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# Hormones and Absence Epilepsy<sup>☆</sup>

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## Introduction

Puberty, menarche, menses, and menopause are often critical times when reproductive hormones may have a profound impact on seizures. An example is the shift from absence to generalized tonic-clonic seizures that has been noted during puberty in some children with childhood absence epilepsy. There is however no direct evidence to substantiate the suggestion that this switch is due to changes in concentration of reproductive hormones. Data from research in laboratory animals show that gonadal hormones can exert a profound influence on neuronal excitability, seizures, and epilepsy. Similarly, environmental stressors are known to facilitate various types of epilepsy; it seems likely, therefore, that also adrenal stress hormones are involved in regulation of seizures.

These observations have led to a focus on corticosteroids and ovarian steroid hormones, estrogen and progesterone—to clarify how these steroid hormones influence seizures in patients under stress and in women with epilepsy. The prevailing view on gonadal hormones is that estrogen is proconvulsant, whereas progesterone is anticonvulsant. The cyclical changes of these ovarian hormones appear to play a key role in the genesis of catamenial seizures. Testosterone also modulates seizure susceptibility, and it has been recently hypothesized that its pro- or anti-convulsant action depends on the concentrations of its specific metabolites.

It seems unlikely that these steroid hormones would exert single and uniform actions given our current understanding of their complex pharmacological actions and physiological relationships. Their modulatory effects on brain excitability and seizures are likely to depend on the endocrine state of a subject, their relative concentration, metabolism, and many other seizure related factors. The complex interaction between progesterone and absence epilepsy is illustrated by two examples: acute pharmacological studies show that progesterone dose-dependently increases the for absence epilepsy highly characteristic spike-wave discharges (SWDs) while during pregnancy when progesterone concentration is high, SWDs are reduced. While the outcomes of the acute study suggest a positive relationship between concentrations of progesterone and the number of absence seizures, the pregnancy study suggests a negative relationship between concentration of progesterone and absence seizures. Clinical and animal research in well validated models, designed with the relevant endocrinological and neurobiological issues in mind, will help advance this field in the future.

## Background

### Hormones and Their Action

Hormones are classically classified according to their chemical structure into peptide hormones (eg, growth hormone, insulin and antidiuretic hormone), steroid hormones (eg, estrogen, progesterone and testosterone), and tyrosine derivatives (eg, thyroxine and adrenaline). Peptide hormones are stored in vesicles and released by exocytosis in response to appropriate stimuli; steroid hormones are lipophilic, not stored in vesicles for secretion, and their rate of release is determined by their rate of synthesis; tyrosine derivatives are synthesized by specific pathways in thyroid and adrenal medulla.

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<sup>☆</sup>*Change History:* January 2016. G van Luijtelaar, EA Tolmacheva, and B Budziszewska updated the text and added some new References.

Circulating hormones of the gonads, adrenals and thyroid gland have many effects on the brain. Some of these effects are permanent, while others are reversible. Many effects occur via stimulation of gene expression through well-characterized intracellular receptors, whereas other effects take place rapidly at the surface of nerve cells via different type of neurotransmitter receptors. Many of these central effects are not confined to reproduction.

Thirty years ago, neurosteroids—steroids which are synthesized in the central and the peripheral nervous systems—were discovered. Neurosteroids are precursors (pregnenolone, dehydroepiandrosterone and their sulfates) and metabolites (allopregnanolone, allotetrahydrodeoxycorticosterone) of steroid hormones which influence the excitability of neurons predominantly by non-genomic mechanisms. Neurosteroids can be synthesized de novo in brain tissue mainly from cholesterol (eg, pregnenolone or dehydroepiandrosterone) or are produced from hormones transported to the brain from peripheral sources (eg, allotetrahydro-pregnanolone or allotetrahydrodeoxycorticosterone). Several of these steroids accumulate in the brain after local synthesis or after metabolism of adrenal or gonadal steroids. Progesterone is also a neurosteroid, and a progesterone receptor has been identified in peripheral and central glial cells. Pregnenolone appears to be among the most quantitatively important neurosteroids in the rat brain, and is a major precursor of the neurosteroid progesterone.

Neurosteroids may have the same action in the brain as steroid hormones; however, they exert their effects mainly through nongenomic mechanisms. Nongenomic steroid effects are principally characterized by their insensitivity to inhibitors of transcription and protein synthesis. A second most obvious reason why neurosteroids have nongenomic effect is the rapid (ie, within minutes) onset of action. These very rapid effects of steroids, mainly affecting intracellular signalling, are incompatible with the genomic model. Instead, the rapid effects of steroid hormones are likely to be mediated through ion-gated neurotransmitter membrane receptors with pharmacological properties distinct from those of the intracellular steroid receptors. Neuroactive steroids may modulate—at low concentrations—the GABA<sub>A</sub> receptor, as allosteric coagonists or antagonists of GABA. The mechanism of action of these neurosteroids appears to be dependent on the specific structure of the GABA<sub>A</sub> receptor (with its subunits forming ligand-gated ion channels). At higher concentrations they can also act as allosteric modulators of neurotransmitter receptors, such as NMDA, glycine, nicotinic, muscarinic and sigma receptors.

