p.172.

Meeren, H.K., Pijn, J.P., van Luijtelaar, E.L., Coenen, A.M., Lopes da Silva, F.H., 2002. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. J. Neurosci. 22, 1480-1495.

Mraovitch, S., Calando, Y., 1995. Limbic and/or generalized convulsive seizures elicited by specific sites in the thalamus. Neuroreport. 6, 519-523.

Nehlig, A., Vergnes, M., Boyet, S., Marescaux, C., 1998. Metabolic activity is increased in discrete brain regions before the occurrence of spike-and-wave discharges in weanling rats with genetic absence epilepsy. Dev. Brain Res. 108, 69-75.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation. II. Motor seizures. Electroenceph. Clin. Neurophysiol. 32, 281-294.

Raos, V., Bentivoglio, M., 1993. Crosstalk between the two sides of the thalamus through the reticular nucleus: a retrograde and anterograde tracing study in the rat. J. Comp. Neurol. 332, 145-154.

Reddy, D.S., Rogawski, M.A., 2002. Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. J. Neurosci. 22, 3795-3805.

Van de Bovenkamp-Janssen, M.C., Korosi, A., Veening, J.G., Scheenen, W.J.J.M., van Luijtelaar, E.L.J.M., Roubos, E.W., 2004. Neuronal parvalbumin and absence epilepsy in WAG/Rij rats. In: van Luijtelaar et al., eds. The WAG/Rij model of absence epilepsy: the Nijmegen-Russian Federation papers, NICI, Nijmegen, pp. 29-36.

Van Luijtelaar, E.L., Coenen, A.M., 1986. Two types of electrocortical paroxysms in an inbred strain of rats. Neurosci. Lett. 70, 393-397.

Van Luijtelaar, E.L., Ates, N., Coenen, A.M., 1995. Role of L-type calcium channel modulation in nonconvulsive epilepsy in rats. Epilepsia 36, 86-92.

Van Luijtelaar, E.L., Weltink, J., 2001. Sleep spindles and spike-wave discharges in rats. In: Sleep-wake in the Netherlands, Vol.12. Eds Alex van Bemmel et al., pp. 81-86.

Young, E.A, Spencer, R.L., McEwen, B.S., 1990. Changes at multiple levels of the hypothalamo-pituitary adrenal axis following repeated electrically induced seizures. Psychoneuroendocrinology 15, 165-172.

Źivanović, D., Bernášková, K., Kaminskij, Yu., Mareš, P., 2003. Action of GABA-B antagonist on cortical epileptic afterdischarges is similar to that of GABA-A antagonist. Physiol. Res. 52, 651-655.

Chapter 5

Absence seizures are reduced by the enhancement of GABA-ergic inhibition in the hippocampus in WAG/Rij rats

Elena A. Tolmacheva and Gilles van Luijtelaar

Abstract. Classical theories on absence epilepsy suggest that spike-wave discharge (SWDs) represent thalamo-cortical oscillations, where an abnormally excitable cortex interacts with thalamus and brain stem reticular formation. The limbic system is generally not included in any theory about the pathogenesis of absence seizures. However, some data demonstrated that the alterations in the limbic system attribute to the expression of absence epileptic phenotype in genetic models of absence epilepsy. The present study investigated whether local intrahippocampal administration of progesterone (a $GABA_A$-mimetic) and tiagabine, an inhibitor of GABA re-uptake, might affect the occurrence of SWDs. Male WAG/Rij rats were implanted with permanent electroencephalograph (EEG) electrodes and bilateral cannulas in the CA1-CA3 region of the dorsal hippocampus. Control rats had bilateral cannulas in the cortical area above the hippocampus. Rats received intracerebral injections of progesterone (5 mg/ml), 45% -cyclodextrin (CD), saline, or tiagabine (2mg/ml). EEG recordings were made before and after injection. Progesterone, CD, and tiagabine administration to the hippocampus reduced SWDs for 60 min following administration without behavioral or electroencephalographic side-effects. Both progesterone administration into the cortex and saline injection into the hippocampus yielded no changes in the occurrence of SWDs. These data suggest that activation of GABA-ergic transmission in the hippocampus has an inhibitory effect on cortico-thalamo-cortical circuits underlying the generation of SWDs and might be critically involved in the regulation of absence seizures.

Keywords: absence seizures, hippocampus, progesterone, tiagabine, GABA, WAG/Rij rats.

1. Introduction

Absence epilepsy is a generalized, non-convulsive form of epilepsy, characterized by spontaneously occurring bursts of bilateral synchronous spike-wave action that are accompanied by a decrease of consciousness. Episodes of this electroencephalographic activity, so-called spike-wave discharges (SWDs), can be recorded by EEG and may appear up to a few hundreds times per day. Mechanisms underlying the generation of SWDs have being explored since the middle of the last century [16]. The most dominant theory, Gloor's classical concept of cortico-reticular epilepsy,

presumes that SWDs represent a thalamo-cortical type of oscillation, where an abnormal excitable cortex interacts with the thalamus and brain stem reticular formation.

Whether a hyperexcitable cortex is indeed a sufficient condition for the occurrence of SWDs was recently tested in WAG/Rij rats [26], which are commonly considered to be a well validated genetic model of absence epilepsy [6]. We found that, in accordance with Gloor's theory, inbred WAG/Rij rats demonstrate higher cortical excitability in comparison with outbred Wistar rats, but not in comparison with non-epileptic inbred control rats of the ACI strain. These data suggest that in addition to a hyperexcitable cortex, the pathogenesis of absence epilepsy may involve other factors. Interestingly, in the same study we found that WAG/Rij rats exhibited a low threshold for the spread of epileptic activity into limbic structures in comparison with Wistar and ACI control rats. This limbic threshold decreased with age and showed an inverse correlation with the number of SWDs, which exhibited an age dependent increase [26]. However, the hippocampus is generally not included in any theory about the pathogenesis of absence epilepsy. Indeed, neither recordings of field potentials, nor single unit activity in the hippocampus in WAG/Rij rats, have indicated involvement of the hippocampus [9,10].

The limbic system and, especially, the hippocampus, is a particular steroid-sensitive area. Steroids hormones are well known to exert powerful effects on the nervous system development and functioning and modulate seizure susceptibility [17, 21]. A series of recent studies in WAG/Rij rats also suggest an important role of steroids hormones, such as progesterone and corticosterone, in the regulation of absence seizures [22, 25, 26, 27]. In an acute experiment it was shown that the effect of progesterone on absence seizures is mediated by its neuroactive metabolite, allopregnanolone, known to facilitate $GABA_A$ receptor inhibitory function [27]. However, neuroactive metabolites of steroid hormones, also known as neurosteroids, are able to modulate not only the GABA-ergic system but also the glutamatergic (NMDA), cholinergic and opioid system [17] - all are also involved in the regulation of absence seizures [6, 7].

The present study investigated whether progesterone, $GABA_A$ – mimetic, and/or tiagabine, a specific GABA (re)uptake inhibitor [13], injected into the hippocampus, an area rich in inhibitory GABA interneurons, would alter the occurrence of absence seizures. We hypothesized that if increased excitability of hippocampal neurons underlies the appearance of absence seizures, then facilitation of GABA-ergic

inhibition (by local administration of progesterone and/or tiagabine) should result in a decrease of SWDs.

2. Materials and methods

2.1. Animals

The present study was performed in male WAG/Rij rats, 5-6 months of age, obtained from the breeding colony at the Department of Biological Psychology, Radboud University Nijmegen. All rats were group-housed prior to surgery and individually following surgery in a temperature-controlled room (21±1°C), on a 12/12-h reversed light cycle (lights off at 8 a.m.). Food and water were available *ad libitum*. All manipulations with animals were approved by the Institutional Animal Care and Use Committee of Radboud University Nijmegen.

2.2. Surgery

Surgery to implant a standard tripolar EEG-electrode set (MS333/1-A, Plastic One, Roanoke, VI, USA) was performed under isoflurane inhalation anesthesia. Electrodes were placed using the following coordinates: AP = + 2.0, L = + 3.0 and AP = -6.0, L = + 4.0 as active electrodes (the ground electrode placed in the cortex of the cerebellum) and two cannulas (C311G, Plastic One). One group of rats (n=16) had cannulas implanted into the CA3 region of the dorsal hippocampus AP = - 3.8; L = ± 2.2; DV= 3.5. In order to ensure that effects of hippocampal manipulation were not due to non-specific effects of microinjection, a second group of rats (n=8) had cannulas aimed to the cortex above the hippocampus (AP = - 3.8; L = ± 2.2; DV=1.5). All stereotaxic coordinates were according to Paxinos and Watson [19]. The assembly of the three electrodes and two cannulas was attached to the skull surface using dental cement and jewelers screws. Following surgery, rats were allowed to recover for at least 2 weeks.

2.3. Drugs

Progesterone (Sigma) (5 mg/ml) was dissolved in 45% 2-hydroxypropyl-γ-cyclodextrin (CD). Tiagabine [(R-)-N- (4,4-di(3-methylthien-2-yl)but-3-enyl) nipecotic acid hydrochloride] (Sigma) (2

mg/ml) was dissolved in saline. The solutions were prepared immediately prior to administration.

2.4. Microinjection procedure

Hippocampal and cortical microinjections were performed through bilateral guide cannulas (C311I, 28-gauge, Plastic One) using injection needles (31-gauge) connected by a polyethylene tube to a 5-µl Hamilton micro syringe. The injection needles were inserted 0.5 mm beyond the tip of the cannulas. Then 1 µl of progesterone, tiagabine, vehicle (45% CD), or saline were injected bilaterally into CA3 of the hippocampus or the cortex above the hippocampus at a rate of 1 µl / 45 sec and needles were left in place for an additional 1 min. Rats were handled daily prior to experimental manipulations and subjected to 2 mock injections in order to habituate the animals to the procedure. Each rat was injected twice, with the order of drug or control injection counterbalanced, group size n=8. Groups were: progesterone in cortex and hippocampus, CD, saline and tiagabine in the hippocampus. The behavior of the animals was monitored regularly, but not quantified.

2.5. EEG recording

Rats were familiarized with the recording leads for at least 3 days prior to the first day of experimental recording. EEG recordings were registered for 30 minutes before, and 2 hrs after, injections, between 10.30 and 13.00. The EEG were amplified and filtered between 1 and 100 Hz, digitized at 200 Hz and stored for off-line analyses. SWDs were quantified in the EEG: the EEG data were pre-processed by a program, which searched in the EEG for the presence of steep and high-voltage activity with a minimal duration of 1 s. The selected periods of aberrant EEG activity were visually inspected to ensure that these periods contained SWDs on the basis of published criteria, and then quantified [5].

2.6. Histology (verification)

Upon completion of experiments, rats were anesthetized and given a microinjection of 2% cresyl violet to determine the site of drug administration. Rats were then exsanguinated with 0.9% saline solution and then perfused with 4% paraformaldehyde (PFA) in 0.1M phosphate buffer

(PB) (pH=7.3). Following perfusion, brains were removed and post-fixed in 4% PFA in 0.1M PB. Brains were later sectioned coronally at the level of cannulas to verify their placement by visual inspection. Only animals with a proper localization of cannulas in the (CA1-CA3) area of hippocampus and in the cortical area above the corpus callosum were included in statistical analyses.

2.7. Statistics

Initial analyses revealed there was no significant order effect, nor an interaction between order and condition. Hence, in all subsequent analyses, order was not included as a factor. The number of SWDs in 30-minute periods was statistically analyzed by two-way ANOVAs, using time and condition as within and between factors, respectively. Orthogonal trends were used in order to show the changes over time. If the interaction between time and group was significant, separate ANOVAs were done to test the difference between groups for each 30 min period before and after an injection, and if appropriate, followed by *post-hoc* T-tests. A P-level of <0.05 was considered to represent a significant effect.

3. Results

3.1. Hippocampal vs cortical administration of progesterone.

There was a significant effect of time (F_{quad} =16.6, df 1,13, $p<0.001$) with decreased SWDs at the first and second 30 minute time periods. There was also a significant interaction between condition and time (F_{quad}=10.03, df 1,13, $p<0.01$), which was due to a greater decrease in SWDs among rats that received progesterone to the hippocampus compared to rats that received progesterone to the cortex. There was a significant difference between groups at the first 30 min (t=2.45, df 13, $p<0.05$) and second - 30-60 min (t=2.30, df 13, $p<0.05$) periods after injection. Progesterone administered to the hippocampus, but not the cortex, decreased SWDs (Figure 1 A).

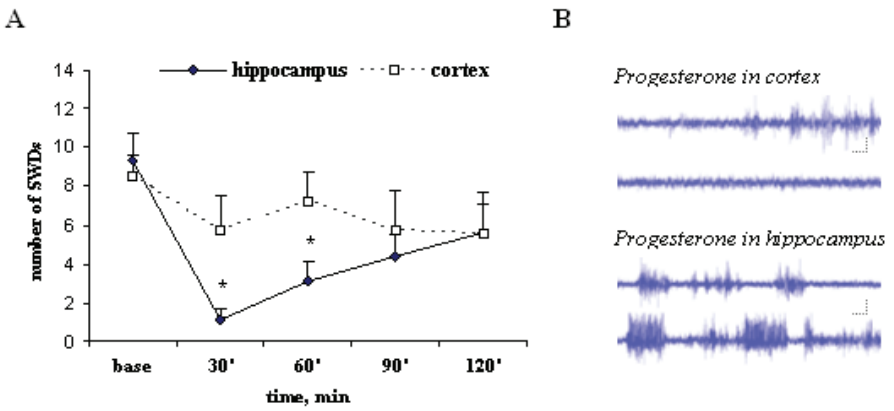

Figure 1. (A) Number (mean ± s.e.m.) of cortical SWDs for five 30 min blocks (1 before (=baseline) and 4 after injection) of rats that received intrahippocampal (n=8) or intracortical (n=7) progesterone injections. The stars indicate a significant difference between the groups (*- $p<0.05$ according to *post-hoc* t-test). (B) Representative examples of EEG recordings at around 30 min after administration of progesterone in cortex and hippocampus in different animals. +/- indicate the polarity of the EEG recordings. Time mark 3 s, amplitude calibration 300 μV.

3.2. Hippocampal injections of progesterone and CD

There was a significant effect of time ($F= 12.33$, df 5,70, $p<0.001$) with a significant quadratic ($F_{quadr} =34.34$, df 1, 14, $p<0.001$) trend (a decrease followed by an increase), but no significant effect of condition. A subsequent paired sampled T-test for each data series showed a significant decrease in the number of SWDs in the first 30 minutes and between 30 and 60 minutes after both progesterone ($t=6.68$ and $t=5.18$, df 7, $p<0.001$) and CD injections ($t=3.5$ and $t=3.4$, df 7, $p<0.01$, Figure 2A). The same was found if the data set were normalized for the base-line score (there was a small non-significant difference between the progesterone and CD group in the base-line).

3.3. Hippocampal injections of tiagabine and saline.

This examination revealed a significant main effect of time ($F=6.34$, df 4, 48, $p<0.001$) and condition ($F=5.08$, df 1,12, $p<0.05$), see also Figure 3A. An orthogonal trend analysis showed a significant quadratic trend in the effect of time ($F_{quadr}=26.5$, df 1,12, $p<0.000$) as well as in the interaction between time and condition ($F_{quad}=7.78$, df 1,12, $p<0.016$).

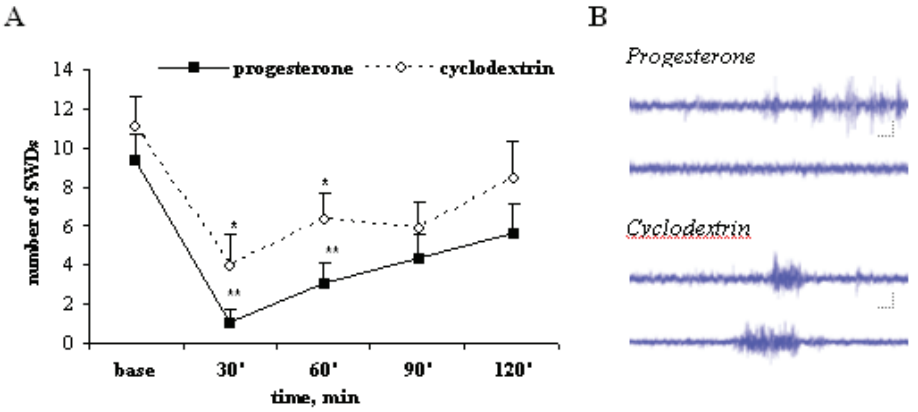

Figure 2. (A) Number (mean ± s.e.m.) of cortical SWDs for five 30 min blocks (1 before (=baseline) and 4 after injection) of rats that received intrahippocampal injections of progesterone (n=8) or cyclodextrin (n=8). The stars indicate a significant decrease compared to the basal level (*- $p<0.01$, ** - $p<0.001$) according to t-test for paired samples). (B) Representative examples of EEG recordings at around 30 min after administration of progesterone and cyclodextrin in different animals. +/- indicate the polarity of the EEG recordings. Time mark 3 s, amplitude calibration 300μV.

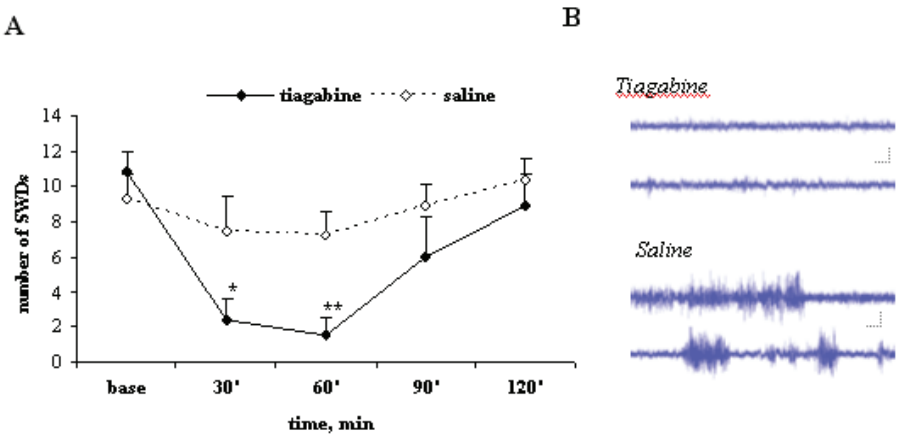

Figure 3. (A) Number (mean ± s.e.m.) of cortical SWDs for five 30 min blocks (1 before (=baseline) and 4 after injection) of rats that received hippocampal tiagabine (n=7) and saline (n=7) injections. The stars indicate a significant difference between the groups (*- $0.05< p <0.06$, **- $p<0.01$ according to post-hoc t-test). (B) Representative examples of EEG recordings at around 30 min after administration of tiagabine and saline in different animals. +/- indicate the polarity of the EEG recordings. Time mark 3 s, amplitude calibration 300 μV.

Tiagabine injected rats tended to have less SWDs in the first 30 minutes ($t=2.76$, df 12, $0.05< p <0.06$), and had significantly less SWDs between 30 and 60 minutes after administration ($t=3.55$, df 12, $p<0.01$) compared to saline injected animals (Figure 3 A).

No behavioral or electroencephalographic side-effects were observed after injections. Representative examples for EEG recordings of different groups at 30 min after administration are presented in Figures 1-3 B.

4. Discussion

The present data suggest that activation of GABA-ergic neurotransmission in the hippocampus might be involved in the modulation of spontaneous absence seizures in genetically epileptic WAG/Rij rats. First, progesterone administration to the hippocampus, but not the cortex, significantly decreased SWDs for 60 minutes. Second, although bothprogesterone and its vehicle, CD, decreased SWDs when administered to the hippocampus, progesterone produced more robust decreases than did CD. Third, tiagabine, but not saline, administration to the hippocampus significantly decreased the occurrence of SWDs. The decrease in SWDs following CD administration was unexpected. However, evidence from both *in vitro* [23] and *in vivo* experiments [28] suggest that cyclodextrins, which are commonly used as solvents for many experimental drugs, may exert their own neuroactive effects. Cyclodextrins have direct effects on $GABA_A$ receptors [23]. Moreover, these cyclic sugar molecules with a hydrophobic core region can sponge neuroactive steroids from endogenous recourses [23]. Both the sponging and the direct effect of cyclodextrins may alter neuronal excitability and account for the reduction of SWDs found after injection of CD into the hippocampus. Hence, although these data support the hypothesis that actions of GABAergic compounds in the hippocampus might be involved in the regulation of absence seizures, the lack of difference between progesterone and CD does not allow us to parse out the effects of progesterone and its solvent. However, the effects of progesterone are also not specific for GABA and may influence a variety of different neurotransmitter systems [17]. Data from our third experiment suggest that drugs specific for activation GABA-ergic transmission can reduce the occurrence of SWDs when administered to the hippocampus. Indeed, the effect of tiagabine, a very specific drug known to inhibit the GABA (re)uptake process, was very prominent and also more similar to that of

progesterone than CD. Taken together, these data suggest that compounds with more specific GABA-ergic activity may have more salient effects on the occurrence of SWDs and that these effects may be due to their actions in the hippocampus.

Interestingly, the anti-seizure (suppression of SWDs) effects of tiagabine (and most likely of progesterone) found in the present experiment are opposite to what has been found in previous studies with systemic injections, in which tiagabine (and progesterone) induced an increase in the number of absence seizures in the same model [5, 26]. However, similarly to our findings, focal bilateral injections of pregnenolone sulphate and allopregnanolone into the peri-oral region of the primary somatosensory cortex also reduced the number and duration of SWDs in WAG/Rij rats [4]. General activation of the GABA-ergic system aggravates absence seizures in both humans and rats [7, 20] and gave a rise to a general postulate regarding absence epilepsy as a condition associated with hyper-function of the GABA-ergic inhibitory system [20]. However, the local enhancement of GABA-ergic inhibition in the reticular thalamic nucleus (RTN) [1, 7] or in the peri-oral region of the somatosensory cortex [4] results in a decrease in SWDs. Hence, the present findings suggest that the hippocampus is another structure, where hypo-, rather than hyper-function of GABA–ergic neurotransmission corresponds to an increased number of SWDs. How, and in which way, this affects the pathogenesis of absence epilepsy needs to be further established.

Cortical SWDs are accompanied by synchronized unit firing in cortex and thalamus and this was never found in the hippocampus in WAG/Rij rats [9, 10] or in any other limbic structure (septum, amygdala, cingular and piriform cortex) in GAERS (Genetic Absence Epileptic Rats from Strasburg) [15]. Therefore, at first glance, the present effects are somewhat surprising. Nevertheless, the contribution of the limbic system in the regulation of SWDs generation might be mediated by the rostral pole of the RTN, which is regarded as part of the limbic system [14]. The RTN is a key structure in the generation of sleep spindles and SWDs, it controls switching from tonic to burst firing mode of thalamo-cortical neurons [3]. The afferents of the middle and caudal parts of the RTN are primarily sensory, while the rostral pole of the RTN is connected with various motor and limbic centres including the hippocampal formation [1]. We suggest that the hippocampus may provide a tonic excitatory input to the rostral part of RTN. However, there is currently no theory to evaluate the extent to which the limbic structures can engage the inhibitory network of the RTN and whether this would have any impact on the occurrence of SWDs.

Interestingly, consistent with our hypothesis about the role of limbic system in absence epilepsy, Deransart et al (2000) showed modulatory effects on absence seizures of dopaminergic neurotransmission in the nucleus accumbens, which is also part of the limbic system. The authors reported that both dopaminergic agonist and antagonist injections in the core of nucleus accumbens resulted in respectively a decrease and an increase in absence seizures without behavioral or electroencephalographic side-effects [8]. The nucleus accumbens receives direct projections from limbic structures, including the hippocampus, and might also play a role in the decrease in the occurrence of SWDs found in the present study. Further studies are required to determine whether activation of GABA-ergic neurotransmission in the hippocampus may indeed enhance dopaminergic activity in the core of the nucleus accumbens, which corresponds to the decreased incidence of SWDs.

Although the mechanisms underlying interactions between the thalamo-cortical and limbic system are unclear, functional alterations in limbic structures are associated with an absence epileptic phenotype in rats. Besides our own data on lower thresholds for limbic type of afterdischarges in WAG/Rij rats [25], Lason and coauthors found elevated levels of α-neoendorphin and up-regulation of the mRNA-encoding prodynorphin in the hippocampus of 6 month old WAG/Rij rats in comparison with younger rats of the same strain and age matched ACI rats [12]. Aker and coauthors [2] found that WAG/Rij rats but also GAERS are more resistant to amygdala kindling. There are also data found in GAERS indicating that at postnatal day 21 (before the occurrence of SWDs), GAERS have higher brain metabolic activation in limbic regions, but not in the thalamo-cortical loop in comparison to non-epileptic control rats [18]. A decreased expression of one of the subunits of the $GABA_A$ receptor [24] as well as an up-regulation of the H-ferritin mRNA was found in the hippocampus of GAERS [11]. All these data show that the pathogenesis of absence epilepsy in WAG/Rij and GAERS involves a variety of alterations in the limbic part of the brain which might be also a consequence of persistent absence seizures.

The present data show that activation of GABA-ergic transmission by tiagabine and progesterone in the hippocampus has an inhibitory effect on cortico-thalamo-cortical circuits. We suggest that hormonal modulation of excitability of hippocampal neurons may play an important role in the pathogenesis of absence epilepsy and that it needs to be investigated

whether this structure might serve as a new putative target for the treatment of absence epilepsy.

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References

[1] Aker, R.G., Ozyurt, H.B., Yananli, H.R., Cakmak, Y.O., Ozkaynakci, A.E., Sehirli, U., Saka, E., Cavdar, S., Onat, F.Y., GABA(A) receptor mediated transmission in the thalamic reticular nucleus of rats with genetic absence epilepsy shows regional differences: functional implications, Brain Res. 1111 (2006), 213-221.

[2] Aker, R.G., Yalanli, H.R., Gurbanova, A.A., Özkaynakçı, A.E., Ateş, N., van Luijtelaar, G., Onat, F.Y., Amygdala kindling in the WAG/Rij rat model of absence epilepsy, Epilepsia 47 (2006) 33-40.

[3] Avanzini, G., de Curtis, M., Marescaux, C., Panzica, F., Spreafico, R., Vergnes, M., Role of the thalamic reticular nucleus in the generation of rhythmic thalamo-cortical activities subserving spike and waves, J. Neural Transm. 35, Suppl. (1992) 85-95.

[4] Citraro, R., Russo, E., Di Paola, E.D., Ibbadu, G.F., Gratteri, S., Marra, R., De Sarro, G., Effects of some neurosteroids injected into some brain areas of WAG/Rij rats, an animal model of generalized absence epilepsy, Neuropharmacol. 50 (2006) 1059-1071.

[5] A.M. Coenen, E.H. Blezer, E.L. van Luijtelaar. Effects of the GABA-uptake inhibitor tiagabine on electroencephalogram, spike-wave discharges and behaviour of rats, Epilepsy Res. 21 (1995) 89-94.

[6] Coenen, A.M., van Luijtelaar, E.L., Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats, Behav Genet. 33 (2003) 635-655.

[7] Danober, L., Deransart, C., Depaulis, A., Vergnes, M., Marescaux, C., Pathophysiological mechanisms of genetic absence epilepsy in the rat, Prog Neurobiol. 55 (1998), 27-57.

[8] Deransart, C., Riban, V., Le, B., Marescaux, C., Depaulis, A., Dopamine in the striatum modulates seizures in a genetic model of absence epilepsy in the rat, Neuroscience 100 (2000), 335-44.

[9] Inoue, M., Duysens, J., Vossen, J.M., Coenen, A.M., Thalamic multiple-unit activity underlying spike-wave discharges in anesthetized rats, Brain Res. 612 (1993) 35-40.

[10] Kandel, A., Bragin, A., Carpi, D., Buzsaki, G., Lack of hippocampal involvement in a rat model of petit mal epilepsy, Epilepsy Res. 23, 2 (1996) 123-127.

[11] Lakaye, B., Thomas, E., Minet, A., Grisar, T., The genetic absence epilepsy rat from Strasbourg (GAERS), a rat model of absence epilepsy: computer modeling and differential gene expression, Epilepsia 43, Suppl 5 (2002) 123-129.

[12] Lason, W., Przewlocka, B., van Luijtelaar, G., Coenen, A., Proenkephalin and prodynorphin mRNA level in brain of rats with absence epilepsy, Neuropeptides 27 (1994) 343-347.
[13] Leach, J.P., Brodie, M.J., Tiagabine, Lancet 351 (1998) 203-207.
[14] Lubke, J., Morphology of neurons in the thalamic reticular nucleus (TRN) of mammals as revealed by intracellular injections into fixed brain slices, J. Comp. Neurol. 329 (1993) 458-471.
[15] Marescaux, C., Vergnes, M., Depaulis, A., Genetic absence epilepsy in rats from Strasbourg - A review, J. Neural Transm. 35, Suppl (1992) 37-69.
[16] Meeren, H., van Luijtelaar, G., Lopes da Silva, F., Coenen, A., Evolving concepts on the pathophysiology of absence seizures: the cortical focus theory, Arch Neurol. 62 (2005) 371-376.
[17] Melcangi, R.C., Panzica, G.C., Neuroactive steroids: old players in a new game, Neurosci. 138 (2006) 733-739.
[18] Nehlig, A., Valenti, M.P., Thiriaux, A., Hirsch, E., Marescaux, C., Namer, I.J., Ictal and interictal perfusion variations measured by SISCOM analysis in typical childhood absence seizures, Epileptic Disord. 6 (2004) 247-253.
[19] Paxinos, G. and Watson, C., The rat brain in stereotaxic coordinates, Academic press, San Diego, CA. (1986).
[20] Peeters, B.W., van Rijn, C.M., Vossen, J.M., Coenen, A.M.. Effects of GABA-ergic agents on spontaneous non-convulsive epilepsy, EEG and behaviour, in the WAG/Rij inbred strain of rats, Life Sci. 45 (1989) 1171-1176.
[21] Rhodes, M.E., Harney, J.P., Frye, C.A., Gonadal, adrenal, and neuroactive steroids' role in ictal activity, Brain Res. 1000 (2004) 8-18.
[22] Schridde, U., van Luijtelaar, G., Corticosterone increases spike-wave discharges in a dose- and time-dependent manner in WAG/Rij rats, Pharmacol Biochem Behav. 78 (2004) 369-75.
[23] Shu, H.J., Eisenman, L.N., Jinadasa, D., Covey, D.F., Zorumski, C.F., Mennerick, S., Slow actions of neuroactive steroids at GABAA receptors, J. Neurosci. 34 (2004) 6667-6675.
[24] Snead 3rd, O.C., Depaulis, A., Banerjee, P.K., Hechler, V., Vergnes, M., The GABAA receptor complex in experimental absence seizures in rat: an autoradiographic study, Neurosci Lett. 140 (1992) 9-12.
[25] Tolmacheva, E.A., Chepurnov, S.A., Chepurnova, N.E., Kochetkov, Y.A., van Luijtelaar, G., Absence seizures during pregnancy in WAG/Rij rats, Physiol Behav. 81 (2004) 623-627.
[26] Tolmacheva, E.A., van Luijtelaar, G., Chepurnov, S.A., Kaminskij, Y., Mares, P., Cortical and limbic excitability in rats with absence epilepsy, Epilepsy Res. 62 (2004) 189-198.
[27] van Luijtelaar, G., Budziszewska, B., Tetich, M., Lason, W., Finasteride inhibits the progesterone-induced spike-wave discharges in a genetic model of absence epilepsy, Pharmacol Biochem Behav. 75 (2003) 889-894.
[28] Wang, M.D., Wahlstrom, G., Backstrom, T., Pregnenolone sulphate and pregnenolone do not interact with 5 beta-pregnanolone- and hexobarbitone-induced anaesthesia in the rat, Br J Anaesth. 78 (1997) 328-331.

Chapter 6

The Hypothalamo-Pituitary-Adrenal axis and stress in the regulation of absence seizures in genetic epileptic rats

Elena A. Tolmacheva, Melly S. Oitzl, Gilles van Luijtelaar

Abstract. In two experiments a putative role of the hypothalamo-pituitary-adrenal (HPA) axis and stress in the pathogenesis of absence epilepsy in WAG/Rij rats was explored. First, basal and acute stress induced plasma corticosterone concentrations were measured in genetically epileptic WAG/Rij, non epileptic inbred ACI and outbred Wistar rats. Second, the number of absence seizures (SWDs) was measured after repeated exposure to foot-shock (FS) stress in WAG/Rij rats.

Three sequential blood samples were collected from tail incision during the dark period of the light-dark cycle before, one week after brain surgery and after acute FS administration in 5-6 months male WAG/Rij, ACI and Wistar rats. Corticosterone was measured in plasma by radioimmunoassay. WAG/Rij rats equipped with EEG electrodes were exposed to three FS series, one series a day. The EEG was recorded 1h before and 2h after each FS series.

WAG/Rij rats tended to have a higher corticosterone plasma level than Wistar rats before and after surgery and after FS exposure, while ACI demonstrated the highest basal and stress induced plasma corticosterone level compared to both other strains. Both inbred (WAG/Rij and ACI) strains showed a depletion of corticosterone following brain surgery. Next, in WAG/Rij rats FS exposure resulted in a decrease (15 min) followed by an increase in SWDs. Repeated exposure to FS reduced initial suppression and increased following aggravation of SWDs. Aggravation in the number of SWDs was also found in the base-line preceding each next FS exposure. The present outcomes indicate that stress, stress anticipation and therefore the HPA axis complex functioning may play a critical role in the pathogenesis of absence epilepsy.

Key words: absence epilepsy, WAG/Rij rats, HPA axis, corticosterone, repeated foot-shocks, stress anticipation.

1. Introduction

Adrenal steroid hormones are known to exert numerous effects on the neuronal excitability and therefore can be critically involved in the pathogenesis of many, if not all, neurological disorders (Joels and de Kloet, 1992). Many reports indicate that stress and increased level of corticosterone may affect seizure susceptibility and underlie the course of epileptic diseases (Bosnjak et al, 2002; Haut et al., 2003; Wiener, 2003). However, in contrary to convulsive epilepsy, little is known about the role of stress and corticosterone in the pathogenesis of absence epilepsy.

Absence epilepsy is a non convulsive type of epilepsy associated with spontaneously occurring bursts of bilateral synchronous spike-wave activity generated in the cortico-thalamo-cortical circuitry. Episodes of spike-wave activity (absence seizures), that are accompanied by a decreased responsiveness and inability to make voluntary movements, usually last 10-20 sec and may appear up to a few hundred times per day (Panayiotopoulos, 2001, Drinkenburg et al., 2003). In a single human study it was reported that children have an enhanced occurrence of absence seizures after stressful events (Bosnjak et al, 2002) that may suggest an involvement of stress and the hypothalamic pituitary adrenal axis (HPA axis) functioning in the pathogenesis of absence epilepsy. Consistent with this data, an acute elevation of corticosterone was shown to enhance absence seizures in genetic epileptic WAG/Rij rats (Schridde and van Luijtelaar, 2004).

Interestingly and relevant to our question, behavioural studies showed that WAG/Rij rats - a well validated genetic model for human absence epilepsy (van Luijtelaar and Coenen, 1986; Coenen and van Luijtelaar, 2003; van Luijtelaar and Sitnikova, 2006), exhibit a number of depressive-like features in their behaviour. They show a decreased or increased ambulation in the open field, increased immobility in Porsolt´s forced swim test and decreased sucrose intake (anhedonia) (Sarkisova et al, 2003, van Luijtelaar et al, 2007), which might also indicate a higher vulnerability to stress in these rats. However, although this hypothesis was already suggested (Sarkisova et al, 2003), the activity of the HPA axis - a major control system of the neuroendocrine stress response in vertebrate organisms, was never investigated in WAG/Rij rats.

The present study was aimed to compare the basal and stress activated functioning of the HPA axis in epilpetic WAG/Rij rats and non-epileptic congenic (inbred) ACI and outbred Wistar rats, which has no or minimal number of SWDs (Inoue et al, 1990). First, we tested whether there are differences in the diurnal pattern of plasma corticosterone level in resting conditions and expected that WAG/Rij rats as a model of depression would have an increased level of corticosterone. In a pilot study, it was shown that implantation of EEG electrodes resulted in the depletion of the basal corticosterone plasma level in subsequent post-operation period. Considering that surgery is shown to activate the HPA axis (De Keyser et al, 2000) and can be regarded as a type of systemic stress, we questioned also whether non-epileptic ACI and Wistar would differ from WAG/Rij rats in their response to this stressor or would also demonstrate a decrease in corticosterone level following surgery. Next, we examined corticosterone

plasma levels in WAG/Rij, Wistar and ACI immediately after a single foot-shock stress exposure considered as an acute mild neurogenic stress (Li et al, 1996) in order to investigate whether the stress reaction was different in genetic epileptic rats.

In the second part of the study, we investigated the effect of the same FS stress on absence seizures in WAG/Rij rats. We tested whether the effect of an acute stress on the occurrence of spike-wave discharges (SWDs) depends on the intensity of the stressor (we used 1, 3 or 10 random FS) and whether this effect would increase or decrease if stress is repeated next day. Considering that Wistar and ACI rats do not have considerable amounts of SWDs, they were not included in this part of the study.

2. Methods

2.1. Animals

The present study was performed with male ACI, Wistar and WAG/Rij rats, 4-5 months of age, all obtained from the breeding colony at the Department of Biological Psychology, Radboud University Nijmegen. All rats were group housed in a temperature-controlled room (21±1°C), on a 12/12-h reversed light cycle (lights off at 8 a.m.) until one-week prior to the first blood sampling in Experiment 1a,b and following surgery in the Experiment 2, then they were individually housed. Food and water were available *ad libitum*. The protocol was approved by the local medical-ethical committee (RU-DEC).

2.2. EEG electrodes implantation

All rats were provided with a standard EEG-electrode set with coordinates: A-P +2.0, L, + 3.0 and A-P -6.0, L, + 4.0 as active electrodes, the earth electrode was placed in the cortex of the cerebellum. Coordinates were according to the atlas of Paxinos and Watson (1986). The surgery was performed under isoflurane inhalation anesthesia. Rats were allowed to recover for at least two weeks following surgery.

2.3. Foot-shocks administration

Animals were individually placed in a Perspex box for foot-shocks (FS) administration. The Perspex box (25x25x40) contained an electrified grid on the floor, through which shocks could be delivered. One, three or ten

scramble electrical shocks (1.5 mA, 1 sec) were given with random (from 1 to 10 sec) inter-shock intervals.

2.4. Blood sampling

Rats were handled and habituated to the blood sampling procedure for one week prior to the experiment. Blood samples were collected using a stress free refined method of tail incision (Fluttert et al., 2000). Blood was collected on ethylenediamin-etetraacetic acid (EDTA) coated microtubes and centrifuged at –10°C within 30 minutes after withdrawal. Plasma samples were stored at –70° C until they were assayed for hormones.

2.5. Hormone assays

Plasma corticosterone immunoreactivity was measured using radioimmunoassay (RIA) kits (ICN Biomedicals). The intra- and interassay coefficients of variations were calculated to be 5.1% and 7.3% respectively, with a detection threshold of 5 pg/ml. Plasma corticosterone levels were assayed with highly specific corticosterone antibodies with minimal detectable level at 0.1 μg /100 ml. The intra- and interassay coefficients of variation were 7.2% and 7.0% respectively.

2.6. Experimental design

Experiment 1a: Basal corticosterone plasma levels were measured in WAG/Rij (n=8), ACI (n=5) and Wistar (n=7) male rats under undisturbed conditions (base-line). Glucocorticoid secretion is known to undergo a daily circadian rhythm; therefore we measured corticosterone level at three different time-points. Three sequential blood samples were collected at 1, 5 hour and 9 hour after light offset (at 9.00 h, 13.00 h, and 17.00 h) one day before and one week after the implantation of the EEG electrodes.