Steroid hormones modulate seizure propensity in all types of epilepsy, including absence. Fluctuations in plasma and brain concentrations of steroid hormones and their neuroactive metabolites, over different stages of the reproductive cycle, play an important role in modulation of neuronal excitability. There is a unique relationship, however, between absence epilepsy and GABA. The pharmacological profile of absence epilepsy is opposite to the profiles typical of other types of seizures. Absences are associated with hyperfunction of thalamic GABAergic inhibitory system, while other seizure types are usually associated with a hypofunction of the GABAergic system. This counter-intuitive relationship between GABA and absence tends to complicate studies investigating the effects of neurosteroid hormones on absence; indeed, neurosteroids that exhibit GABA-mimetic properties are pro-epileptic: (ie, they exacerbate absence seizures). Further, pharmacological studies have shown that whereas systemic administration of GABA-mimetic drugs enhance absence seizure activity, locally-administration of the same drugs may have opposite effects and suppress spike-wave discharges (SWDs). This observations make it difficult to predict the effect of steroids hormones such as progesterone (known to modulate the GABAergic system) on the occurrence of absence. It is necessary, therefore, to investigate local effects of these hormones directly.

*Method:* The majority of the studies reported here were carried out on WAG/Rij rats, a genetic model of absence epilepsy. GAERS (genetic absence epileptic rats from Strasbourg, originally described by Christian Marescaux and Marguerite Vergnes and Wistar Albino Glaxo rats bred in Rijswijk (WAG/Rij), first described by Gilles van Luijtelaa and Anton Coenen, are most commonly used to study intrinsic, network and behavioral features of absence epilepsy. All individuals of now both inbred strains show an age-dependent increase in the number of SWDs. The SWDs are accompanied by small but clear clinical signs typical of absence epilepsy. Both rat strains are well characterized and well validated models of absence epilepsy.

We have examined the relation between absence episodes and hormone levels in rats that are implanted with EEG electrodes in the cortex; effects of stressors and hormonal manipulations are assessed based on the incidence or number and duration of SWDs in freely moving animals over the course of a few or many days. Blood samples are taken, to determine circulating hormonal levels, via the jugular vein (in chronic studies) or via incisions at the end of the rat's tail (in acute studies); up to 300  $\mu$ L collected for these analyses (via RIA).

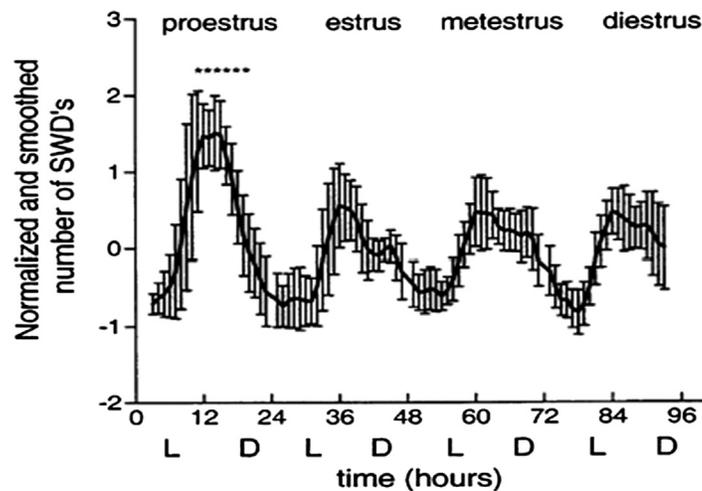
## **Recent Results**

### **Progesterone and Related Neurosteroids**

The most widely studied steroid hormone is progesterone. We addressed the question of whether (and how) progesterone affects absence seizure in two ways:

First, we examined the time course of absence seizures over the ovarian cycle in adult female WAG/Rij rats. In this way, we could establish the extent of physiological fluctuations of SWDs as a function of the fluctuations of the ovarian hormones progesterone and estradiol. These hormones are known to be elevated at specific days and hours of the ovarian cycle. The data of the long term EEG recording of SWD in female WAG/Rij rats are presented in [Fig. 1](#).

These data show a consistent circadian pattern for the number of SWDs, with an acrophase during the first hours of the dark period and a nadir during the first hours of the light period. While this circadian distribution of SWDs is in full agreement with outcomes of earlier studies, it also shows a newly-observed additional increase in SWDs at the shift from the light to the dark phase



**Figure 1** Hourly number of spike-wave discharges in the 4 days of the estrous cycle in WAG/Rij rats ( $n = 8$ ). The hourly number (mean and S.D.) of spike-wave discharges was normalized (mean, 0; variance, 1) per subject and smoothed by a centered moving averaging filter with span 4. Note the day effect at the first few hours of the dark period at proestrus.

of the proestrus day. The latter finding strongly suggests that a physiological increase in the concentration of plasma progesterone on the proestrus day enhances SWDs. The outcome of an acute pharmacological study points in the same direction. Progesterone rapidly enhances, in a dose- and time-dependent manner, the number of SWDs. Similarly, an increase in SWDs during proestrus days was found in a rat model for atypical absences (adult rats show atypical SWDs after an injection of the cholesterol synthesis inhibitor AY9944), and an acute injection of progesterone increased SWDs in these animals. The relevance of these data is twofold: First, changes in progesterone in physiological concentrations affect the number of spontaneously occurring SWDs.

Second, experimentally-administered progesterone increases SWDs in two different models of absence epilepsy—which is in agreement with a single case study in humans which showed increased absence occurrence in a woman when she was given progesterone. The direction of the effect of progesterone is in contrast to the often found anti-epileptic action of progesterone on other seizure types, both in animal models and in catamenial seizures.

Subsequent research was designed to establish whether the effects of progesterone were due to the classical genomic mechanism or to receptor activation at the cell membrane. Finasteride, an inhibitor of  $5\alpha$ -reductase which inhibits the synthesis of neurosteroids that have agonistic activity at GABA<sub>A</sub> receptors (eg, allopregnanolone, tetrahydrodeoxycorticosterone (THDOC), androsterone) alone did not affect the number of SWDs in a 24 h recording period. However, finasteride did antagonize the increase in number of SWDs induced by systemic progesterone in this rat model. In contrast, injection of RU 38486, an antagonist of intracellular progesterone receptors, had no effect on SWDs and did not block the stimulatory effect of progesterone. These findings demonstrate that progesterone has two types of effects: The normally-occurring SWDs during baseline periods were not affected by finasteride and RU 38486, and are most likely controlled by genomically-mediated mechanisms and the biological clock. However, the SWDs induced by acute administration of progesterone were blocked by finasteride, suggesting that this increase was due to non-genomic effects of progesterone, most likely those of allopregnanolone or  $3\alpha$ -OH-dihydroprogesterone, both potent endogenous positive modulators of CNS GABA<sub>A</sub> receptor function. Similar outcomes and conclusions have been presented by Persad and co-workers in the atypical absence seizure model.

Interestingly, ovariectomy has little effects on SWDs; therefore it seems that the circadian rhythm of SWDs is not controlled by swings in plasma concentration but by central effects. The increase in SWDs during proestrus, however, coincides with increased plasma levels of progesterone; how SWDs are specifically enhanced during the proestrus hours remains to be established.

The effects of two neurosteroids which are synthesized in the brain from progesterone (allopregnanolone) or from cholesterol (pregnenolone sulphate) have been tested on absences in order to explore the mechanism of action of progesterone. Allopregnanolone potently increased SWDs dose-dependently in WAG/Rij rats. This action is most parsimoniously explained by its action in stimulating the classical GABA<sub>A</sub> receptor. In support of this hypothesis is the recent report that isopregnanolone, an isomer of allopregnanolone which is devoid of GABA<sub>A</sub> agonistic activity, when injected into the brains of WAG/Rij rat, has no effect on SWDs. Ganaxolone is a synthetic analogue of allopregnanolone. It is, like allopregnanolone, a potent positive allosteric modulator of GABA<sub>A</sub> receptors. Ganaxolone has a robust anticonvulsant profile in a variety of animal models of epilepsy, is orally active and lacks hormonal side effects. Another advantage is that tolerance to the anticonvulsive action does not develop following chronic therapy. However, ganaxolone has been found to increase SWDs when injected into thalamic nuclei, and this is in full agreement with the effects of systemic administered allopregnanolone. Although the systemic effect of ganaxolone has not yet been tested in genetic absence epilepsy models (only in an absence seizure model where it aggravates SWDs), we predict that it will increase SWDs in the genetic absence models and that ganaxolone should not be given to patients with absence seizures.

Pregnenolone sulphate increases SWDs, although this was only found after the highest dose. The increase can be explained by its agonistic effects on the NMDA receptor, although other mechanisms could not be excluded, such as an action through its conversion via progesterone to allopregnanolone.