Experiment 1b: Stress induced corticosterone plasma levels were measured in WAG/Rij (n=8), ACI (n=5) and Wistar (n=7) male rats before the implantation of the EEG electrodes. Sequential blood samples were collected before and at 5, 20 and 60 min after FS administration. First, we took the first blood sample from the rats in the home cage. Thereafter the animals were individually placed in the Perspex box and after 15 minutes of confinement they received a series of 3 FS. After the second blood sample

(at 5 min after FS administration) was collected, the rats returned to their home cages. Next two samples (at 20 and 60 min after FS administration) were collected, when animals were in their home cages. The experiment was carried out between the 2rd and the 3rd hour after light onset, when corticosterone level in plasma is quite stable.

Experiment 2*:* WAG/Rij rats (n=21) with implanted electrodes were tested in an experiment with FS administration. First, all the rats were placed individually into a Perspex recording box to get familiarized with the experimental conditions (adaptation) and on the next day, the rats were again placed into this Perspex box for 3 hours for the recording of the basal EEG Day 0. On Day 1, the rats were placed into the same Perspex box and after one hour of confinement, they received a series of 1, 3 or 10 FS (3 groups). On Day 2 and 3, the whole procedure was repeated with different number of FS. Overall, rats were delivered 1, 3 and 10 FS (group 1); 3, 10 and 1 FS (group 2); 10, 1 and 3 FS (group 3). The cortical EEG was recorded 1h before and 2h after each series of FS. The experiments were carried out between the 2rd and the 5th hour after light offset (between 11.00 and 14.00). SWDs were analyzed for 15 min episodes and for base-line the data of the four 15 min episodes were pooled.

2.7. EEG analysis

The EEG were amplified and filtered between 1 and 100 Hz, digitized at 200 Hz and stored for off-line analyses. The EEG data were processed by a program, which searched in the EEG for the presence of series of high-voltage activity with a minimal duration of 1 s. The selected periods of aberrant EEG activity were visually inspected on the basis of standard published criteria, whether these periods contained SWDs (van Luijtelaar and Coenen, 1986).

2.8. Statistics

Experiment 1a. A three-way ANOVA for repeated measurements with time (3 levels) and operation (2 levels: pre-post) as within-subjects factors and strain (3 levels) as between-subjects factor followed by orthogonal trend analysis and post-hoc pairwise comparisons was used for statistical evaluation and description of circadian fluctuations in basal plasma corticosterone levels before and after operation in the three strains of rats.

Experiment 1b. A two-way ANOVA for repeated measurements with time (4 levels) as within- subjects factor and strain (3 levels) as between-subjects factor followed by orthogonal trend analysis and post-hoc pairwise comparisons was used to test and describe a time-course of stress induced plasma corticosterone level in different strains.

Experiment 2. An omnibus ANOVA (with order as between subjects factor) and days (four levels), time (12 15 minute blocks) and FS (4 levels, 0, 1, 3, 10) was not possible considering a lack of sufficient df. Therefore, we decided to analyse the effects of FS and days in separate ANOVAs. Since there was no main effect of order, no first order interactions with order, and only a quite low F-value ($F=1.54$, df 36,414, $p<0.05$) for the second order interaction, we removed in all subsequent analyses the factor order from our data analysis for reasons of parsimoniously.

The level of statistical significance was set at 5 % (two tailed tests) for all variables.

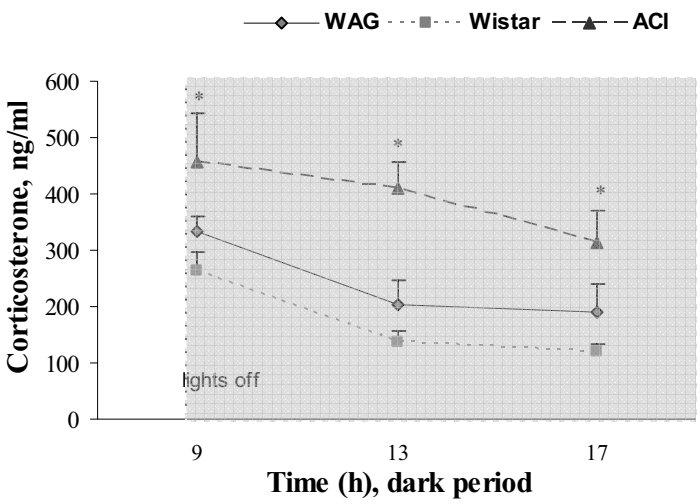
3. Results

3.1. Basal corticosterone plasma levels before and after surgery in WAG/Rij, ACI ands Wistar rats (Figure 1 A,B)

Basal plasma corticosterone was measured at three different time points during the dark period for WAG/Rij, ACI and Wistar male rats before and after operation. A similar diurnal pattern ($F_{time}=29.13$, df=2,34, $p<0.001$; $F_{lin}=54.96$, df=1,17, $p<0.001$) with the highest levels after light-offset and a gradual decrease over subsequent time points ($p<0.01$) was found in all three strains of rats both before and after operation.

The ANOVA revealed a main significant effect of strain ($F_{strain}=5.40$, df=2,17, $p<0.01$) and a post-hoc comparison showed that ACI rats had a higher corticosterone plasma level than both WAG/Rij and Wistar rats ($p<0.05$ and $p<0.01$). No significant difference in basal corticosterone plasma level was found between WAG/Rij and Wistar rats.

A



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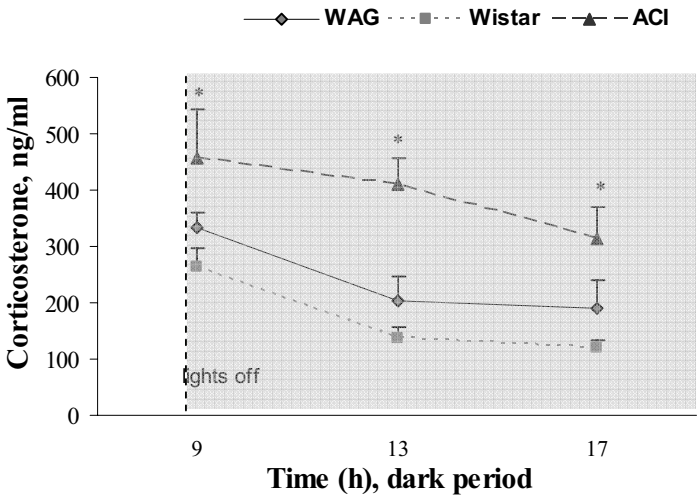

Figure 1. Diurnal fluctuations of plasma corticosterone levels (ng/ml) in inbred epileptic WAG/Rij and non-epileptic inbred ACI and outbred Wistar male rats over the dark phase of one day before (A) and one week after (B) brain surgery. The stars indicate a significant difference between ACI rats and the two other strains (*- $p<0.05$ compared to WAG/Rij rats, $p<0.01$ compared to Wistar rats).

Finally, the ANOVA showed a significant main effect of operation ($F_{operation}$=13.84, df=1,17, p<0.01), which was differential depending on the strain of rats ($F_{operation*strain}$ = 8.76, df=2,17, p<0.01). One-way ANOVA revealed a main significant effect of strain (F_{strain}=13.84, df 2,17, p<0.01) in the difference (delta) between pre and post-surgery corticosterone level and T-tests showed a significant decrease in circulating corticosterone levels after surgery in WAG/Rij and ACI rats (T=2.64, df=7, p<0.05; T=7.55, df=4, p<0.01, respectively) and a tendency to increase in Wistar rats (see also Figure 2).

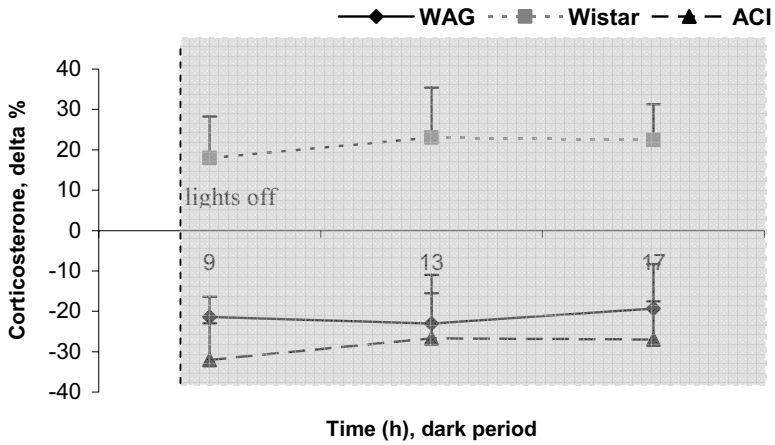

Figure 2. The difference (delta) in % in corticosterone plasma level in inbred epileptic WAG/Rij and non-epileptic inbred ACI and outbred Wistar male rats before and after brain surgery.

3.2. Corticosterone plasma level after FS administration in WAG/Rij, ACI and Wistar rats (Figure 3)

As expected, FS administration resulted in a temporal increase in corticosterone level in plasma (F_{time}=66.21, df=3,54, p<0.001; F_{quadr}=164.09, df=1,20, p<0.001). Post-hoc comparison confirmed an aggravation in plasma corticosterone level at 5 min (p<0.001), further increase from 5 to 20 min (p<0.01) and no significant difference in corticosterone level between base-line and 60 min after FS administration.

The ANOVA revealed a main significant effect of strain (F_{strain}=11.46, df=2,18, p<0.001) and, consistent with the outcomes of the previous experiment, ACI rats had a higher corticosterone plasma level than WAG/Rij and Wistar rats over all measured time-points after FS exposure (p<0.05 and p<0.001).

In this experiment, the basal corticosterone level in WAG/Rij rats before FS exposure (as measured during the light phase of the light-dark cycle) was higher than in Wistar rats (p<0.01) and did not differ from ACI (F=5.92, df=2,18, p<0.01).

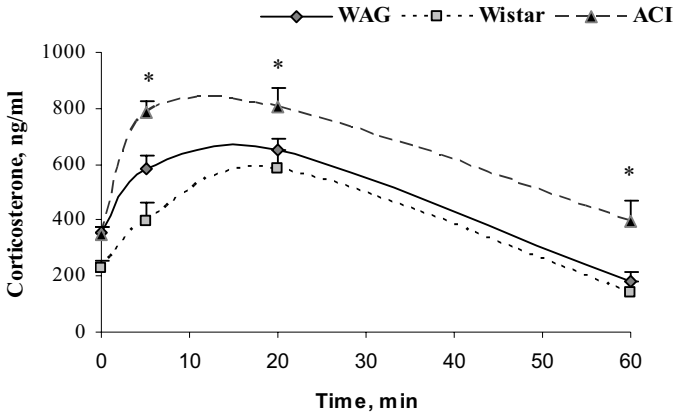

Figure 3. Corticosterone plasma level in inbred epileptic WAG/Rij and non-epileptic inbred ACI and outbred Wistar male rats.before and at 5, 20 and 60 min after exposure to foot-shock stress in The stars indicate a significant difference between ACI rats and the two other strains (*- p<0.05 compared to WAG/Rij rats, p<0.001 compared to Wistar rats).

3.3. Effect of FS administration on the occurrence of SWDs (Figure 4)

Exposure to FS changed the absence seizure susceptibility in WAG/Rij rats (F_{time}=7.41, df=6,150, p<0.001; F_{day}=4.35, df=3,75, p<0.01) with differential responses depending on day and time after the FS ($F_{time*day}$=2.14, df=18, 450, p<0.01). Significant biphasic changes in the number of SWDs over time were revealed by orthogonal trend analysis (F_{quadr}=4.44, df=1,25, p<0.05). The initial decrease at the first 15 min after FS administration was followed by an increase between 15 and 60 min after FS exposure and a subsequent decrease back to the base-line level.

A day-to-day increase in the occurrence of SWDs was found between 15-30 minutes (F_{day}= 3.57, df=3,75, p<0.001; F_{lin}=12.24, df=1.25, p<0.05),

between 30-45 minutes (although only a trend - F_{lin}=4.34, df 1,25. p<.05) and between 45-60 min (F_{day}=5.35, df=3,75, p<0.01; F_{lin}=8.60, df=1,25, p<0.01) after FS administration. An occurrence of SWDs in the first 0-15 minutes interval was characterized by a significant cubic orthogonal trend (F_{day}= 2.75, df=3,75, p<0.001; F_{cub}=6.93, df=1.25, p<0.05), characterizing a decrease from Day 0 to Day 1, followed by a slow increase from Day 1 to Day 3. All these findings illustrate that after each next FS series (across the days) the initial suppression of SWDs is reduced, while the following aggravation of SWDs become more prominent.

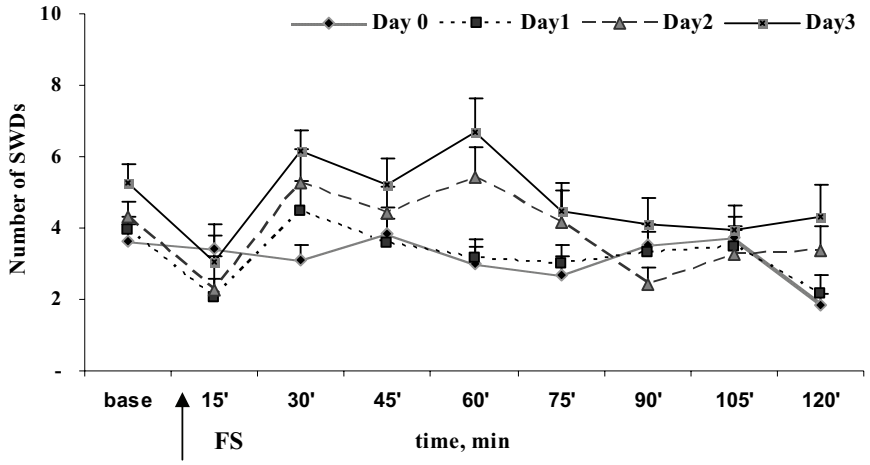

Figure 4. The number of SWDs in base conditions (Day 0) and before (base) and 120 min after foot-shock (FS) exposure at the first (Day 1), the second (Day 2) and the third (Day 3) experimental days in WAG/Rij male rats.

Interestingly, a day-to-day increase in the number of SWDs was also present in the occurrence of SWDs preceding FS administration (F_{day}=3.74, df=3,75, p<0.05; F_{lin}=8.82, df=1,25, p<0.05). Moreover, an ANOVA performed for the data (number of SWD following FS) normalized to the level in the occurrence of SWDs preceding the FS exposure failed to show a main significant effect of day. This suggests that a day effect characterized by an increase in the number of SWDs after each next FS series is at most determined by the changes occurring in the period preceding FS stress exposure.

Finally, there was a significant interaction between the number of FS and time ($F_{number\ of\ FS*time} = 1.91$, df=12,276, $p<0.05$). A significant effect of the number of FS was found in the first 15 min after FS administration ($F_{number\ of\ FS} = 4.18$, df 3,83, $p<0.01$), in which presentation of 10 FS was more effective in suppressing SWDs than 1 FS (see also Figure 5). Consistently, due to a following aggravation in SWDs, between 15-30 minutes the rats that received 1 and 3 FS showed already more SWDs than the unshocked group ($F_{number\ of\ FS} = 3.17$, df 3,83, $p<0.05$). No significant effect of the number of FS was found between 30-45, 45-60 min suggesting an equal increase in the number of SWDs found at these episodes regardless the number of FS.

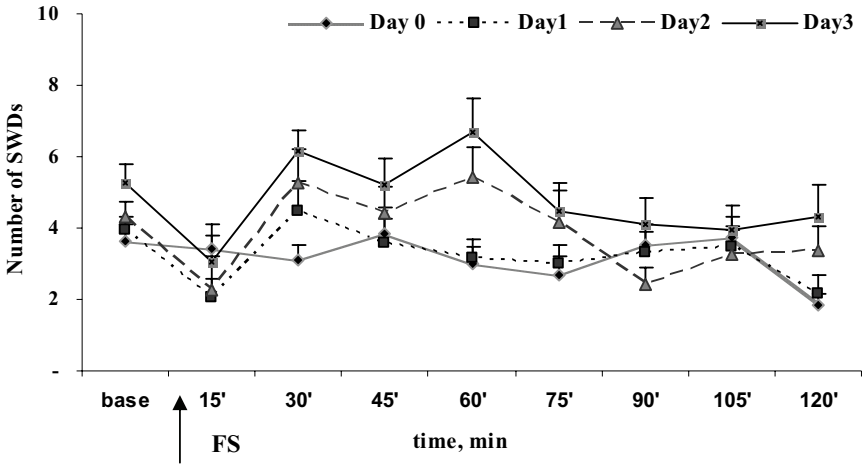

Figure 5. The number of SWDs and before (base) and 120 min after exposure of 1, 3 or 10 foot-shocks (FS) in WAG/Rij male rats.

4. Discussion

First of all, the rats of all three strains demonstrated a similar diurnal pattern with the highest corticosterone plasma level occurring at the beginning of the dark part of the light-dark cycle and showed a slow decline over this period, consistent with data from others (Koehl et al, 1999). Epileptic WAG/Rij rats tended to have a higher corticosterone level than non-epileptic Wistar rats, but it did not reach a significant value in this part of the study. Inbred non-epileptic ACI rats exhibited the highest plasma

corticosterone level compared to both Wistar and WAG/Rij rats over all measured time-points. ACI rats are more agitated than albino rats and their show a low ambulation score in a bright lit open field (de Bruin et al., 2001). Interestingly, the circadian distribution of absence seizures in WAG/Rij rats has a similar diurnal pattern with the only difference that the peak in SWDs occurs 3-4 hours after the light off-set (van Luijtelaar and Coenen, 1988).

Exposure to FS disturbed circadian secretion rhythm and resulted in an increased plasma corticosterone with maximum between 5-20 min after exposure and return to the starting level between 20 and 60 min in all three strains. Similarly, WAG/Rij rats tended to have slightly higher corticosterone in plasma after stress exposure than Wistar rats, whereas ACI rats again had the highest corticosterone response compared to both Wistar ands WAG/Rij rats. Interestingly, in this experiment conducted during the light phase of the light-dark cycle, in the base-line conditions before the FS exposure WAG/Rij rats had a significantly higher corticosterone level in plasma compared to Wistar and did not differ from ACI. Based upon these data, we can conclude that inbred WAG/Rij rats tend to have a slightly higher plasma corticosterone than outbred Wistar rats, but the highest corticosterone secretion is characteristic for inbred ACI rats.

The two inbred strains (ACI and WAG/Rij) also differed from the outbred strain in the plasma corticosterone response one week following brain surgery: corticosterone was decreased in ACI and WAG/Rij rats, while no differences in corticosterone levels before and after operation were found in Wistar rats. Surgical stress is known to induce acute activation of the HPA-axis in rats for at least 24 hours (De Keyser et al, 2000). A depletion in the corticosterone levels, found one week following brain surgery in both inbred strains WAG/Rij and ACI, may indicate a lower resistance to this acute systemic stress and implies a higher vulnerability of the HPA axis in both inbred strains. However, the mechanisms of this 'recovery' process accompanied by an inversed reaction of the HPA axis needs to be further investigated.

In the next experiment (conducted only in WAG/Rij rats), we demonstrated that exposure to FS stress had a biphasic effect on the number of absence seizures: a quick decrease in the first 15 minutes was followed by an increase in SWDs. A similar biphasic effect, an initial 15 min decrease followed by an increase in SWDs, was observed after an acute systemic injection of corticosterone (Schridde and van Luijtelaar, 2004). Consistent with this is also the fact that both mild (saline injection) and

severe acute stress (immobilization) concomitant with corticosterone release are known to lead to an aggravation in the number of SWDs in WAG/Rij rats (De Bruin et al, 2000; unpublished data). The initial suppression in SWDs was dependent on the number of FS and was largest after 10 FS, while the subsequent aggravation in SWDs was irrespective of the number of FS and was the same in all three conditions. Conversely, we found a reduction in the initial suppression and an increase in further aggravation in SWDs from day to day, when FS exposure was repeated. Remarkably, this observation is consistent with the classical theory on emotions of Solomon and Corbit (1974). They described dynamics in opponent reactions towards emotional stimuli as consequence of repeated stimulation: the initial decrease in SWD becomes shorter over days, while the opponent reaction, the aggravation, becomes larger over time.