Pregnancy is an interesting hormonal condition and epidemiological studies show an increase in seizure frequency in up to one third of the woman with epilepsy due to hormonal and pharmacokinetic changes affecting the action of antiepileptic medication. Progesterone is chronically enhanced during pregnancy, to a concentration much higher than  $17\beta$ -estradiol. In our study in pregnant WAG/Rij rats, SWDs were reduced during the first 18 days of pregnancy. Two days before delivery, SWDs increased significantly while the plasma concentration of progesterone decreased. These data suggest a negative relationship between plasma levels of progesterone and SWD in pregnant females, while the outcomes of the 96 h recording during the ovarian cycle and the acute pharmacological study demonstrate a positive relationship between plasma or brain concentrations of progesterone and absence seizures. The fact that diametrically opposite relationship between absence seizures and progesterone exist is a beautiful illustration that the relation between hormones and seizure type may be completely opposite during a different hormonal state, in this case pregnancy. However, the exact reasons remain to be elucidated. It is well known that fluctuations in the level of neurosteroids have consequences for changes in the expression of genes for specific GABA<sub>A</sub> receptor subunits. They can also affect posttranslational modification and they can do this differently and on their own way during the estrus cycle, during pregnancy, puberty and across lifetime. It is proposed that action of some of these neurosteroids is dependent on the specific structure of the GABA<sub>A</sub> receptor with its subunits forming ligand-gated ion channels. One examples is given on how changes in fluctuating brain levels and subunit expression influences SWD occurrence. This comes from the work of Pisu and colleagues. They noticed that presymptomatic WAG/Rij rats had higher plasma levels of allopregnanolone, as well in cortex and thalamus compared to age matched control Wistar rats. The age related increase in SWDs, typical for absence epilepsy, is accompanied by a decrease in allopregnanolone in the thalamus. An age dependent increase of  $3\alpha,5\alpha$ -TH-DOC was found in plasma and in the cortex, while the age dependent decrease was typical for the thalamus. These changes in brain concentrations from 2 to 6 month were accompanied by an increase in WAG/Rij rats in the expression of  $\alpha 4$  and  $\delta$  in thalamic relay nuclei. Both  $\alpha 4$  and  $\delta$  subunits are found in extrasynaptic GABA<sub>A</sub> receptors, where they facilitate tonic inhibition. Increased tonic inhibition of thalamic neurons is a major neurobiological underpinning of the occurrence of SWDs, as was established in the GAERS absence model. Therefore, we propose that the age dependent increase in  $\alpha 4$  and  $\delta$  GABA subunits increases tonic inhibition in thalamic relay neurons which at its turn contributes to the age dependent increase in SWDs. Changes in hippocampal GABA<sub>A</sub> receptor subunit expression in particular in the  $\delta$  and  $\gamma 2$  subunit were found at day 18 of pregnancy in mice by Western blot analyses, which rebounds to base-line levels by 48 post-partum. These type of changes in subunit expression are accompanied by changes in tonic and phasic inhibition, as has been established by Maguire and Mody, 2008. Also estrous cycle-related changes in  $\delta$  subunits mediating tonic inhibition and susceptibility for seizures were described, although not for the for absence epilepsy relevant cortex and thalamus.

#### **Local Versus Systemic Injection of Progesterone in WAG/Rij Rats**

Progesterone is known to exert its effects in the cortex, thalamus and limbic system. Another clue about how to understand this opposite action of progesterone during pregnancy comes after the discovery of a cortical focus in absence epilepsy. This finding showed that SWDs are elicited in a part of the somatosensory cortex, and it was predicted that local focal injections of GABA-mimetics (such as tiagabine) and modulators of the GABA<sub>A</sub> receptor should suppress SWD—in contrast to the effects of systemic studies of GABA mimetic drugs (muscimol, THIP, SKF 89976, vigabatrin, tiagabine) which enhance SWDs. It quickly became clear that focal injection into this zone in the somatosensory cortex suppresses SWDs (compared to effects of systemic injections), not only for GABA-mimetic drugs but also for antiepileptic drugs such as phenytoin. This results has led De Sarro and co-workers to compare the effects of local injections of neurosteroids into various regions in the thalamo-cortical loop (including the focal area in the peri-oral region of the somatosensory cortex) in WAG/Rij rats. Allopregnanolone and ganaxolone, both with a potent enhancement of the GABA-response, injected in the thalamic VPM or VPL, increased SWD, similar to the systemic effects of allopregnanolone. The neurosteroid with a GABA antagonistic profile, pregnenolone sulphate, decreased SWDs after injections into the VPM, was ineffective in the VPL, and increased SWDs in the RTN. These regional differences are currently not well understood but demonstrate that the effects of neurosteroids on SWDs are region specific. Most interesting is that allopregnanolone and ganaxolone suppressed SWDs dose-dependently when administered into the peri-oral region of the somatosensory cortex, in contrast to what has been found after systemic injections. Pregnenolone sulphate showed the opposite effects of allopregnanolone and ganaxolone when administered into this region of the cortex.