An increase in SWDs over days was also present in the base-line, before FS administration, when animals were placed in the Perspex box, which is obviously linked to an anticipatory response (classical Pavlovian fear conditioning). The anticipatory response is capable to activate the HPA-axis and regulate glucocorticoid release response under conditions in which physical challenges may be predicted (Herman et al, 2003). Indeed, we found that on the second and the third day, corticosterone level was already significantly increased before FS administration (unpublished data). Interestingly, the hippocampus and prefrontal cortex known to be the key-structures in contextual fear conditioning (Antoniadis and MacDonald, 2000; Maren and Quirk, 2004) as well in the regulation of the HPA axis (Herman et al, 2003) are also involved in the regulation of SWDs in WAG/Rij rats (Tolmacheva and van Luijtelaar, 2007; Midzyanovskaia et al., 2006). Based on this, we suggest that the aggravation of SWDs over days preceding and following repeated FS exposure might be linked to stress induced activation of limbic structures and even underlie the predisposition of these rats to develop a depressive like state described by Sarkisova and coauthors (2003). Further investigation of the mechanisms underlying this increased aggravation in the occurrence of SWDs might be relevant to understand the development of depression concomitant to absence epilepsy in this model.

Finally, acute stress is known to increase plasma and brain concentrations of corticosteroids and neuroactive steroids, which modulate GABA-ergic neurotransmission (Barbacia et al, 1997, 2001). The initial reaction after acute stress, an immediate decrease in SWD, is associated with a depression in GABA-ergic neurotransmission (Barbaccia et al., 1997, 2001), likely mediated by glucocorticoid metabolites (Stromberg et al, 2005). However, the initial alerting phase is followed by a release of

neurosteroids (such as pregnenolone, allopregnanolone, 3a5a-THDOC) facilitating GABA–ergic neurotransmission (Barbaccia et al., 1997, 2001; Stromberg et al, 2005) and SWD are increased. These neurosteroids are thought to balance excitatory and inhibitory inputs in complex neural circuits (Stromberg et al, 2005; Saalman et al, 2006). Considering that GABA-ergic neurotransmission is intimately involved in the generation of SWDs (Coenen and van Luijtelaar, 2003), changes in the number of SWDs after stress may reflect an interplay between excitatory and compensatory inhibitory processes in cortico-thalamic systems and/or the other systems involved in the regulation of SWDs, such as limbic and striatal system (Tolmacheva and van Luijtelaar, 2007; Deransart et al, 2001). In addition to neurosteroids, the role of stress induced activation of noradrenergic as well as the other catecholaminergic systems (Midzyanovskaya et al, 2006; Goldstein et al, 2005) known to modulate the incidence of SWDs (Sitnikova and van Luijtelaar, 2005) can also not be ruled out.

In conclusion, the present study characterized basal and stress-induced corticosterone levels in plasma in inbred epileptic WAG/Rij, inbred non-epileptic ACI and outbred non-epileptic Wistar rats. We showed that ACI rats have the highest corticosterone plasma level in both resting and stress conditions, while WAG/Rij rats tend to show higher corticosterone than Wistar. Next, it was shown that plasma corticosterone level can deplete as a long-term consequence of operation in both inbred strains. In the second experiment, we demonstrated that both stress and anticipation of stressful stimuli aggravate the incidence of absence seizures in WAG/Rij rats. Based upon these data, and considering that stress is part of daily life we suggest that the HPA axis functioning may play a role in the pathogenesis of absence epilepsy. Consistently, in a single human study it was reported that children have enhanced numbers of absence-type seizures after stressful events (Bosnjak et al., 2002). Further investigation of the mechanisms involved in the regulation of anticipatory stress response may bring a new target in the treatment of absence epilepsy and prevent the development of concomitant depression.

References

Antoniadis, E.A., McDonald, R.J., 2000. Amygdala, hippocampus and discriminative fear conditioning to context. Behav Brain Res. 109, 141-142.

Antonijevic, I.A., 2006. Depressive disorders -- is it time to endorse different pathophysiologies? Psychoneuroendocrinology 31, 1-15.

Bosnjak, J., Vukovic-Bobic, M., Mejaski-Bosnjak, V., 2002. Effect of war on the occurrence of epileptic seizures in children. Epilepsy Behav. 3, 502-509.

Barbaccia, M.L., Roscetti, G., Trabucchi, M., Purdy, R.H., Mostallino, M.C., Concas, A., Biggio, G., 1997. The effects of inhibitors of GABAergic transmission and stress on brain and plasma allopregnanolone concentrations. Br. J. Pharmacol. 120,1582-1588.

Barbaccia, M.L., Serra, M., Purdy, R.H., Biggio, G., 2001. Stress and neuroactive steroids. Int. Rev. Neurobiol. 46, 243-272.

Coenen, A.M., van Luijtelaar, E.L., 2003. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. Behav. Genet. 33, 635-655.

de Bruin, N.M., van Luijtelaar, E.L., Cools, A.R., Ellenbroek, B.A.,2001. Dopamine characteristics in rat genotypes with distinct susceptibility to epileptic activity: apomorphine-induced stereotyped gnawing and novelty/amphetamine-induced locomotor stimulation. Behav Pharmacol. 12, 517-525.

De Keyser, F.G., Leker, R.R., Weidenfeld, J., 2000. Activation of the adrenocortical axis by surgical stress: involvement of central norepinephrine and interleukin-1. Neuroimmunomodulation. 7, 182-188.

Deransart, C., Riban, V., Le, B., Marescaux, C., Depaulis, A., 2000. Dopamine in the striatum modulates seizures in a genetic model of absence epilepsy in the rat. Neuroscience 100, 335-344.

Drinkenburg, W.H., Schuurmans, M.L., Coenen, A.M., Vossen, J.M., van Luijtelaar, E.L., 2003. Ictal stimulus processing during spike-wave discharges in genetic epileptic rats. Behav. Brain Res. 143, 141-146.

Fluttert, M., Dalm, S., Oitzl, M.S., 2000. A refined method for sequential blood sampling by tail incision in rats. Lab. Anim. 34, 372-378.

Gallagher, B.B., Murvin, A., Flanigan, H.F., King, D.W., Luney, D., 1984. Pituitary and adrenal function in epileptic patients. Epilepsia 25, 683–689.

Goldstein, L.E., Rasmusson, A.M., Bunney, B.S., Roth, R.H., 1996. Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. Neurosci. 16, 4787-4798.

Haut, S.R., Vouyiouklis, M., Shinnar, S., 2003. Stress and epilepsy: a patient perception survey. Epilepsy Behav. 4, 511-514.

Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C., Cullinan, W.E., 2003. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front. Neuroendocrinol. 24, 151-158.

Inoue, M., Peeters, B.W., van Luijtelaar, E.L., Vossen, J.M., Coenen, A.M., 1990. Spontaneous occurrence of spike-wave discharges in five inbred strains of rats. Physiol Behav. 48, 199-201.

Joels, M., de Kloet, E.R., 1992. Control of neuronal excitability by corticosteroid hormones. Trends Neurosci. 15, 25-30.

Koehl, M., Darnaudery, M., Dulluc, J., Van Reeth, O., Le Moal, M., Maccari, S., 1999. Prenatal stress alters circadian activity of hypothalamo-pituitary-adrenal axis and

hippocampal corticosteroid receptors in adult rats of both gender. J. Neurobiol. 40, 302-315.

Li et al, 1996 - neurogenic stress

Maren, S., Quirk, G.J., 2004. Neuronal signalling of fear memory. Nat Rev Neurosci. 5, 844-52.

Mello Ade, A., Mello, M.F., Carpenter, L.L., Price, L.H., 2003. Update on stress and depression: the role of the hypothalamic-pituitary-adrenal (HPA) axis. Rev Bras Psiquiatr. 25, 231-238.

Midzyanovskaya, I.S., Kuznetsova, G.D., van Luijtelaar, E.L., van Rijn, C.M., Tuomisto, L., Macdonald, E., 2006. The brain 5HTergic response to an acute sound stress in rats with generalized (absence and audiogenic) epilepsy. Brain. Res. Bull. 69, 631-638.

Panayiotopoulos, C.P., 2001. Treatment of typical absence seizures and related epileptic syndromes. Paediatr Drugs. 3, 379-403.

Paxinos, G., Watson, C., 1986. The rat brain in stereotaxic coordinates, Academic Press, San Diego, CA.

Pritchard, P.B., Wannamaker, B.B., Sagel, J., Nair, R., DeVillier, C., 1983. Endocrine function following complex partial seizures. Ann. Neurol. 14, 27–32.

Reddy, D.S., Rogawski, M.A., 2002. Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. J. Neurosci. 22, 3795-3805.

Saalmann, Y.B., Morgan, I.G., Calford, M.B., 2006. Neurosteroids involved in regulating inhibition in the inferior colliculus. J. Neurophysiol. 96, 3064-3073.

Sarkisova, K.Y., Midzianovskaia, I.S., Kulikov, M.A., 2003. Depressive-like behavioral alterations and c-fos expression in the dopaminergic brain regions in WAG/Rij rats with genetic absence epilepsy. Behav Brain Res. 144, 211-226.

Schridde, U., van Luijtelaar, G., 2004. Corticosterone increases spike-wave discharges in a dose- and time-dependent manner in WAG/Rij rats. Pharmacol. Biochem. Behav. 78, 369-375.

Solomon, R.L., Corbit, J.D., 1974. An opponent-process theory of motivation. I. Temporal dynamics of affect. Psychol. Rev. 81, 119-145.

Stromberg, J., Backstrom, T., Lundgren, P., 2005. Rapid non-genomic effect of glucocorticoid metabolites and neurosteroids on the gamma-aminobutyric acid-A receptor. Eur. J. Neurosci. 21, 2083-2088.

Tolmacheva, E.A., van Luijtelaar, G., Chepurnov, S.A., Kaminskij, Y., Mares, P., 2004. Cortical and limbic excitability in rats with absence epilepsy. Epilepsy Res. 62, 189-198.

Tolmacheva, E.A., van Luijtelaar G., 2007. Absence seizures are reduced by the enhancement of GABA-ergic inhibition in the hippocampus in WAG/Rij rats, Neuroscience Letters, 000-000...

van Luijtelaar, E.L., Coenen, A.M., 1988. Circadian rhythmicity in absence epilepsy in rats. Epilepsy Res. 2, 331-336.

van Luijtelaar, G., Sitnikova, E., 2006. Global and focal aspects of absence epilepsy: The contribution of genetic models. Neurosci. Biobehav. Rev. 30, 983-1003.

van Luijtelaar, G., Sarkisova, K.Y., Midzyanovskaya, I.S., Tolmacheva, E.A. 2007 "Stress vulnerability and depressive symptoms in genetic epileptic rats" In New research on Epilepsy and Behavior, eds Hollaway, K.J. Nova, Ca, USA.

Chapter 7

GENERAL DISCUSSION

7.1. The hypothalamo-pituitary-gonadal system in the regulation of absence seizures in WAG/Rij rats

The main objective of the first part of this thesis was to investigate the role of ovarian hormonal milieu in the regulation of absence seizures in WAG/Rij rats. Rats of this strain are considered to be a genetic model with predictive, face and construct validity for human absence epilepsy (Coenen and van Luijtelaar, 2003). In Chapter 1 the main principles in the regulation and functioning of the hypothalamo-pituitary-gonadal system, as well as the main facts concerning the role of ovarian steroid hormones in the regulation of neuronal excitability and seizure susceptibility were described and reviewed. A few recent studies have demonstrated that membrane effects of the ovarian steroid hormones are important in the regulation of typical absence seizures in humans and rats (Grunewald et al, 1992; Budziszewska et al, 1999; van Luijtelaar et al, 2001; 2003). Based on the fact that acute injections of progesterone exacerbate the number of spontaneous occurring absence seizures, or spike-wave discharges (SWDs), in both male and female WAG/Rij rats, a functional role of progesterone in exacerbating SWDs in generalized absence epilepsy was suggested. It was also hypothesized that progesterone and its neuroactive derivative allopregnanolone could be a strong candidate to account for the changes in the number of SWDs over circadian and estrous cycle (van Luijtelaar et al, 2001, 2003). However, the latter still remains to be investigated.

In contrary to these acute approach studies, we focused on the occurrence of absence seizures in conditions characterized by chronic or long-lasting changes in the steroid hormonal milieu. The first series of experiments, as presented in Chapter 2, were aimed to investigate the occurrence of absence seizures during pregnancy. Pregnancy is characterized by an increase in endogenous secretion of ovarian steroid hormones and, in particular, progesterone. The data showed that chronic elevation of progesterone during pregnancy was accompanied by a clear decrease in the occurrence of SWDs. Moreover, withdrawal from progesterone before the delivery triggered a robust increase in both number and duration of SWDs. After delivery progesterone concentration increased, while the number of SWDs seemed to return to the base-line level. Overall the dynamics of changes in the number of SWDs during pregnancy negatively correlated with the dynamics of progesterone plasma

concentration in female WAG/Rij rats. Thus the present data showed that the effect of chronic changes in the hormonal milieu during pregnancy was opposite to the effect of an acute hormonal challenge with progesterone.

In the next series of experiments, we tried to model the situation of chronic elevation of progesterone beyond the pregnancy study and tested the occurrence of SWDs in WAG/Rij rats after daily repeated progesterone administration (Chapter 3). The data demonstrated a 60-90 min increase in the occurrence of SWDs after the first progesterone exposure, supporting previous findings on effects of progesterone in this model (Budziszewska et al, 1999, van Luijtelaar et al, 2001). However, no significant effect of progesterone on SWDs was found after the second progesterone injection. This observation suggests that a single administration of progesterone in a dose of 20 mg/kg is already enough to make rats tolerant to the subsequent exposure of this hormone. The lack of effects in a group that received repeated successive injections of cyclodextrine suggests that tolerance was developed to progesterone and not to the injection procedure per se. In support to the present data, an acute tolerance development was demonstrated after 60-90 min of continuous exposure to allopregnanolone in an anaesthesia model (Zhu et al, 2004).

There are also several other recently published data indicating that neuroactive steroids may have a tolerance liability similar to that of benzodiazepines (Czlonkowska et al, 2001; Zhu et al, 2004; Birzniece et al, 2006; Turkmen et al, 2006). It was shown that neurosteroids not only interact directly with $GABA_A$ receptors but also regulate the expression of genes that encode the subunits of this receptor (Yu et al, 1996; Barbaccia et al, 2001). The acute development of tolerance was shown to be associated with rapid changes in the subunit composition of the $GABA_A$ receptor, resulting in a reduced sensitivity of this receptor to allosteric modulation (Birzniece et al, 2006; Maguire and Mody, 2007). Another non-genomic mechanism that might be involved in the development of tolerance to neurosteroids is phosphorylation, which is capable to alter the sensitivity of the receptor to allosteric steroid modulation independent of its subunit composition (Koksma et al, 2003; Harney et al, 2003).

The development of tolerance to progesterone and its metabolites might also take place during pregnancy, when the endogenous secretion of this hormone is chronically increased. This process should help the organism to adjust to the new hormonal level and underlie the lack of exacerbating effects of progesterone and its metabolites on absence seizures in this period. However, it is still difficult to answer, based on what we found, whether there is a relationship between the basal steroid hormonal milieu and the occurrence of epileptic seizures.

To approach this question, we further investigated the occurrence of absence seizures following ovariectomy, which eliminates the main peripheral resource of gonadal steroids (see also Chapter 3). We hypothesized that if there is a relationship between hormonal milieu and the occurrence of absence seizures, the withdrawal of ovarian steroids hormones after ovariectomy should alter the occurrence of SWDs. In contrast to what has been expected, our data have revealed that a chronic decrease in circulating ovarian steroids after ovariectomy does not affect either the occurrence or the duration of absence seizures in WAG/Rij rats for up to a 35 days period after surgery (Chapter 3). Ovariectomy of WAG/Rij rats at 3 months of age had also no significant effects on number, mean and total duration of SWD at 6 months of age (van Luijtelaar et al, 1996). Rather similar data were reported for the pilocarpine model for temporal lobe epilepsy, in which chronic diminution of ovarian hormones did not change the incidence of status epilepticus, although a more rapid progression to the status development was observed (Scharfman et al, 2005). The lack of any changes in the occurrence of absence seizures after ovariectomy implies that steroid hormonal milieu is not critically involved in seizure control in base-line, resting conditions. In support, it has been previously found that injection of finasteride, which inhibits the conversion from progesterone to allopregnanolone, blocks an acute increase in SWDs induced by progesterone, while it has no effect on the normal number of SWDs in the base-line conditions (van Luijtelaar et al, 2001).

On the other hand, considering that progesterone has an imperative impact on the GABA-ergic transmission there should also be compensatory responses to sustained levels of inhibition in the brain. Indeed, a number of adaptive changes in subunit composition underlying specific binding associated with $GABA_A$ receptors (Wilson and Biscardi, 1992), as well as in density of dopamine receptors were found in different brain structures such as striatum and prefrontal cortex in rats following ovariectomy (Bosse and Di Paolo, 1995). Conversely, even though depletion in ovarian steroids hormonal milieu is not critically involved in the regulation of absence seizures in resting conditions, it might be critical when there is a challenge in the environment that requires additional excitatory and inhibitory recourses.

To test this suggestion, we investigated the occurrence of absence seizures in ovariectomized and sham-operated animals after repeated exposure to stressful stimuli (Chapter 3). We used a foot-shock (FS) stress paradigm, which is considered to represent an acute mild stress. Consistent with our primary hypothesis, we found that ovariectomized rats showed a larger increase in spike-wave activity after repeated exposure to FS stress, as

well as in conditions in which FS exposure can be anticipated (or stress anticipation reaction). Thus, although the basal number of SWDs in the resting conditions was not changed after ovariectomy, the lack of steroid hormones led to a larger aggravation in the incidence of absence seizures in a stressful situation. Similarly and in further support, it was recently demonstrated that prepuberal rats (25-28 days of age), that have lower progesterone and estradiol plasma concentrations, demonstrate a prolonged adrenocorticotropic hormone (ACTH) and corticosterone response to stress compared to adult rats (>65 days) (Romeo and McEwen, 2004; Romeo et al, 2006).

Overall, the outcomes of the last experiment suggest that there is an important interaction between the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axis in seizure control, and that animals with an intact hypothalamic-pituitary-adrenal axis have a stronger resistance to repeated stressful situations. Could this factor play a critical role in the pathogenesis of absence epilepsy? To answer this question, in the next chapter (Chapter 4) we characterized some of the basic parameters in the hypothalamic-pituitary-adrenal axis function in WAG/Rij rats in comparison with non-epileptic ACI and Wistar rats and further investigated the effect of acute and chronic stress on the occurrence of absence seizures.

7.2. The characteristics of the hypothalamo–pituitary-adrenal system and the effect of stress on absence seizures

The hypothalamo-pituitary-adrenal (HPA) axis is a major system responsible for the neuroendocrine response to stress in all vertebrate organisms. In Chapter 1 we described the main principles in the regulation and functioning of the hypothalamic-pituitary-adrenal system and reviewed the facts concerning the role of stress and adrenal steroid hormones in seizure susceptibility. It has been reported that children have an enhanced occurrence of absence seizures after stressful events (Bosnjak et al, 2002). This suggests that stress and the hypothalamic pituitary adrenal axis (HPA axis) function might be also involved in the pathogenesis of absence epilepsy. Consistently, an acute elevation of corticosterone was shown to enhance absence seizures in WAG/Rij rats (Schridde and van Luijtelaar, 2004) and footshock stress elevates SWD (this thesis). On the other hand, behavioural studies show that WAG/Rij rats exhibit a number of depressive-like features in their behaviour, such as a decreased or increased ambulation in the open field, increased immobility in Porsolt's forced swim test and

decreased sucrose intake (anhedonia) (Sarkisova et al, 2003, van Luijtelaar et al, in press). It is suggested that these depressive-like symptoms may indicate a higher vulnerability to stress in these animals that could be related to the impaired regulation and/or functioning of the HPA axis.

The fourth chapter of this thesis had two research objectives. First, to characterize the basic HPA axis parameters, such as basal and stress-induced plasma corticosterone concentration in inbred epileptic WAG/Rij, as well as in non-epileptic inbred ACI and outbred Wistar rats. And second, to establish the effects of repeated stress exposure as well as the role of stress intensity on absence seizures in male WAG/Rij rats. Our data demonstrated that WAG/Rij rats tended to show a higher basal plasma corticosterone concentration than Wistar rats, while the highest corticosterone concentration was characteristic for ACI rats. A similar situation was found after exposure to acute stress. Plasma concentrations of corticosterone quickly rose and were elevated for around 30-40 minutes. Thereafter, at around 50-60 minutes after exposure to foot shock stress they returned to the base-line level. Over all time-points WAG/Rij rats demonstrated slightly higher plasma corticosterone concentrations than Wistar rats, while ACI rats always showed the highest values.

Next, we found that in both inbred strains plasma corticosterone concentration can deplete as a long-term consequence of a brain surgery, while no differences in plasma corticosterone concentration before and after operation were found in Wistar rats. Surgical stress is known to induce acute activation of the HPA-axis in rats for at least 24 hours (De Keyser et al, 2000). Based upon these results, we suggest that depletion in plasma corticosterone concentration in the two inbred strains one week after surgery might be part of a 'recovery' process following the acute activation of the HPA axis. The longer duration of this process in WAG/Rij and ACI rats might indicate a higher vulnerability of the HPA axis to systemic stress induced by brain surgery in these animals.

We investigated the occurrence of absence seizures after repeated exposure to FS stress in a second series of experiments. The data demonstrated that exposure to FS stress had a biphasic effect on the number of absence seizures: a quick decrease in the first 15 minutes was followed by an increase in SWDs lasting 45-60 min. A similar biphasic effect, an initial 15 min decrease followed by an increase in SWDs, was also observed after an acute systemic injection of corticosterone (Schridde and van Luijtelaar, 2004). We also found a reduction in the initial suppression and an increase in further aggravation in SWDs from day to day, when FS exposure was repeated. Remarkably, this observation is consistent with the classical theory on emotions of Solomon and Corbit (1974). They described

two opponent processes in reaction towards emotional stimuli: an initial and fast response (in our case a short lasting decrease in SWD) was followed in time by an larger opponent reaction (in our case a prolonged increase) and showed that after repetition of the stimuli the initial reaction goes down, while the following opponent reaction becomes larger.

Stress induced activation of the HPA axis can alter seizure susceptibility by releasing adrenal steroids hormones such as cortisol and corticosterone both associated with a depression in GABA-ergic neurotransmission (Concas et al, 1985; Barbaccia et al, 1997; Biggio et al,et al, 2007). However, in the next phase a depression in GABA-ergic neurotransmission is followed by a quick rise in the level of 3α,5α-reduced derivatives of progesterone, such as allopregnanolone and deoxycorticosterone, known to facilitate GABA–ergic inhibition (Barbaccia et al, 1998, 2001; Stromberg et al, 2005; Biggio et al, 2007). The latter rise of neurosteroids level is thought to balance excitatory and inhibitory inputs in complex neural circuits (Stromberg et al, 2005; Saalman et al, 2006) and counteract the initial excitatory effects of glucocorticoid metabolites. Hence, the biphasic dynamics in the incidence of absence seizures after FS exposure may reflect an interplay between excitatory and opponent compensatory inhibitory processes in cortico-thalamic systems and/or in other systems involved in the regulation of SWDs. Along with this, stress is also known to activate noradrenergic as well as other catecholaminergic systems (Midzyanovskaya et al, 2006), which are thought to play a role in the regulation of absence seizures in WAG/Rij rats (Buzsáki et al, 1991; Midzyanovskaya et al, 2006; Sitnikova and van Luijtelaar, 2004). In particular, clonidine, a presynaptic a2 agonists, facilitates the occurrence of SWDs (Buzsáki et al,et al, 1991; Sitnikova and van Luijtelaar, 2004). Therefore, activation of noradrenergic system might also participate in the initial suppression of SWDs induced by FS stress.

Interestingly, however, an aggravation in the number of SWDs from day to day was also found in the base-line period preceding exposure to FS stress and each next day this aggravation became more prominent. Similarly, in the experiment with repeated progesterone injections, it was found that the effect of acute administration of progesterone was aggravated by two preceding injections of cyclodextrine. As was mentioned in Chapter 1, the HPA axis can be activated not only upon a real, but also upon a predicted threat of any psychological of physiological challenge disturbing homeostasis. Consistently, our data indicate that not only stress, but also anticipation of stressful stimuli is capable to aggravate the incidence of absence seizures in WAG/Rij rats, and that the lack of ovarian steroid hormones enhances this aggravation.

7.3. Excitability of cortical and limbic systems in genetically epileptic WAG/Rij rats

The second part of this thesis was aimed to investigate the excitability of cortical and limbic systems in WAG/Rij rats. On the one hand, the cortex and the limbic system structures are known to be the major targets for steroid hormones in the brain (McEwen et al, 1983; McEwen, 2004; Rhodes and Frye, 2005). On the other, the cortex plays a leading role in various theories on the origin of absence epilepsy (Meeren et al, 2005; van Luijtelaar and Sitnikova, 2006) and hyperexcitability of the cortex is classically regarded as a precondition for the transformation of sleep spindles into SWDs (cortico-reticular theory - Gloor et al, 1988; Kostopoulos, 2000). Therefore, in Chapter 5 a local rhythmic electrical stimulation of the sensori-motor cortex in freely moving rats was used to investigate whether genetic epileptic rats are interictally endowed with a more excitable cortex. WAG/Rij rats of 3 months (when not all rats have SWDs and the incidence is relatively low) and 6 months of age (when all rats have seizures and their incidence is high) were compared with non-epileptic ACI and Wistar rats of an appropriate age in a stimulation protocol measuring various threshold values for cortical and limbic excitability. A detailed description of this protocol is at disposal because the stimulation protocol was routinely used to study the ontogeny of cortical epileptic afterdischarges (Mareš et al, 2002). An experimental design included two different types of controls. A group of old epileptic WAG/Rij rats with hundreds of SWDs per day was compared, first, with a group of young adult WAG/Rij rats, which do not have SWDs yet, and, second, with age-matched groups of ACI rats - an inbred strain with a minimal incidence of SWDs (ACI) (Inoue et al, 1990; de Bruin et al, 2001), and outbred Wistar rats. This design allowed us to establish whether differences between the groups could be attributed to the development of genetic epileptic phenotype, age effects, strain effects and inbred versus outbred strain differences.

In contrast to what has been expected, there was no difference in excitability between the WAG/Rij and ACI rats as measured by various types of electrophysiological variables, such as the threshold for movements elicited by individual stimuli and the threshold for evoking epileptic afterdischarges which are accompanied by clonic seizures. Moreover, ACI rats tended to show even lower thresholds for different types of phenomena related to cortical excitability than WAG/Rij rats. Thus, it can be concluded that an increased cortical excitability is not a sufficient condition for the brain to develop absence seizures. On the other hand, it could also be that

the appearance of absence seizures is preceded by a local, rather than a general increase in cortical excitability. This latter scenario fits into the recently proposed new theory on the focal origin of absence seizures (Meeren et al, 2002; 2005; van Luijtelaar and Sitnikova, 2006).

Another striking finding was that WAG/Rij rats showed the lowest threshold for the limbic type of after-discharges (indicating a spread of the epileptic activity into the limbic system), compared to both Wistar and ACI rats. Moreover, this threshold decreased with age in all animals and there was a negative correlation between the threshold value and the amount of spike-wave activity. The limbic system is generally not included in theories about the pathogenesis of absence epilepsy and neither field potentials recordings nor single unit activity in WAG/Rij rats showed any sign of an involvement to the generation of SWDs (Kandel et al, 1996; Inoue et al, 1993). Thus, the present results give the first indication that the excitability of the limbic system is related to the occurrence of SWDs in WAG/Rij rats and, furthermore, that a higher incidence of SWDs corresponds to a higher excitability of the limbic system. In support, a series of studies by Deransart and coauthors (1998; 2001) demonstrate that the basal ganglia and, in particular, the nucleus accumbens (which is also part of the limbic system), are involved in the remote control of thalamo-cortical oscillations in GAERS and can play a critical role in the pathogenesis of absence epilepsy.

Considering that the limbic system is the major target area for all steroid hormones in the brain, it is relevant to test whether local administration of steroid hormones into the limbic system would have an effect on the occurrence of absence seizures. We have chosen to test the effect of progesterone – a $GABA_A$ mimetic - in the hippocampus, which is one of the most investigated parts of the limbic system involved in the control of autonomic, cognitive, and emotional functions. We hypothesized that if the hippocampus is involved in one or another way in the control of absence seizures, an increase of progesterone, facilitating the GABA-ergic transmission in the hippocampus, would alter and, most likely (in agreement with the outcomes of the previous experiment) would suppress the occurrence of absence seizures. Indeed, in the previous experiment we found that a higher limbic excitability corresponded to a higher number of SWDs. Therefore, if we facilitate the GABA-ergic transmission in the hippocampus, this should dampen limbic excitability and, if there is a functional relationship, should lead to suppression of spike-wave activity.

The outcomes of this experiment, described in Chapter 6, showed that intrahippocampal administration of both progesterone and its solvent (cyclodextrine) resulted in a reduction of spontaneous SWDs during 60 min (for cyclodextrine) and 120 min (for progesterone). There was no decrease

in the occurrence of SWDs after a sham injection, while an injection of tiagabine - a very specific drug known to inhibit the GABA (re)uptake process - resulted in a very prominent suppression, more similar to that of progesterone than to cyclodextrine. Taken together, these data suggest that compounds with a more specific GABA-ergic activity may have salient suppressive effects on the occurrence of SWDs and that these effects may be due to their actions in the hippocampus.

7.4. Systemic versus local effects of progesterone: region-specific (or differential) effects of GABA-ergic inhibition on the occurrence of absence seizures

Interestingly, the reduction in the occurrence of SWDs found after hippocampal administration of progesterone and tiagabine (Chapter 6) is diametrically opposite to what has been found earlier in experiments with systemic i.p. injections of progesterone (Budzsizewska et al, 1999) and tiagabine (Coenen et al, 1995). These drugs injected systemically show a dose-dependent increase in the number of absence seizures and their pro-epileptic effect is consistent with a general postulate regarding absence epilepsy as a condition associated with a hyper-function of the GABA-ergic inhibitory system (Peeters et al, 1989; Marescaux et al, 1992; Coenen and van Luijtelaar, 2003). This postulate is based on the evidence that intraperitoneal administration of $GABA_A$-mimetics aggravates seizures in all models of generalized non-convulsive epilepsy in rodents, as well as in cats (Vergnes et al, 1984; Peeters et al, 1989; Marescaux et al, 1992; Snead, 1994). Aggravation of the occurrence of absence seizures can also be found when $GABA_A$-mimetics are injected bilaterally into the thalamic relay nuclei (Liu et al, 1991; Marescaux et al, 1992b), while either systemic or local intrathalamic $GABA_A$ antagonists (picrotoxin or bicuculline) injections reduce spontaneous SWDs in GAERS (Liu et al, 1991; Marescaux et al, 1992c). At high doses, intraperitoneal or intrathalamic injections of $GABA_A$-mimetics induce permanent SWDs with a reduced frequency (5–6 c/s) or isolated spikes on a flat EEG background.

In contrast, when $GABA_A$ mimetics such as muscimol, are applied bilaterally into the reticular thalamic nucleus, they suppress SWDs in GAERS, probably by preventing oscillatory activities in this nucleus (Liu et al, 1991). A more precise recent pharmacological study in GAERS has revealed that administration of bicuculline, a $GABA_A$ receptor antagonist, into the caudal part of the reticular thalamic nucleus (RTN) produces an

increase in the duration of SWDs, while injections into the rostral part produces a decrease in both the duration and the number of SWDs (Aker et al, 2006). Next, the focal bilateral injections of pregnenolone sulphate and allopreganonolone – both $GABA_A$ mimetics - into the peri-oral region of the primary somatosensory cortex reduced the number and duration of SWDs in WAG/Rij rats, similar to muscimol injection into the RTN (Citraro et al, 2006). Finally, local microinjections of muscimol into the mediodorsal thalamic nucleus, which is a part of the prefrontal cortex–nucleus accumbens–thalamus loop involved in the remote control of absence seizures (Deransart et al, 1998, 2000), are also shown to suppress SWDs in GAERS (Riban et al, 2004).

Taken together, these data elaborate the general postulate on hyperfunction of GABA-ergic system and point to the region-specific role of the GABA-ergic neurotransmission in generation of absence seizures. In particular, there are a number of structures, such as peri-oral region of the primary somatosensory cortex, the rostral part of RTN, the mediodorsal nucleus of the thalamus and the hippocampus, where an enhancement of GABA-ergic neurotransmission results in a suppression of spontaneous absence seizures.

7.5. The limbic system and remote control of absence seizures: a new conceptual framework on the role of steroid hormones in absence epilepsy

An absence seizure is an epileptic phenomenon generated in the cortico-thalamo-cortical circuitry, which involve three different targets for steroid hormones action: the cortex, thalamic relay nuclei and the reticular thalamic nuclei. In addition to this, a considerable amount of evidence supports the existence of other sub-cortical networks, such as for example the system of basal ganglia, involved in the remote control of the thalamo-cortical loop and the generation of absence seizures (Depaulis et al, 1998; Deransart et al, 1998, 2001; Paz et al, 2006). This control system is defined as structures distinct from the epileptogenic zone, that is, without any paroxysmal activity and not necessary for seizure generation. However, the pharmacological modulations within this control circuit can modify the occurrence of seizures (Depaulis et al, 1994). In particular, injection of low doses of dopaminergic agonists into the nucleus accumbens suppresses SWDs in GAERS (Deransart et al, 2000). The outcomes of the experiments described in Chapter 4, 5 and 6 of this thesis rise the possibility that also the

limbic system, and, in particular, the circuitry formed by the hippocampus and the prefrontal cortex can provide a remote control of oscillations generated in the cortico-thalamo-cortical loop. In particular, in the experiment with hippocampal injections of tiagabine and progesterone it was shown that an enhancement of GABA-ergic inhibition in the hippocampus leads to suppression in SWDs in WAG/Rij rats (Chapter 6). Consistent with this, it was recently found that there is an increase in synchronization in the hippocampus during spike-wave seizure activity (Perez Velazquez et al, 2007).

The model presented in Figure 1 is an attempt to integrate the classical cortico-thalamo-cortical loop with the limbic system circuitry (the hippocampus - the prefrontal cortex ensemble) and the hypothalamic-pituitary-adrenal hormonal system. In this model, we portrayed three putative mechanisms (pathways) that could underlie the interaction between the specific and non-specific (related to the limbic system) thalamo-cortical circuits and account for the effect of pharmacological manipulations with the GABA-ergic system in the hippocampus on absence seizures.

First of all, it shows that there could be a direct projection from the hippocampus to the rostral RTN, which is known to be a part of the limbic system receiving projections from various motor and limbic centres including the hippocampal formation (Lubke 1993; Aker et al, 2006). Interestingly, direct projections from the RTN to the hippocampus are currently described (Filiz Onat, personal communication). On the other hand, the hippocampus gives also direct projections to the ventral tegmental area and nucleus accumbens (Morgane et al, 2005) and can be directly involved in the regulation of the ascending midbrain dopaminergic system known to play a prominent role in the modulation of absence seizures through activation of the basal ganglia system (Deransart et al, 1998, 2000; De Bruin et al, 2001).

Finally, there could also be an indirect interaction between the hippocampus and cortico-thalamo-cortical system via a release of the HPA axis related hormones such as CRH, ACTH and corticosterone, all known to exert neuroactive effects and to affect seizure succeptibility. In addition, there could also be an interaction on the cortical level, which remains to be investigated.

Next, in the proposed model we also illustrate the differential (region-specific) involvement of the GABA-ergic neurotransmission in the genesis of absence seizures, as evident from the data on acute effects of progesterone applied locally in different parts of the thalamo-cortical (Citraro et al, 2006) and the limbic (Chapter 6 of this thesis) systems.

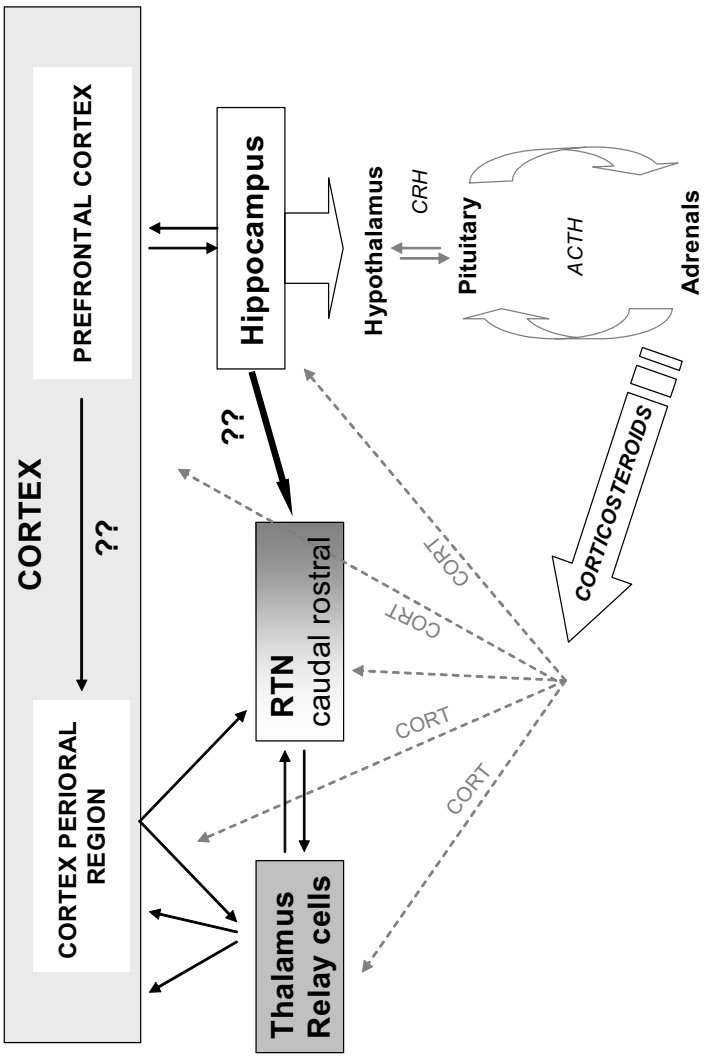

Figure 1. A model integrating the classical cortico-thalamo-cortical loop with the limbic system circuitry (the hippocampus - the prefrontal cortex ensemble) and the hypothalamic-pituitary-adrenal hormonal system.
The dark color indicate the structures (thalamic relay cells, the rostral part of the RTN), in which progesterone (or allopregnanolone) application aggravates the occurrence of absence seizures; the white (perioral region op the somatosensory cortex, hippocampus, caudal part of the RTN), in which it suppresses absence seizures. Whether progesterone in the prefrontal cortex would affects the occurrence of seizures, remains to be investigated. *RTN- reticular thalamic nucleus.*

This is all consistent with the data described in the previous paragraph. The final physiological effect of the hormone in the brain is assumed to be a function of the relative effects of this hormone in different brain regions. Considering that facilitation of GABA ergic neurontransmission play a pivotal role in the acute effect of progesterone (Majewska et al, 1986; Rhodes et al, 2004; Melcangi and Panzica, 2006), in the framework of our model it can be suggested that the acute exacerbating effect of progesterone on absence seizures after systemic intraperitoneal administration could be mediated by its effect on the relay thalamic nuclei. This direct effect of progesterone on the cortico-thalamo-cortical circuitry seems to dominate all the other local effects such as in the peri-oral region of the somato-sensory cortex and in the limbic system. This particular activation profile may also explain the situation of an acute endogenous release of progesterone at the early proestrus day of the ovarian cycle, which is also accompanied by an increase in the occurrence of SWDs (van Luijtelaar et al, 2001).