In all, the data show that neurosteroids like allopregnanolone, that potentiate the effects of GABA on GABA<sub>A</sub> receptor, can have different effects, depending on the part of the brain. Local cortical administration in the cortex blocks SWDs, while injection into thalamic relay nuclei enhances SWDs. Another example illustrating such differences is seen with local injections of progesterone and tiagabine (a GABA-reuptake inhibitor). Local cortical and hippocampal injections were effective in suppressing SWDs, while systemic or thalamic injections of these drugs enhanced SWDs. Moreover, cortical injections outside the peri-oral cortical regions were without any effect on SWDs. These data demonstrate that the same neuroactive steroid may have different or even opposite consequences on absence seizure activity, dependent on the location of where the neurosteroids are active. They also suggest that the hippocampus, although not involved in the initiation and spread of absence seizures, may modulate absences seizure activity in the cortico-thalamic circuits.

It can be concluded that progesterone modulates SWD both by genomic and non-genomic effects; the non-genomic effects might occur via its metabolite allopregnanolone with its GABA mimetic effects. Lifetime changes in the concentration of neurosteroids induces changes in GABA<sub>A</sub> receptor subunit composition, which might be different in different brain regions. It is possible that changes in subunit expression during the ovarian cycle and during pregnancy might also contribute to the complex and sometimes opposite relations between progesterone and SWDs.

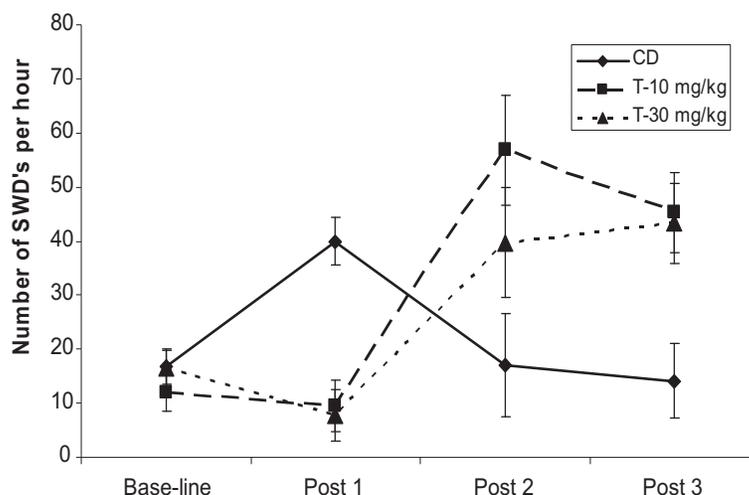
### 17 $\beta$ -Estradiol

A second important neuroactive steroid hormone is 17 $\beta$ -estradiol. Beside an action via classical intracellular receptors on gene transcription, estrogen interacts with specific receptors at the plasma membrane of neurons, and activation of these receptors activates intracellular signal transduction pathways. Estrogen has been shown to regulate the activation of some G-protein-coupled receptors (such as opioid, GABA<sub>B</sub> and dopamine D<sub>2</sub>) and ionotropic receptors (such as 5-HT<sub>3</sub> and nicotinic). Estrogens also activate the *N*-methyl-D-aspartate (NMDA) excitatory neurotransmitter receptors in the hippocampus. It also affects the synthesis of DA. Considering the multiple action of estrogens, its action on absence seizures is difficult to predict.

We observed no difference in the number of SWDs during the first few hours of the light period between proestrus and the three other days of the cycle in female WAG/Rij rats in a 96 h EEG study, suggesting that 17 $\beta$ -estradiol has no effect on SWDs. Outcomes of pharmacological studies confirmed that 17 $\beta$ -estradiol does not affect absence seizures; in addition, tamoxifen, an antagonist of 17 $\beta$ -estradiol intracellular receptor, did not affect SWDs. In many other seizure models (eg, kainic acid, ethyl chloride, kindling, and pentylenetetrazole), estrogens increase the number of seizures and decrease the threshold for electroshock-induced seizures. Interestingly, 17 $\beta$ -estradiol reduced absence seizures in the atypical absence seizure model.