In contrast, the suppression of SWDs during pregnancy could be due to the effects of ovarian steroid hormones in the limbic system, rather than in the cortico-thalamo-cortical system. Indeed, the limbic system is a primary target for the genomic effects of steroid hormones (McEwen et al, 1983; McEwen, 2004), which could take place during pregnancy when the steroid hormone synthesis is chronically elevated. In support, an increase in the affinity of $GABA_A$ receptors for [^{3}H] muscimol in the forebrain was found on days 15-19 of gestation in rats (Majewska et al, 1989). Moreover, the density of central benzodiazepines binding sites was increased in the hippocampus (on day 19), while it was decreased in the hypothalamus and pituitary (Gavish et al, 1987). The lack of acute exacerbating effect of progesterone in the thalamus during pregnancy could be due to the development of tolerance through the reorganization of the $GABA_A$ receptor subunit composition (Birzniece et al, 2006; Maguire and Mody, 2007).

In the experiments with repeated foot-shock exposure it was found that not only exposure to stress, but also anticipation of stressful stimuli, was capable to aggravate the incidence of absence seizures in WAG/Rij rats. Anticipation of stress or, in other terms, contextual fear conditioning, is a cognitive process that is thought to take place in the hippocampus and the prefrontal cortex and is based on the extinction and the further evaluation of fear in the lateral amygdala (Antoniadis and MacDonald, 2000; Maren and Quirk, 2004). The circuitry formed by these structures is also capable to activate the HPA axis in conditions, in which stressful stimuli could be anticipated (Herman et al, 2003). Prediction or anticipation of aversive events was also found to be associated with a depression of the ascending midbrain dopaminergic system (Schults, 2007). Consistently, aggravation of

SWDs preceding FS exposure could be mediated either via activation of the HPA axis and corticosterone release (corticosterone facilitates the occurrence of SWD, Schridde and van Luijtelaar, 2004), and/or via suppression of dopaminergic neurotransmission in midbrain neurons (dopamine receptors agonists applications to both the shell and the core of the nucleus accumbens are shown to suppress SWDs, Deransart et al, 1998; 2000). Both mechanisms could account for an aggravation of SWDs preceding FS exposure.

Alternatively, the loss of steroid hormones induced by ovariectomy might result in the reduction of GABA-ergic inhibition in the limbic system, which might lead to a higher excitability of this system towards anticipation of repeated stress. Interestingly, the difference between the ovariectomized and sham-operated animals was still hardly present after the first acute exposure to stress, while it became more salient when this stress was further repeated. Chronic stress is known to induce sensitization in the limbic system and repeated stress exposure results in an increased numbers of c-Fos–positive nuclei response in the number of limbic structures such as hypothalamus, dentate gyrus, medial prefrontal cortex and central and medial amygdala (Gerrits et al, 2006). In the framework of our model we can suggest that the loss of steroid hormones could result in the reduction in GABA-ergic inhibitory mechanisms in the limbic system or/and upregulation of the specific subunits of $GABA_A$ receptors (Wilson and Biscardi, 1992; Smith et al, 2007), that might underlie a more prominent anticipatory reaction in ovariectomized females.

The model presented in this paragraph shows that the role of ovarian steroids in absence epilepsy could be closely related to the role of the limbic system and, in particular, to the circuitry formed by the hippocampus, the prefrontal cortex and the amygdala, which is known to be involved in stress anticipation response. Along with the basal ganglia system, the limbic system could also be a part of a major circuitry that provides a remote control of oscillations generated in the cortico-thalamo-cortical loop mediating the influence of chronic changes in ovarian steroid hormonal milieu. An involvement of the limbic system in the remote control of absence seizures provides additional insight on the hormonal regulation of absence seizures and helps to understand controversial effects of ovarian steroid hormones described in the present thesis.

7.6. Experimental perspectives

Our proposal in the previous paragraph model (Fig.1) is necessarily a simplification, since neuroactive steroid hormones are able to modulate not only the GABA-ergic system but also the glutamatergic (NMDA), cholinergic and opioid system, which are shown to be involved in the occurrence of absence seizures (Coenen et al, 1992; Lason et al, 1994; Berdiev et al, 2002). These neurotransmitter systems are not taken into consideration in our model and this is a challenge for further research to integrate the modulatory effects of various neurotransmitters in the proposed model. For example, it would be beneficial to test the role of midbrain dopaminergic system including the role of ventral tegmental area and the nucleus accumbens in the increase of SWDs in stress-sensitive conditions.

Next, an intriguing question that remains to be further investigated is the precise mechanism underlying the biphasic dynamics in the number of SWDs after repeated exposure to acute stress. Acute stress induces activation of noradrenergic, as well as the other catecholaminergic systems and opioids in epileptic rats (Goldstein et al, 1996; Midzyanovskaya, 2006; Midzyanovskaya et al, 2006). Considering that these systems are also involved in the regulation of absence seizures (Sitnikova and van Luijtelaar, 2005; Lason et al, 1994), they might also contribute to the stress-induced changes in the incidence of SWDs.

Another relevant issue concerns the relationship between the circadian rhythmicity of progesterone and its central metabolites such as allopreganolone and circadian changes in the number of absence seizures. Although, we found that the basal ovarian steroid hormones milieu is not critically involved in the regulation of the occurrence of SWDs in base-line, resting conditions, it is still possible that circadian fluctuations in allopregnanolone concentration in the brain might be coupled to cluster organized circadian dynamics of SWDs (Midzyanovskaya et al, 2006). Therefore, it could be beneficial to record the EEG simultaneously with the local microdialysis measurement of progesterone and allopregnanolone in the thalamus and the limbic system.

Another experiment could be aimed to investigate the development of tolerance. Although we suggested that acute tolerance might develop already after the first injection, it could be additionally tested whether a second injection with progesterone, given two hours later, would increase SWDs. It could also be a challenge to investigate the expression of the subunit composition of $GABA_A$ receptors in different brain structures, in

particular in the thalamus and in the limbic system, before and during pregnancy in WAG/Rij rats.

Finally, the most interesting and relevant experiment would be to investigate in more details the relationship between the limbic and the thalamo-cortical system as proposed in our model. For example, it can be proposed to investigate whether there are direct projections from the hippocampus to the rostral part of the RTN. If so, it would give the most direct and parsimonious explanation for the hippocampal control of SWD. Retrograde tracing studies to describe the neuroanatomical projections between the RTN and various limbic centers including the hippocampus could be suggested to answer this question.

7.7. Clinical relevance

There is a number of other reasons that makes the topic of steroid hormones in general and in relation to epilepsy in particular interesting and relevant. Many people are daily exposed to stress, that transiently alters the concentration of some neuroactive steroids; woman have changing levels of steroid hormones during their ovarian cycle and during pregnancy, and also at puberty and transition period there are large fluctuations in steroid hormones.

The first outcome of this thesis that might have clinical relevance is that a chronic decrease in the number of absence seizures can be found during pregnancy in genetically epileptic rats. Therefore it seems that pregnancy is accompanied by a natural decrease in seizure susceptibility and mothers might not need any special treatment against seizure activity in this period. The latter is particularly important considering that most of antiepileptic drugs are known to have side-effects leading to the development of malformations in the fetus.

The next issue concerns the fact that the neuroendocrine gonadal system is often adversely affected in people with epilepsy (Klein and Herzog, 1998). Reproductive abnormalities associated with epilepsy are thought to involve hypothalamic disturbances, particularly to the Gonadotropin Releasing Hormone (GnRH) network, resulting in altered secretion of GnRH and subsequent gonadal steroid hormones (Fawley et al, 2006). Abnormal afferents to the hypothalamus and/or changes in neurotransmitter and hormone concentrations following seizures could be one of the reasons underlying alterations in GnRH release. Conversely, reproductive disorders can be also caused by antiepileptic drugs interfering

with a normal functioning of the HPG axis. Based upon our findings, we suggest that disturbed regulation of the HPG system can also result in higher stress vulnerability and lead to an increased aggravation in the incidence of seizures, i.e. absence seizures in stress-sensitive conditions. Therefore, the appropriate assessment and, if necessary, further correction of the HPG axis could be an important part of a more comprehensive management in antiepileptic therapy.

Finally, the last, but perhaps the most relevant outcome of this thesis concerns the relationship between convulsive and non-convulsive forms epilepsy and, in particular, the transition during puberty between these two forms, which occur in 30-40% of adolescents suffering from childhood absence epilepsy (Hirsch et al, 1994; Panayiotopoulos, 1999). Although, understanding of the mechanisms underlying this transition would have a very important clinical relevance, it still remains largely unknown. Moreover, it is not clear how generalized absence epilepsy associated with a hyperfunction of the GABA-ergic inhibitory system (Peeters et al, 1989; Liu et al, 1991; Marescaux et al, 1992) can transform to convulsive forms of epilepsy mainly characterized by a hypofunction of the GABA-ergic neurotransmission. In the framework of our model we suggest that an increased excitability of the limbic system and, in particular of the hippocampus, might represent a common mechanism that can worsen seizure control in both types of epilepsy and could mediate the transition from one type of epilepsy to another. This transition takes place during puberty - a period, in which major changes in steroid hormonal milieu occur. It is also shown that in this transitional period response to stressful events become exacerbated (Modesti et al, 2004). The higher excitability of limbic system associated with higher vulnerability to stress in adolescents with absence epilepsy, might predispose them to develop other types of epilepsy, such as, for example, temporal lobe epilepsy. Therefore, it is important to protect the child with absence epilepsy from highly stressful situations in general and especially during puberty. In support, in a single human study it was reported that children have enhanced numbers of absence-type seizures after stressful events (Bosnjak et al, 2002). Therefore, in more general terms, it could also be recommended as a part of therapeutical treatment, to learn children with absence epilepsy how to cope with stress and how to behave in stressful situations. Further investigation of the mechanisms involved in the regulation of limbic excitability and anticipatory stress response, may bring new targets in the treatment of absence epilepsy.

Conclusions

1. A chronic endogenous increase in progesterone as during pregnancy is accompanied by a major suppression, while withdrawal from progesterone before delivery is followed by an aggravation in the occurrence of absence seizures. The direction of this effect is diametrically opposite to the acute effect of progesterone shown to exacerbate the occurrence of absence seizures.

2. Single exposure to progesterone in a dose of 20 mg/kg is sufficient to induce the development of tolerance towards the acute exacerbating effect of progesterone on absence seizures. Repeated mild stress associated with an injection procedure causes an aggravation of the effect of progesterone on absence seizures.

3. Acute exposure to foot-shock stress induces a biphasic effect on absence seizures: an initial suppression is followed by an aggravation in the occurrence of absence seizures, which is presumable related to the activation of the HPA axis. When exposure to stress is daily repeated, the initial suppression in the occurrence of SWDs become shorter, while the aggravation becomes larger. Both outcomes are in agreement with the two opponent process theory on emotions of Solomon and Corbit (1974).

4. The plasma corticosterone concentration in WAG/Rij rats is not significantly different from Wistar rats in resting conditions and after an acute stress exposure. However, both inbred strains (WAG/Rij and ACI) rats show a depletion in plasma corticosterone level one week after brain surgery, which indicate a higher vulnerability of these animals to this type of stress.

5. WAG/Rij rats show an increasing anticipation response towards a stressful situation, as evident from a day-to-day aggravation of SWDs preceding FS exposure.

6. The diminution of ovarian steroid hormones following ovariectomy does not affect the occurrence of absence seizures in WAG/Rij rats in base-line (resting) conditions. However, ovariectomized females show a more prominent day-to-day aggravation in absence seizures after repeated foot-shock administration indicating a tight relationship between the HPA and the HPG system in seizure control in stress-sensitive conditions.

7. Genetically epileptic WAG/Rij rats do not show a higher cortical excitability in the interictal period compared to non-epileptic inbred ACI rats, as could be inferred from the classical cortico-reticular theory on absence epilepsy. However, WAG/Rij rats have the lowest threshold for the spread of the epileptic activity into the limbic system. Moreover, this threshold correlates positively with the expression of spike-wave activity in WAG/Rij rats, suggesting that this system might also be involved in the regulation of absence seizures.

8. The enhancement of GABA-ergic inhibition in the hippocampus results in a suppression of absence seizures. This confirms our hypothesis that the limbic system and, in particular, the hippocampus might be involved in the control of thalamo-cortical system and plays a role in pathogenesis of absence epilepsy.

Epilogue

The data of the present thesis suggest that although in resting conditions the ovarian steroid hormonal milieu is not critically involved in the regulation of absence seizures, the presence of ovarian hormones becomes significant for the occurrence of absence seizures when the organism is challenged. In the present thesis it is demonstrated that an increase in endogenous secretion of ovarian steroid hormones during pregnancy is accompanied by a major suppression in the occurrence of spike-wave activity, while a diminution of ovarian steroids following ovariectomy results in a higher aggravation of absence seizures in stress-sensitive conditions. It is also shown that the acute and chronic changes in steroid hormonal milieu may have opposite effect on seizure susceptibility. In addition to this, it is found that genetically epileptic WAG/Rij rats show an impaired function of the limbic system. This might imply that the limbic circuitry, which is not considered in any classical theory on absence epilepsy, can play an important role in the remote control of absence seizures and mediate the effects of the ovarian steroid hormonal milieu on seizure control. Finally, we propose that changes in the limbic system at puberty can form the basis for either the remission, or transition from absence epilepsy to less benign seizure types.

References

Aker, R.G., Ozyurt, H.B., Yananli, H.R., Cakmak, Y.O., Ozkaynakci, A.E., Sehirli, U., Saka, E., Cavdar, S., Onat, F.Y., GABA(A) receptor mediated transmission in the thalamic reticular nucleus of rats with genetic absence epilepsy shows regional differences: functional implications, Brain Res. 1111, (2006) 213-221.

Barbaccia, M.L., Serra, M., Purdy, R.H., Biggio, G., Stress and neuroactive steroids, Int Rev Neurobiol. 46, (2001) 243-272.

Berdiev, R.K., Chepurnov, S.A., Chepurnova, N.E., van Luijtelaar, E.L.J.M., Coenen A.M.L., Effects of neuropeptide galanin on spike-wave discharges in WAG/Rij rats. In: Kuznetsova, G.D., Coenen, A.M.L., Chepurnov, S.A., van Luijtelaar, E.L.J.M., Eds. "The WAG/Rij model of absence epilepsy: the Nijmegen-Moscow research." Nijmegen: Nijmegen University Press, (2000) 71-78.

Biggio, G., Concas, A., Follesa, P., Sanna, E., Serra, M., Stress, ethanol, and neuroactive steroids. Pharmacol Ther. (2007).

Birzniece, V., Turkmen, S., Lindblad, C., Zhu, D., Johansson, I.M., Backstrom, T., Wahlstrom, G., GABA(A) receptor changes in acute allopregnanolone tolerance, Eur. J. Pharmacol. 535, (2006) 125-134.

Bosnjak, J., Vukovic-Bobic, M., Mejaski-Bosnjak, V., Effect of war on the occurrence of epileptic seizures in children. Epilepsy Behav. 3, (2002) 502-509.

Bosse, R., Di Paolo, T., Dopamine and GABAA receptor imbalance after ovari-ectomy in rats: model of menopause. J Psychiatry Neurosci. 20, (1995) 364-371.

Budziszewska, B., van Luijtelaar, G., Coenen, A., Leskiewicz, M., Lason, W., Effects of neurosteroids on spike-wave discharges in the genetic epileptic WAG/Rij rat, Epilepsy Res. 33 , (1999) 23-29.

Coenen, A.M.L., Drinkenburg, W.H.I.M, Inoue, M., van Luijtelaar, E.L.J.M.,. Genetic models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. Epilepsy Res. 12: 75-86, 1992.

Concas A., Follesa P., Barbaccia, M.L, Purdy, R.H., Biggio, G., Physiological modulation of $GABA_A$ receptor plasticity by progesterone metabolites. Eur. J. Pharmacol. 30, (1999) 225-235.

Citraro, R., Russo, E., Di Paola, E.D.,. Ibbadu, G.F, Gratteri, S., Marra, R., De Sarro G, Effects of some neurosteroids injected into some brain areas of WAG/Rij rats, an animal model of generalized absence epilepsy, Neuropharmacol. 50, (2006) 1059-1071.

Coenen, A.M., van Luijtelaar, E.L., Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats, Behav Genet. 33, (2003) 635-655.

Czlonkowska, A.I., Krzascik, P., Sienkiewicz-Jarosz, H., Siemiatkowski, M., Szyndler, J., Maciejak, P., Bidzinski, A., Plaznik, A., Tolerance to the anticonvulsant activity of midazolam and allopregnanolone in a model of picrotoxin seizures, Eur. J. Pharmacol 425, (2001) 121-127.

Danober, L., Deransart, C., Depaulis, A., Vergnes, M., Marescaux, C., Pathophysiological mechanisms of genetic absence epilepsy in the rat, Prog Neurobiol. 55, (1998) 27-57.

De Bruin, N.M., van Luijtelaar, E.L., Cools, A.R., Ellenbroek, B.A., Dopamine characteristics in rat genotypes with distinct susceptibility to epileptic activity: apomorphine-induced stereotyped gnawing and novelty/amphetamine-induced locomotor stimulation. Behav. Pharmacol. 12, (2001) 517-525.

DeKeyser, F.G., Leker, R.R., Weidenfeld, J., Activation of the adrenocortical axis by surgical stress: involvement of central norepinephrine and interleukin-1. Neuroimmunomodulation. 7, (2000) 182-188.

Deransart, C., Vercueil, L., Marescaux, C., Depaulis, A., The role of basal ganglia in the control of generalized absence seizures. Epilepsy Res. 32, (1998) 213-223.

Deransart, C., Riban, V., Le, B., Marescaux, C., Depaulis, A., Dopamine in the striatum modulates seizures in a genetic model of absence epilepsy in the rat, Neuroscience 100, (2000) 335-344.

Deransart, C., Depaulis, A., The control of seizures by the basal ganglia? A review of experimental data. Epileptic Disord. 4 Suppl 3, (2002) 61-72.

Follesa, P., Floris, S., Tuligi, G., Mostallino, M.C., Concas, A., Biggio, G., Molecular and functional adaptation of the GABA(A) receptor complex during pregnancy and after delivery in the rat brain. Eur. J. Neurosci. 10, (1998) 2905-2929.

Fuchs, E., Flugge, G., Czeh, B., Remodeling of neuronal networks by stress, Front Biosci. 11, (2006) 2746-2758.

Gerrits, M., Bakker, P.L., Koch, T., Ter Horst, G.J., Eur J Neurosci. 23, (2006) 1747-1756.

Gloor, P., Fariello, R.G., Generalized epilepsy: some of its cellular mechanisms differ from those of focal epilepsy, Trends Neurosci. 11, (1988) 63-68.

Goldstein, L.E., Rasmusson, A.M., Bunney, B.S., Roth, R.H., 1996. Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. Neurosci. 16, 4787-4798.

Grunewald, R.A., Aliberti, V., Panayiotopoulos, C.P. Exacerbation of typical absence seizures by progesterone. Seizure 1, (1992) 137-138.

Harney, S.C., Frenguelli, B.G., Lambert, J.J., Phosphorylation influences neurosteroid modulation of synaptic GABAA receptors in rat CA1 and dentate gyrus neurones, Neuropharmacology 45, (2003) 873-883.

Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C., Cullinan, W.E., Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front Neuroendocrinol. 24, (2003) 151-158.

Inoue, M., Duysens, J., Vossen, J.M., Coenen, A.M., Thalamic multiple-unit activity underlying spike-wave discharges in anesthetized rats, Brain Res. 612, (1993) 35-40.

Kandel, A., Bragin, A., Carpi, D., Buzsaki, G., Lack of hippocampal involvement in a rat model of petit mal epilepsy, Epilepsy Res. 23, (1996) 123-127.

Koksma, J.J., van Kesteren, R.E., Rosahl, T.W., Zwart, R., Smit, A.B., Luddens, H., Brussaard, A.B., Oxytocin regulates neurosteroid modulation of GABA(A) receptors in supraoptic nucleus around parturition, J. Neurosci. 23, (2003) 788-797.

Kostopoulos, G.K., Spike-and-wave discharges of absence seizures as a transformation of sleep spindles: the continuing development of a hypothesis. Clin Neurophysiol. 111 Suppl. 2, (2000) 27-38.

Lason, W., Przewlocka, B., Coenen, A., Przewlocki, R., van Luijtelaar, G., Effects of mu and delta opioid receptor agonists and antagonists on absence epilepsy in WAG/Rij rats. Neuropharmacol. 33, (1994) 161-166.

Liu, Z., Vergnes, M., Depaulis, A., Marescaux, C., Evidence for a critical role of GABAergic transmission within the thalamus in the genesis and control of absence seizures in the rat, Brain Res. 545, (1991) 1-7.

Lubke, J., Morphology of neurons in the thalamic reticular nucleus (TRN) of mammals as revealed by intracellular injections into fixed brain slices, J. Comp. Neurol. 329, (1993) 458-471.

Maguire, J., Mody, I., Neurosteroid synthesis-mediated regulation of GABA(A) receptors: relevance to the ovarian cycle and stress. J. Neurosci. 27, (2007) 2155-2162.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor, Science 232, (1986) 1004-1007.

Majewska, M.D., Ford-Rice, F., Falkay, G., Pregnancy-induced alterations of GABAA receptor sensitivity in maternal brain: an antecedent of post-partum 'blues'? Brain Res. 482, (1989) 397-401.

Marescaux, C., Vergnes, M., Bernasconi, R., $GABA_B$ receptor antagonists: potential new anti-absence drugs, J. Neural. Transm. 35, Suppl (1992a) 179-188.

Marescaux, C., Vergnes, M., Depaulis, A., Genetic absence epilepsy in rats from Strasbourg - A review, J. Neural Transm. 35, Suppl (1992b) 37-69.

McEwen, B.S., Davis, P., Gerlach, J., Progestin receptors in the brain and pituitary gland. In: C.W. Bardin, J. Mauvais-Jarvis, E. Mil-Grom. Progesterone and progestins. NewYork: Raven Press, (1983), 59-76.

McEwen, B.S., Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. Ann. N. Y. Acad. Sci. 1032, (2004) 1-7.

McEwen, B.S., Effects of adverse experiences for brain structure and function, Biol Psychiatry. 48, (2000) 713-714.

Meeren, H.K.M., Cortico-thalamic mechanisms underlying generalized spike-wave discharges of absence epilepsy. A lesional and signal analytical approach in the WAG/Rij rat. PhD thesis, NICI, Radboud University Nijmegen, 2002.

Meeren, H., van Luijtelaar, G., Lopes da Silva, F., Coenen, A., Evolving concepts on the pathophysiology of absence seizures: the cortical focus theory. Arch. Neurol. 62, (2005) 371-376.

Melcangi, R.C., Panzica, G.C., Neuroactive steroids: old players in a new game, Neurosci. 138 (2006) 733-739.

Midzyanovskaya, I., Strelkov, V., Rijn, C., Budziszewska, B., van Luijtelaar, E., Kuznetsova, G., Measuring clusters of spontaneous spike-wave discharges in absence epileptic rats. J. Neurosci. Methods. 154, (2006) 183-189.

Midzyanovskaya, I.S., Kuznetsova, G.D., van Luijtelaar, E.L., van Rijn, C.M., Tuomisto, L., Macdonald, E., The brain 5HTergic response to an acute sound stress in rats with generalized (absence and audiogenic) epilepsy. Brain Res. Bull. 69, (2006) 631-638.

Midzyanovskaya, I.S., Absence and mixed forms of epilepsy in WAG/Rij rats: characteristics and brain aminergic modulations. PhD thesis, NICI, Radboud University Nijmegen, 2006.

Modesti, P.A., Pela, I., Cecioni, I., Gensini, G.F., Serneri, G.G., Bartolozzi, G.. Changes in blood pressure reactivity and 24-hour blood pressure profile occurring at puberty. Angiology. 45, (1994) 443-450.

Morgane, P.J., Galler, J.R., Mokler, D.J., A review of systems and networks of the limbic forebrain/limbic midbrain. Prog. Neurobiol. 75, (2005) 143-160.

Paz, J.T., Polack, P.O., Slaght, S., Deniau, J.M., Mahon, S., Charpier, S., Propagation and dynamic processing of cortical paroxysms in the basal ganglia networks during absence seizures. In "Generealized seizures: from clinical phenomenology to underlying systems and networks" Eds: Hirsch, E., Andermann, F., Chauvel, P., Engel, J., Lopes de Silva, F., Luders, H.; John Libbey Eurotext, UK, 2006, pp. 120-126

Peeters, B.W., van Rijn, C.M., Vossen, J.M., Coenen, A.M.. Effects of GABA-ergic agents on spontaneous non-convulsive epilepsy, EEG and behaviour, in the WAG/Rij inbred strain of rats, Life Sci. 45, (1989) 1171-1176.

Perez Velazquez, J.L., Huo, J.Z., Dominguez, L.G., Leshchenko, Y., Snead III, O.C., Typical versus Atypical Absence Seizures: Network Mechanisms of the Spread of Paroxysms. Epilepsia 48(8) (2007) 1585-1593.

Rhodes, M.E., Harney, J.P., Frye, C.A., Gonadal, adrenal, and neuroactive steroids' role in ictal activity, Brain Res. 1000, (2004) 8-18.

Rhodes, M.E., Frye, C.A., Actions at GABA(A) receptors in the hippocampus may mediate some antiseizure effects of progestins, Epilepsy Behav. 6, (2005) 320-327.

Riban, V., Pereira de Vasconcelos, A., Pham-Le, B.T., Ferrandon, A., Marescaux, C., Nehlig, A., Depaulis, A., Modifications of local cerebral glucose utilization in thalamic structures following injection of a dopaminergic agonist in the nucleus accumbens--involvement in antiepileptic effects? Exp. Neurol. 188, (2004) 452-460.

Romeo, R.D., McEwen, B.S., Stress and the adolescent brain. Ann. N. Y. Acad. Sci. 1094, (2006) 202-214.

Romeo, R.D., Lee, S.J., McEwen, B.S., Differential stress reactivity in intact and ovariectomized prepubertal and adult female rats. Neuroendocrinology. 80, (2004) 387-393.

Stromberg, J., Backstrom, T., Lundgren, P., Rapid non-genomic effect of glucocorticoid metabolites and neurosteroids on the gamma-aminobutyric acid-A receptor, Eur J Neurosci. 21, (2005) 2083-2088.

Saalmann, Y.B., Morgan, I.G., Calford, M.B., Neurosteroids involved in regulating inhibition in the inferior colliculus, J Neurophysiol. 96, (2006) 3064-3073.

Sarkisova, K.Y., Midzianovskaia, I.S., Kulikov, M.A, Depressive-like behavioral alterations and c-fos expression in the dopaminergic brain regions in WAG/Rij rats with genetic absence epilepsy. Behav. Brain Res. 144, (2003) 211-226.

Scharfman, E., Goodman, J.H.,. Rigoulot, M.A, Berger, R.E., Walling, S.G., Mercurio, T.C., Stormes, K., Maclusky, N.J., Seizure susceptibility in intact and ovariectomized female rats treated with the convulsant pilocarpine. Exp Neurol. 196, (2005) 73-86.

Schridde, U., van Luijtelaar, G., Corticosterone increases spike-wave discharges in a dose- and time-dependent manner in WAG/Rij rats, Pharmacol Biochem Behav. 78, (2004) 369-375.

Schultz, W., Behavioral dopamine signals. Trends Neurosci. 30, (2007) 203-210.

Snead 3rd, O.C., Depaulis, A., Banerjee, P.K., Hechler, V., Vergnes, M., The GABAA receptor complex in experimental absence seizures in rat: an autoradiographic study, Neurosci Lett. 140, (1992) 9-12

Solomon, R.L., Corbit, J.D., An opponent-process theory of motivation. I. Temporal dynamics of affect. Psychol. Rev. 81, (1974) 119-145.

Smith, S.S., Shen, H., Gong, Q.H., Zhou, X., Neurosteroid regulation of GABA(A) receptors: Focus on the alpha4 and delta subunits. Pharmacol Ther.

Turkmen, S., Lofgren, M., Birzniece, V., Backstrom, T., Johansson, I.M., Tolerance development to Morris water maze test impairments induced by acute allopregnanolone, Neuroscience 139, (2006) 651-659.

van Luijtelaar, E.L.J.M., Coenen, A.M.L., The WAG/Rij model for generalized absence seizures. In: J. Manelis et al (Ed.), Advances in Epileptology, Vol. 17, Raven Press, 1989, 78-83.

van Luijtelaar, G., Budziszewska, B., Jaworska-Feil, L., Ellis, J., Coenen, A., Lason, W., The ovarian hormones and absence epilepsy: a long term EEG study and pharmacological effects in a genetic absence epilepsy model, Epilepsy Res. 46, (2001) 225-239.

van Luijtelaar, G., Budziszewska, B., Tetich, M., Lason, W., Finasteride inhibits the progesterone-induced spike-wave discharges in a genetic model of absence epilepsy, Pharmacol. Biochem. Behav. 75, (2003) 889-894.

van Luijtelaar, E.L., Dirksen, R., Vree, T.B., van Haaren, F., Effects of acute and chronic cocaine administration on EEG and behaviour in intact and castrated male and intact and ovariectomized female rats. Brain Res. Bull. 40, (1996) 43-50.

van Luijtelaar, G., Sarkisova, K.Y., Midzyanovskaya, I.S., Tolmacheva, E.A., Stress vulnerability and depressive symptoms in genetic epileptic rats. In: New research on Epilepsy and Behavior, eds Hollaway, K.J. Nova, Ca, USA pp 000-000..2007

van Luijtelaar, G., Sitnikova, E., Global and focal aspects of absence epilepsy: The contribution of genetic models, Neurosci. Biobehav. Rev. 30, (2006) 983-1003.

Vergnes, M., Marescaux, C., Depaulis, A., Mapping of spontaneous spike and wave discharges in Wistar rats with genetic generalized non-convulsive epilepsy, Brain Res. 523, (1990) 87-91.

Weizman, R., Dagan, E., Snyder, S.H., Gavish, M., Impact of pregnancy and lactation on $GABA_A$ receptor and central-type and peripheral-type benzodiazepine receptors. Brain Res. 752, (1997) 307-314.

Wilson, M.A., Biscardi, R., Effects of gender and gonadectomy on responses to chronic benzodiazepine receptor agonist exposure in rats, Eur J Pharmacol. 215, (1992) 99-107.

Zhu, D., Birzniece, V., Backstrom, T., Wahlstrom, G., Dynamic aspects of acute tolerance to allopregnanolone evaluated using anaesthesia threshold in male rats, Br. J. Anaesth. 93, (2004) 560-567.

Summary

Summary

In the General Introduction (**Chapter 1)**, the main principles in the regulation and functioning of the hypothalamo-pituitary-adrenal (HPA) and the hypothalamo-pituitary-gonadal (HPG) hormonal systems, as well as the main facts concerning the role of steroid hormones in the regulation of seizure susceptibility are described.

The first series of experiments (**Chapter 2)**, were aimed to investigate the occurrence of absence seizures during pregnancy. Pregnancy is characterized by an increase in endogenous secretion of ovarian steroid hormones, in particular, progesterone. Acute pharmacological studies showed that systemic administration of progesterone leads to a dose-dependent increase in the number of spike-wave discharges in WAG/Rij rats. By contrast, we showed that a high plasma progesterone concentration during pregnancy was accompanied by a decrease, rather than in increase, in spike-wave discharges. Thus the effect of chronic changes in the hormonal milieu during pregnancy was found to be opposite to the effect of an acute challenge with progesterone.

In the next series of experiments in **Chapter 3**, the occurrence of spike-wave discharges after daily repeated administration of progesterone was tested. The outcomes revealed that a single administration of progesterone in a dose of 20 mg/kg enhances indeed spike-wave discharges, but it is also sufficient to make rats tolerant to subsequent exposure to this hormone. In addition, it was demonstrated that the effects of acute administration of progesterone were aggravated by two preceding injections of cyclodextrine which was used as a control. It was suggested that mild stress accompanying the injection was the underlying reason for the increase. Further in the same chapter (**Chapter 3**), the occurrence of absence seizures was investigated following ovariectomy, which eliminates the peripheral source of gonadal steroid hormones. The data showed no difference in either the occurrence or the duration of absence seizures between the ovariectomized and the sham-operated WAG/Rij females for up to 35 days period after surgery. Interestingly, however, ovariectomized rats showed a larger increase in spike-wave activity after repeated exposure to stress induced by foot shocks, as well as in conditions in which foot shock exposure was anticipated. These results suggest that there is a remarkable interaction between the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axis in the control of absence seizures.

In **Chapter 4,** some of the basic parameters of the hypothalamic-pituitary-adrenal axis function in epileptic WAG/Rij male rats in

comparison to non-epileptic control rats (ACI and Wistar strains) were characterized. It was found that WAG/Rij rats tended to show a higher basal plasma corticosterone concentration than Wistar rats, while the highest corticosterone concentration was characteristic for ACI rats. A similar relation between strains and plasma levels was found after exposure to acute stress.

Next in **Chapter 4**, it was shown that an exposure to foot shock stress had a biphasic effect on the number of absence seizures: a quick decrease in the first 15 minutes was followed by an increase in discharges lasting 45-60 min. Remarkably, it was demonstrated that if an exposure to foot shock was repeated, the initial suppression was getting reduced, while the subsequent aggravation in spike-wave discharges became larger. An aggravation in the number of discharges from day to day was also found in the base-line period, while each following day also this aggravation became more and more prominent. Hence, not only exposure to stress, but also the anticipation of stressful stimuli is capable to increase the incidence of absence seizures in WAG/Rij rats, and the lack of ovarian steroid hormones (as shown in Chapter 3) enhances this aggravation.

The second part of this thesis was aimed to investigate the excitability of the cortical and the limbic system – the two major target areas for steroid hormones in the brain - in WAG/Rij rats. The cortex plays a leading role in various theories on the origin of absence epilepsy and a hyper-excitability of the cortex is classically regarded as a precondition for the transformation of sleep spindles into spike-wave discharges. In the following study in **Chapter 5,** a local rhythmic electrical stimulation of the sensorimotor cortex in freely moving rats was used to investigate whether genetic epileptic rats are inter-ictally endowed with a more excitable cortex. In contrast to what was expected, there appeared not to be a difference in excitability between WAG/Rij and ACI rats, as measured by various types of electrophysiological variables such as the threshold for movements elicited by individual stimuli and the threshold for evoking epileptic afterdischarges accompanied by clonic seizures. Importantly, however, it was found that WAG/Rij rats are characterized by the lowest threshold for the spread of epileptic activity into the limbic system compared to both Wistar and ACI rats. Moreover, a negative correlation between this threshold and the amount of spike-wave activity was found.

Then in **Chapter 6**, it was tested whether a local administration of steroid hormones into the limbic system would have an effect on the occurrence of absence seizures. We have chosen to test the effect of progesterone (a $GABA_A$ mimetic) and tiagabine (a GABA reuptake

inhibitor) in the hippocampus, which is one of the important parts of the limbic system involved in the control of autonomic, cognitive, and emotional functions. The outcomes showed that hippocampal administration of both progesterone, tiagabine, but also the vehicle used for the administration of progesterone (cyclodextrine), but not the solvent for tiagabine results in a reduction of spontaneous spike-wave discharges during 60 min (for cyclodextrine) and 90-120 min (for progesterone and tiagabine). Progesterone administration into the cortex yielded no changes in the occurrence of discharges. The data suggest that activation of GABA-ergic neurotransmission in the hippocampus has an inhibitory effect on cortico-thalamo-cortical circuits underlying the generation of spike-wave discharges. Furthermore, the hippocampus might be critically involved in the regulation of absence seizures.

In the General Discussion (**Chapter 7),** all the major findings of the thesis were outlined and a model, integrating the classical cortico-thalamo-cortical loop with the limbic system circuitry (the hippocampus and the prefrontal cortex ensemble) was presented. In this model, putative mechanisms that could underlie the interaction between the specific and non-specific (related to the limbic system) thalamo-cortical circuits were portrayed. In the framework of this model and on the base of the evidence from the experimental data, it was hypothesized that the role of steroid hormonal milieu in absence epilepsy could be intimately related to the limbic system, in particular, to the circuitry formed by the hippocampus, the prefrontal cortex and the amygdala. It was postulated that the limbic system, along with the basal ganglia system, could be a part of a major circuitry that provides a remote control of oscillations generated in the cortico-thalamo-cortical loop.

Overall, the data of the present thesis showed that the ovarian steroid hormonal milieu were not critical for the regulation of absence epilepsy under base-line, resting, conditions. However, the presence of ovarian hormones became critical when there was either an endogenous (pregnancy) or exogenous (stress) challenge for the organism. In addition to this, it was found that genetically epileptic WAG/Rij rats showed an impaired function of the limbic system and that the limbic circuitry, which is not considered in any classical theory on absence epilepsy, could play an important role in the remote control of absence seizures. Considering that the limbic system is a major target for the effects of steroid hormones of the hypothalamus-pituitary-adrenal and hypothalamus-pituitary-gonadal axis, the involvement of the limbic system in the remote control of absence seizures provides an additional perspective on the role of how steroid hormones play a role in the pathogenesis of absence epilepsy. This helps to understand the controversial

effects of the acute and chronic effects of ovarian steroid hormones described in the present thesis. These data also provide an insight why at puberty some children with absence epilepsy eventually start to experience tonic-clonic convulsions.

Samenvatting

In de Algemene Introductie van **Hoofdstuk 1** zijn de belangrijkste regulatieprincipes van het hormonale hypothalamus-hypofyse-gonaden systeem en van het hypothalamus-hypophyse-bijnier systeem beschreven. Ook is de rol van steroid hormonen bij de gevoeligheidsregulatie van epileptische aanvallen besproken. In de eerste serie experimenten, gepresenteerd in **Hoofdstuk 2**, wordt het voorkomen van epileptische absence aanvallen tijdens de zwangerschap van ratten onderzocht. Eerdere farmacologische studies in acute experimenten hebben laten zien dat progesteron aanleiding geeft tot een dosis-afhankelijke toename van het aantal epileptische ontladingen in WAG/Rij ratten. Tijdens de zwangerschap neemt de endogene secretie van steroid hormonen in het ovarium toe, in het bijzonder van progesteron. Interessant is daarbij dat een hoge plasma progesteron concentratie, zoals tijdens de zwangerschap, juist gepaard gaat met een afname van het aantal epileptische ontladingen. In dit hoofdstuk is dus aangetoond dat de effecten van chronische veranderingen van progesteron in het hormonale milieu omgekeerd zijn aan die van acute hormonale veranderingen.

In de volgende serie experimenten, beschreven in **Hoofdstuk 3**, is het optreden van piek-golf ontladingen na een herhaalde dagelijkse injecties van progesteron, bestudeerd. De uitkomsten geven aan dat een enkele toediening van progesteron, in een dosis van 20 mg/kg, inderdaad een verhoging geeft van het aantal aanvallen, maar ook al voldoende is om ratten tolerant te maken voor een volgende toediening. Ook is aangetoond dat een acute toediening van progesteron de aanvallen verergeren als deze voorafgegaan wordt door enkele voorafgaande injecties van cyclodextrine, een stof die als controle gebruikt wordt. Gedacht wordt, dat de milde stress die gepaard gaat met het toedienen van het oplosmiddel, daarvoor verantwoordelijk is. In hetzelfde hoofdstuk is onderzocht wat er met piek-golf ontladingen gebeurt na het wegnemen van het ovarium, een ingreep die de perifere bron van de gonadale steroid hormonen wegneemt. De uitkomsten tonen echter aan dat er zowel in de aantallen als in de duur van de aanvallen geen verschil is tussen vrouwtjesratten waarbij het ovarium is weggenomen als de ‘sham’ geopereerde WAG/Rij vrouwtjes, zeker tot een periode tot 35 dagen na de operatie. Interessant is wel dat de ratten zonder ovarium een grotere toename vertonen van epileptische activiteit na een herhaaldelijke blootstelling aan stress, veroorzaakt door electrische voetschokken. Dit is ook al het geval in condities waarbij blootstelling aan elektrische schokken

verwacht wordt, voorafgaand aan het toedienen van de dagelijks herhaalde voetschokken. Deze gegevens wijzen op een belangrijke interactie tussen de hypothalamus-hypophyse-gonaden as en de hypothalamus–hypophyse-bijnier as bij de aanvalscontrole.