### Testosterone

There are no documented papers on the relationship between testosterone and absence epilepsy. Testosterone may have anti- or proconvulsant effects, depending on the dose, the animal's age, and the seizure type. The variable actions of testosterone can be partly explained by the metabolism of testosterone. Testosterone is metabolized via aromatization to 17 $\beta$ -estradiol (which is not effective, see above), and testosterone can also be reduced to 5 $\alpha$ -dihydrotestosterone which is then converted to 3 $\alpha$ -androstenediol, the latter is a powerful and positive modulator of the GABA<sub>A</sub> receptor. Therefore a relatively late increase in SWDs after systemic administration of testosterone can be predicted based on the GABA-mimetic effects of this metabolite. The first metabolite, 5 $\alpha$ -dihydrotestosterone, is known to mimic the action of NMDA antagonists, it may reduce absence seizures considering that all NMDA antagonists reduce absence seizures. The results of systemic injections of testosterone in 6 months old male WAG/Rij rats are presented in Fig. 2. The solvent cyclodextrine (CD) increased SWDs in the first hour, but this increase was not found for testosterone. Testosterone did not show the increase in SWDs in comparison with the solvent at the first hour post drug administration, at post 2 and 3 testosterone showed a clear increase in number of SWDs while CD was no longer active. Considering these outcomes, we propose that testosterone is first active through its metabolite 5 $\alpha$ -dihydrotestosterone and only later to 3 $\alpha$ -androstenediol, which have anti- and pro-absence action on SWDs, respectively. The increase of SWDs by



**Figure 2** Effects of systemic injections of testosterone in WAG/Rij rats. Number and SEM of SWDs per hour during the base-line (pre-injection), and in the 1st, 2nd and 3rd hour following systemic injection with testosterone (10 or 30 mg kg<sup>-1</sup>) of solvent CD (cyclodextrin). Note that in the first hour post injection CD enhanced SWDs, and testosterone had less SWDs than CD alone, CD was no longer effective in the second and third hour post administration, while both testosterone groups showed an increase in SWDs. Courtesy of Alexandra Badura.

the solvent might be due to the small injection stress or by intrinsic effects through sponging neuroactive steroids from endogenous resources which alters neuronal excitability.

### **Hypothalamic-Pituitary-Adrenal (HPA)-Axis**

Steroid hormones, secreted by the adrenal glands in response to stress, rapidly modulate brain function and behavior as well as cellular responses in general. The stress response in rats emerges within a few minutes after exposure to a stressor (footshocks) or after a systemic injection of a glucocorticoid. Acute stress is known to increase circulating and brain levels of allopregnanolone and THDOC. Stress also modulates neurotransmission in the limbic system eg, the hippocampus by a rapid and transient increase in the intracellular levels of inhibitory neurotransmitter GABA and the excitatory amino acids aspartate and glutamate. Foot shocks and systemic injections of corticosteroids in WAG/Rij rats produced first a short decrease followed by an increase in SWDs. We've speculated that the short lasting decrease in SWDs is due to activation of sympathetic nervous system and an increase in peripheral adrenalin and to the enhancement of catecholamines in the central nervous system—known to accompany arousal and activation and suppress SWDs. We propose that the subsequent increase in SWDs might be explained by the increase of glutamate in the hippocampus, since opposite effects (reduced SWDs) were obtained after the administration of GABA-mimetics in the hippocampus. Another possibility is that the proabsence effect seen after the initial decrease in SWDs after footshock stress could be ascribed to the conversion of deoxycorticosterone in neuroactive metabolites such as THDOC or to a stress induced increase in allopregnanolone level, ie, an enhancement of the concentration of GABA<sub>A</sub> agonistic neurosteroid. However, both possibilities need experimental verification. Interestingly, experiments with mild daily footshock presentation induced a similar pattern—a short lasting suppression of SWDs was followed by a return to base-line or an increase. The data of this study are presented in Fig. 3. The initial decrease in SWDs on day 1 after the footshocks subsided over days, with a subsequent increase in SWDs that became larger over days. These data show that acute stress has a biphasic effects on SWDs and it is very likely that corticosteroids play a role in the increase of SWDs through their action in the cortico-thalamo-cortical circuit and or hippocampus. The increase in SWDs over days before the presentation of the daily footshocks suggests that the rats anticipate adverse stimulation.

The data show also that the increase in SWDs after repeated footshock exposure is elevated in ovariectomized rats. This observation has been attributed to the fact that ovarian and HPA-axis hormones both contribute in the coping response to stressors.

A final observation is that there is high comorbidity between epilepsy and depression. Similar pathophysiological pattern might be present in both disorders and glucocorticoids are indeed involved in both disorders. Approximately 50% of people with major depression have an hyperactive HPA axis, which is thought to arise from an impairment of negative feedback inhibition of the HPA axis by glucocorticoids. Similar to this group of depressive patients, WAG/Rij rats showed elevated resting corticosterone concentrations and a particular stress-induced corticosterone response profile, which might also indicate deficient feedback inhibition.

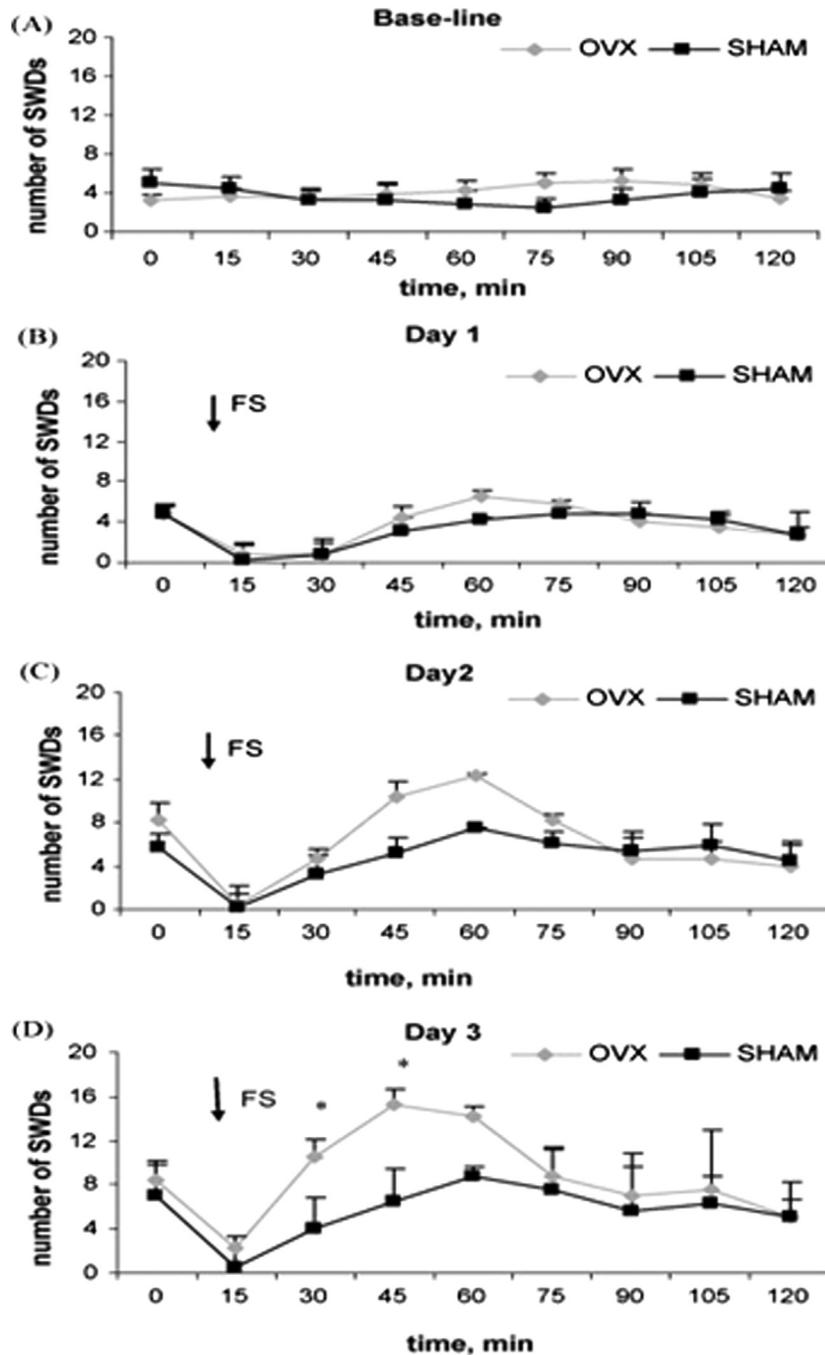
### **Other Hormones, Peptides and Absence Epilepsy**

Although the thalamus is not the origin of absence seizures, it is definitely involved in the amplification and spread of these seizures. There are many neuropeptides hormones and peptides that can alter rhythmic thalamic activity, including somatostatin, cholecystokinin, neuropeptide Y, nociceptin/orphanin FQ, substance P, and vasoactive intestinal peptide. These peptides have all been localized within the thalamus, along with their respective receptors, and have been shown to alter the excitability of thalamic neurons by changing intrinsic properties of thalamic cells (such as resting membrane potential, input resistance, membrane conductance, action potential firing mode and synaptic transmission). One peptide hormone, PrRP (Prolactin-releasing peptide—which releases prolactin from pituitary cells), has been investigated in vivo and in vitro in experiments on GAERS. This peptide inhibits rhythmic activity in the reticular thalamic nucleus in slice preparations, and it influences non-REM sleep and SWDs in intact animals. Both effects may emerge through action on PrRP receptors in the reticular thalamic nucleus (RTN); the mRNA for this receptor is expressed in the RTN and present on GABAergic neurons in the RTN. Considering these effects in RTN neurons, one might predict that PrRP has anti-absence effects—and this effect has indeed been found. A second peptide hormone, TRH (Thyrotropin-releasing hormone), administered i.c.v. decreased dose-dependently the number and mean duration of SWDs in WAG/Rij rats, and also has mild anti-absence effects.