In **Hoofdstuk 4** worden enige basale parameters van het hypothalamus-hypophyse-bijnier systeem van mannelijke epileptische WAG/Rij ratten onderzocht, in vergelijking met niet-epileptische stammen als de ACI en de Wistar stam. In het bijzonder werd gevonden dat WAG/Rij ratten een hogere basale plasma corticosteron concentratie hebben dan Wistar ratten, terwijl de hoogste concentratie corticosteron voorkomt bij ACI ratten. Eenzelfde verschil is gevonden na blootstelling aan acute stress.

Vervolgens is aangetoond dat blootstelling aan stress van voetschokken een bifasisch effect heeft op het aantal absences. Een snelle afname in de eerste 15 minuten wordt gevolgd door een toename van aanvallen die 45 tot 60 minuten aanhoudt. Van belang is dat de initiële aanvalsreductie na een voetschok, overgaat in een toename als de voetschok herhaaldelijk wordt toegediend. Van dag tot dag is een toename van aanvallen gezien, ook in het spontane voorkomen en deze verergering van de epileptische situatie is aanzienlijk. De conclusie is dat niet alleen de daadwerkelijke blootstelling aan stress, maar zelfs de anticipatie op het aanbieden van stressvolle stimuli, de epilepsie verergert. Voorts is geconcludeerd dat een tekort aan ovariële steroid hormonen, zoals beschreven in het vorige hoofdstuk, deze verergering verder opjaagt.

Het tweede deel van het proefschrift is gewijd aan het bestuderen van de gevoeligheid van het corticale en het limbische systeem van de WAG/Rij rat: de twee belangrijke doelgebieden voor steroid hormonen in het brein. De cortex speelt een leidinggevende rol in diverse theorieën over het ontstaan van absence epilepsie, en een overgevoeligheid van de cortex wordt vaak gezien als een preconditie voor de omvorming van slaapspoelen in piek-golf ontladingen. In het onderzoek, beschreven in **Hoofdstuk 5**, wordt een locale ritmische electrische stimulatie van de sensorimotorische cortex van vrijbewegende ratten toegepast om na te gaan of genetisch epileptische ratten, interictaal, een meer gevoelige cortex hebben. Tegen de verwachting in, bleek er geen verschil te zijn in de corticale gevoeligheid tussen WAG/Rij en ACI ratten. De corticale gevoeligheid is vastgesteld met electrofysiologische variabelen, zoals de drempelwaarde voor bewegingen die uitgelokt worden door stimuli en de drempelwaarde voor het opwekken van epileptische ontladingen die gepaard gaan met clonische aanvallen. Opvallend is echter dat WAG/Rij ratten, in vergelijking met zowel Wistar als ACI ratten, de laagste drempelwaarde voor de spreiding van de

epileptische activiteit naar het limbische systeem laten zien. Bovendien is er een negatieve correlatie gevonden tussen de hoogte van de drempelwaarde en de hoeveelheid piek-golf ontladingen.

In **Hoofdstuk 6** is getest of een locale toediening van steroid hormonen in het limbische systeem effect heeft op het optreden van epileptische aanvallen. Er is gekozen om dit effect te onderzoeken met progesteron (een $GABA_A$ mimeticum) en de effecten zijn vergeleken met tiagabine (een GABA-heropname remmer). Beide stoffen zijn aangebracht in de hippocampus, een van de meest onderzochte delen van het limbisch systeem dat betrokken is bij autonome en cognitieve functies, alswel bij de regulatie van emoties. De uitkomsten demonstreren dat toediening in de hippocampus van zowel progesteron, van tiagabine als van het progesteron oplosmiddel cyclodextrine resulteert in een vermindering van spontane piek-golf ontladingen gedurende 60 minuten (voor cyclodextrine) en 90-120 minuten (voor progesteron en tiagabine). Zowel progesteron toediening in de motorische cortex als een saline injectie in de hippocampus levert geen veranderingen in de mate van optreden van ontladingen. De gegevens suggereren dan ook dat een activatie van de GABA-erge transmissie in de hippocampus een inhibitoir effect heeft op de cortico-thalamo-corticale circuits die betrokken zijn bij de generatie van epileptische aanvallen ofwel bij de regulatie van de aanvallen.

In de Algemene Discussie in **Hoofdstuk 7** zijn de belangrijke bevindingen van dit proefschrift in een model ondergebracht. Dit model integreert het klassieke cortico-thalamo-cortical circuit en het circuit van het limbisch systeem (de hippocampus en het prefrontale cortex ensemble) met het hormonale systeem. In het model zijn de mogelijke mechanismen geschetst die aan de basis zouden kunnen staan van de interactie tussen de specifieke (thalamo-corticale) en de niet-specifieke (limbische) circuits. In het kader van dit model en de evidentie van de experimentele gegevens, wordt getheoretiseerd dat bij absence epilepsie de rol van het hormonale steroid milieu nauw gerelateerd is aan het limbisch system, en in het bijzonder aan het circuit dat gevormd wordt door de hippocampus, de prefrontale cortex en de amygdala. In dit hoofdstuk wordt tevens gepostuleerd dat naast het systeem van de basale ganglia system, het limbische system onderdeel zou kunnen zijn van het belangrijke circuit dat op afstand controle uitoefent op de oscillaties die gegenereerd worden door het cortico-thalamo-corticale systeem.

In het algemeen tonen de gegevens die in dit proefschrift beschreven zijn aan dat onder omstandigheden van rust het ovariële hormonale steroid milieu niet kritisch betrokken is bij de regulatie van absence epilepsie. Dit verandert echter ingrijpend wanneer er hetzij een endogene (zwangerschap)

verandering optreedt, dan wel een exogene (stress door electrische schok, anticipatie van stress) verandering in het organisme. Daarbij komt de vondst dat de genetisch epileptische WAG/Rij rat een verstoorde functie van het limbisch system laat zien, en dat het limbisch system, dat in geen enkele theorie van absence epilepsie voorkomt, een aanzienlijke rol kan spelen in het op afstand controleren van de aanvallen. Gegeven het feit dat het limbisch systeem een belangrijk doelwit voor steroid hormonen is, levert de betrokkenheid van het limbisch systeem bij de aanvalscontrole aanvullende inzichten in de rol van steroid homonen bij absence epilepsie. Op deze wijze kunnen de tegenstrijdige gegevens van acute en chronische effecten van ovariële steroid hormones die in dit proefschrift beschreven zijn, begrepen worden. Ook verschaffen deze gegevens inzicht in het overgangsproces van typische absence aanvallen naar juvenile absences en tonisch-clonische aanvallen; een proces dat bij kinderen in de puberteit kan optreden.

Автореферат

Данная диссертационная работа посвящена изучению роли стероидных гормонов в регуляции абсансных судорог у крыс линии WAG/Rij. В общем введении (**Глава 1**) описаны основные пирнципы в регуляции гипоталамо-гипофизарно-адреналовой и гипоталамо-гипофизарно-гонадальной систем, а также основные данные о роли стероидных гормонов в регуляции судорожной чувствительности.

В первой серии экспериментов (**Глава 2**) исследовали частоту абсансных судорог у крыс линии WAG/Ri во время беременности. Беременность является состоянием, характеризуемым повышенной секреции овариальных стероидных гормонов, в частности, прогестерона. Ранее, в остром фармакологическом эксперименте было выявлено, что системное введение прогестерона ведет к дозозависимому увеличению количества спайк-волновых разрядов у крыс линии WAG/Rij. В данной главе показано, что высокое плазменное содержание прогестерона во время беременности сопровождается снижением, а не повышением спайк-волновых разрядов. Таким образом, результат хронических изменений в гормональном статусе может быть противоположен немедленному эффекту острого введения того или иного гормона.

В следующей серии экспериментов **(Глава 3**) была исследована частота спайк-волновой активности после повторного (ежедневного) введения прогестерона. Полученные результаты показали, что введение прогестерона в дозе 20 мг/кг действительно приводит к усилению спайк-волновой активности, но при этом вызывает быстрое развитие толерантности, т.е. отсутсвие какого-либо эффекта при повторном введении этого гормона. Кроме этого, было показано, что эффект системного введения прогестерона может быть усилен предварительным введением раствора циклодекстрина используемго в качестве контроля. Предположено, что мягкий стресс сопроваждающий процедуру инъецирования мог являться причиной данного поотенциирующего эффекта. Далее в этой же главе была исследована частота спайк-волнвой активности после операции овариэктомии, при которой удаляется основной перифирический источник половых стероидных гормонов. Полученные данные не выявили разницы в спайк-волновой активности между овариэктомированными и ложнооперированными самками на протяжении 35 дней после операции. Интересно, что овари-

эктомированные самки демонстрировали большее увеличение в спайк-волновой активности при повторном стрессорном воздействии 'foot-shock', а также в условиях его ожидания, что указывает на взаимодействие между гипоталамо-гипофизарно-адреналовой и гипоталамо-гипофизарно-гонадальной системами.

В **Главе 4** были характеризованы некоторые из основных параметров гипоталамо-гипофизарно-адреналовой системы у крыс эпилептической линии WAG/Rij по сравнению с крысами неэпилептических линий (ACI и Wistar). Было покзано, что крысы линии WAG/Rij имеют тенденцию к более высокомй уровню кортикостерона по сравнению с крысами линии Wistar, тогда как самый высокий уровень кортикостерона характерен для крыс линии ACI. Сходные результаты были получены для параметров стресс-вызванного содержания кортикостерона в плазме.

Далее, в той же **главе 4** было продемонстроировано, что предъявление 'foot-shock'стресса вызывает двухфазное изменение в динамике частоты появления абсансных судорог: за резким снижением частоты судорог в течение первых 15 минут следовало учеличение числа разрядов на протяжение 45-60 минут. При повторном предъявлении данного стрессорного воздействия, первичное снижение становилось короче и мнее выраженным, а последующее учеличение, наоборот, длиннее и более выраженным. Повышение частоты появления абсансных судорог было найдено также и в период предшествующий непосредственному предъявлению стрессорного воздействия. Таким образом, не только предъявление стрессорного воздействия, но и его психологическое ожидание повышает частоту абсансный судорог у крыс линии WAG/Rij, причем нехватка овариальных гормонов (как было покзано в Главе 3) существенно усиливает выраженность данной реакции.

Вторая часть данной диссертационной работы была посвящена исследованию возбудимости кортикальной и лимбической системы – двух главных мишеней действия стероидных гормонов в мозге - у крыс линии WAG/Rij. Кора больших полушарий играет ведущую роль в различных теориях о происхожении абсансной эпилепсиии. Согласно классическим представлениям, гипервозбудимость коры ведет к трансформации сонного веретена в спайк-волновой разряд и является необходимым прелусловием его возникновения. В следующем эксперименте (**Глава 5**), при помощи метода локальной электрической стимуляции сенсомоторной коры свободно движущегося животного было исследовано характеризует ли крыс линии WAG/Rij повышенная

возбудимость коры в интериктальный период. Вопреки всем ожиданиям, результаты данного исследования не выявили достверного различия между крысами линии WAG/Rij и ACI по таким характеристикам возбудимости коры как порог появления непроизвольных моторных движений во время стимуляции, а также порог появления вызванного эпилептического послеразряда, сопровождаемого моторными судорогами. Интересно, однако, что по сравнению с крысами неэпилептических линий Wistar и ACI генетически эпилептических крыс линии WAG/Rij характеризовал более низкий порог появления лимбического типа послеразряда, свидетельствующий о распространении эпилептической активности в лимбическую систему. Кроме того, была найдена негативная корреляция между величиной порога и количеством спайк-волновой активности.

Далее, в **Главе 6**, был исследован эффект локальной администрации стероидных гормонов в лимбическую систему на абсансные судороги. Исследовали эффект прогестерона (ГАМК-миметик) и тиагабина (блокатор обратного захвата ГАМК) апплицированных в гиппокамп - одну из важнейших структур лимбической системы, участвующую в регуляции автономных, когнитивных и эмоциональных функций. Результаты исследования показали, что внутри-гиппокампальная аппликация прогестерона, тиагабина, а также циклодекстрина (использованного для растворения прогестерона), но не физиологического раствора (использованного для растворения тиагабина) ведет к подавлению спайк-волновой активности на протяжении 60 минут (для циклодекстрина) и 90-120 мин (для прогетсерона и тиагабина) после введения. Введение прогестерона в кору над гиппокампом никаких изменений в частоте спайк-волновых разрядов не вызывало. Полученны данные свидетельствуют о том, что активация тормозной ГАМК-эргической системы гиппокампа тормозит генерацию спайк-волновых разрядов в кортико-таламо-кортикальной системе. Данный результат свидетельствует о том, что гиппокам может принимать участие в регуляции спайк-волновых разрядов.

В общем обсуждении (**Глава 7**) суммированы все основные результаты данной диссертации и представлена модель, интегрирующая таламо-кортикальную и лимбическую (префронтальная кора и гиппокамп) системы. Предложены механизмы взаимодействия между специфическими и неспецифическими таламо-кортикальными нейронными сетями. В рамках данной модели и на основе известных экспериментальных данных, выдвинута гипотеза о

том, что роль стероидных гормонов в абсансной эпиепсии тесно связана с лимбической системой, в особенности структурами гиппокампа, префронтальной коры и амигдалы. Предположено, что наряду с системой базальных ганглиев, лимбическая система может участвовать в ‘дистанционной’ регуляции осцилляций генерируемых в таламо-кортикальной системе.

Таким образом, результаты данной диссертационной работы показали, что плазменное содержание овариальных стероидных гормонов не оказывает существенного влияния на частоту возникновения абсансных судорог в нормальных условиях. Тем не менее, присутствие этих гормонов становится важным в ситуации либо эндогенной (беременность), либо экзогенной нагрузки на организм. Показано, что крыс линии WAG/Rij характеризует более высокая возбудимость лимбической системы и что эта система может быть вовлечена в регуляцию частоты возникновения абсансных судорог. Учитывая то, что лимбическая система является одной из основных мишеней для действия стероидных гормонов в мозге, участие структур лимбической системы в регуляции спайк-волновой активности существенно расширяет наши представления о роли стероидных гормонов в патогенезе абсансной эпилепсии. Это позволяет объяснить противоречивые результаты полученные при острых и хронических изменениях в гормональном статусе, а также понять почему во время пубертатного периода некоторые из детей с абсансной эпилепсией начинают испытывать приступы более тяжелых, тонико-клонических судорог.

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Elena A. Tolmacheva, October 2007

Curriculum Vitae

Elena Alexandrovna Tolmacheva

was born on 5th of March, 1980 in Ivanteyevka, Moscow Region, USSR (Russia). After completion of her secondary school (1987-1996) including an advanced study in mathematics and physics at the A. N. Colmogorov school at Moscow State University, she entered the Biological Faculty of M.V. Lomonosov Moscow State University. She studied at the Department of Human and Animal Physiology and specialized in neurophysiology and epileptology. Her bachelor and master thesis were supervised by Prof. **Serguei A. Chepurnov**. In 2001 she graduated ('cum laude') with a Master of Science degree in Physiology and received the Gold Medal of Excellence of Moscow State University. In 2001 she became a postgraduate student at the Department of Human and Animal Physiology to work on the project "The role of steroid hormones in the regulation of absence seizures in WAG/Rij rats". In the same year she did a research project on cortical excitability in WAG/Rij rats at the Institute of Physiology, Academy of Sciences of the Czech Republic under supervision of Prof. Pavel Mares. Later on, in 2003, she received a Huygens Scholarship to do part of her experiments at the Dept. Biological Psychology, Institute for Cognition and Information (NICI), Radboud University Nijmegen, The Netherlands under supervision of Dr. Gilles van Luijtelaar. After that she was offered to participate in a 'sandwich' PhD project between Radboud University of Nijmegen and Moscow State University and to continue her research on steroid hormones and absence epilepsy in the financial framework of this program.

List of publications

Full publications in international journals:

Chepurnov S.A., Chepurnova N.E., Tolmacheva E.A., Kochetkov Ya.A., Luijtelaar E.L.J.M., Coenen A.M.L., Progesteron, estradiol and Spike And Wave discharges during the pregnancy of WAG/Rij rats – model of absence epilepsy.// *Russian Journal of Physiology* (formerly I.M.Sechenov Physiological Journal), 88, 6: 557-65, 2002.

Tolmacheva E. A., Chepurnov S. A., Chepurnova N. E., Kochetkov Ya. A., van Luijtelaar E.L.J.M. Absence seizures during pregnancy in WAG/Rij rats. *Physiology and Behavior*, 81(4): 623-627, 2004.

Tolmacheva E.A., van Luijtelaar G., Chepurnov S.A, Kaminskij Y., Mares P. Cortical stimulation in rats with genetic absence epilepsy. *Epilepsy Research*, 62: 189-198, 2004.

Tolmacheva E.A., van Luijtelaar G. Absence seizures are reduced by the enhancement of GABA-ergic inhibition in the hippocampus in WAG/Rij rats. *Neuroscience Letters,* 416(1): 17-21, 2007.

Tolmacheva E.A., van Luijtelaar G. Ovarian steroid hormones in the regulation of basal and stress induced absence seizures. *Journal of Steroid Biochemistry and Molecular Biology*, 104(3-5):281-288, 2007.

Tolmacheva E.A., Oitzl M.S., van Luijtelaar G. Hypothalamo-pituitary-adrenal axis and stress in the regulation of absence seizures in genetic epileptic rats (*under preparation for submission*).

Publications in books:

Tolmacheva E. A., Chepurnov S. A., Chepurnova N. E.,Kochetkov Ya. A., van Luijtelaar E.L.J.M. Absence seizures and progesterone in WAG/Rij rats: effects of pregnancy. In "The WAG/Rij model of absence epilepsy: The Nijmegen-Russian Federation papers", editors: Gilles van Luijtelaar, Galina D. Kuznetsova, Anton Coenen and Serguei A. Chepurnov, Nijmegen University Press, p.139-147, 2004.

Tolmacheva E.A., van Luijtelaar G., Chepurnov S.A, Mares P. Genetic absence rats have a lower threshold for limbic type of afterdischarges: a cortical stimulation study. /In "The WAG/Rij model of absence epilepsy: the Nijmegen-Moscow papers", editors: Gilles van Luijtelaar, Galina D. Kuznetsova, Anton Coenen and Serguei A. Chepurnov, Nijmegen University Press, pp.225-235, 2004.

van Luijtelaar, G., Sarkisova, K.Y., Midzyanovskaya, I.S., Tolmacheva, E.A., Stress vulnerability and depressive symptoms in genetic epileptic rats. In: New research on Epilepsy and Behavior, eds Hollaway, K.J. Nova, Ca, USA, 2007.

Abstracts in international journals:

Tolmacheva E.A., van Luijtelaar G., Chepurnov S.A, Mares P. Genetic absence rats have a lower threshold for limbic type of afterdischarges: a cortical stimulation study. // Abstract from the 5th European Congress on Epileptology Madrid, 6-10 October 2002, Epilepsia, 2002, V.43, Suppl.8, p.84.

Chepurnov S.A., Tolmacheva E.A., Kochetkov Ya.A., Luijtelaar E.L.J.M., Coenen A.M.L. Progesteron, estradiol and spike and wave discharges during the pregnancy of WAG/Rij rats – model of absence epilepsy // Abstract from the 5th European Congress on Epileptology Madrid, 6-10 October 2002, Epilepsia, 2002, V.43, Suppl.8, p.84.

Tolmacheva E.A., Oitzl M. S., Chepurnov S.A., van Luijtelaar E.L.J.M. Activity of the hypothalamus-pituitary-adrenal axis in female absence epileptic rats. // Abstract from the 6th International Congress on Epileptology, Lissabon, 2003 *Epilepsia,* 44, Suppl.8 p.107.