Neuropeptide (NP) Y, a likely endogenous anti-epileptic substance with possible antiepileptogenic effects, was shown to dose-dependently suppress SWDs in GAERS. Subsequent studies with specific NP Y receptor agonists showed that NP Y<sub>2</sub> receptors are more important than Y<sub>1</sub> and Y<sub>5</sub> in mediating this effect, so that Y<sub>2</sub> receptor agonists may represent targets for novel drugs against generalized epilepsies.

### **Future Challenges**

In order to understand the relation between steroid hormones and absence seizures, it is necessary to find the factors involved in thalamic GABA hyperfunction responsible for SWDs as measured during baseline periods, and the changes that occur in SWDs during different hormonal states such as pregnancy, puberty and various phases of the ovarian cycle. The hyperfunction and its changes can be due to elevated concentration of neuroactive steroid hormones in the cortex, thalamus and hippocampus, and/or



**Figure 3** The effects of daily footshocks on the number of SWDs in a 2 h recording period. Note that the number of SWDs are immediately decreased after the shock and return to pre-shock levels, that the number of SWDs increase over days both pre and after footshocks, and that the increase over days (A to D) post footshocks becomes larger over days. The latter effect is pronounced in ovariectomized females. Tolmacheva, E.A., van Luijtelaar, G., 2007b. The role of ovarian steroid hormones in the regulation of basal and stress induced absence seizures. *J. Steroid Biochem. Mol. Biol.* 104, 281–288.

to local concentrations of GABA-mimetic agents such as allopregnanolone, THDOC and  $3\alpha$ -androstenediol. The concentrations of allopregnanolone and THDOC were already determined in cerebral cortex and thalamus and compared between presymptomatic and symptomatic WAG/Rij and control Wistar rats of different age ie, before the onset of seizures, and when the seizures were fully developed. An age-dependent increase in the level of these neurosteroids was found in cerebral cortex, but not in thalamus, where WAG/Rij rats showed a decrease in allopregnanolone and THDOC. However, levels were determined on the whole thalamus. Since it has been found that the effects of GABA-mimetics injected into the reticular thalamic nucleus are opposite to the effects of these

drugs after their injections into thalamic relay nuclei, it is important to determine levels of  $3\alpha,5\alpha$ -TH PROG and  $3\alpha,5\alpha$ -TH DOC separately in these thalamic sub regions in WAG/Rij and non-epileptic control Wistar rats in order to specify their role in epileptogenesis. Also determination the level of their precursors (progesterone, corticosterone) and  $3\alpha$ -androstenediol in these thalamic regions as well as in cerebral cortex and other SWD modulating brain regions should help to clarify the role of neurosteroids in SWD regulation and epileptogenesis.

Additionally, the local concentration of enzymes that metabolize steroid hormones to the positive neurosteroid GABA modulators (eg.  $5\alpha$ -reductase and  $3\alpha$ -hydroxysteroid oxidoreductase) should be compared in different hormonal conditions in WAG/Rij rats, and between WAG/Rij and age matched control rats.

It would be challenging to investigate whether the GABA hyperfunction is caused by action of GABA-mimetic neurosteroids (even at their unchanged level) on presynaptic, postsynaptic or extrasynaptic GABA<sub>A</sub> receptors, the composition of the receptor subunits, and the phosphorylation status of the receptors under different hormonal conditions. These data should be compared between WAG/Rij and control rats. It is known that the ability of neurosteroids to affect GABA receptor function depends on the GABA<sub>A</sub> subunit expression. In some brain regions, extrasynaptic GABA<sub>A</sub> receptors, especially those containing delta subunits are more sensitive to neurosteroids than synaptic receptors. Also, phosphorylation of GABA<sub>A</sub> receptors regulates the interaction of neurosteroids with this receptor.

The changes that occur in Childhood Absence Epilepsy, around puberty, are not understood. Therefore, it would be useful to develop a model for the transition from childhood absence epilepsy to other generalized (tonic-clonic) epilepsies.

It is challenging to study further the interactions between steroid hormones, neurosteroids and glucocorticoids in response to stress (acute/chronic) in WAG/Rij and control rats. This stress response is relevant to our understanding of absence seizures, considering that the hormonal state changes dramatically in females – and that the way in which they cope with stress is highly dependent on these endocrinological changes. Although there are no sex differences in the rodent absence models regarding the incidence of SWDs, the different coping strategies in female and male rats and their effects on seizures might be worth to investigate. Moreover, acute stress increases the concentration of neuroactive steroids (allopregnanolone, THDOC) in plasma and brain, and neurosteroids in turn inhibit the release of both Corticotropin-releasing hormone (CRH) and Gonadotropin-releasing hormone (GnRH). How and in which way allopregnanolone inhibits HPA-axis activity and also glucocorticoid-mediated gene transcription and how this affects absence activity deserves to be investigated.

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