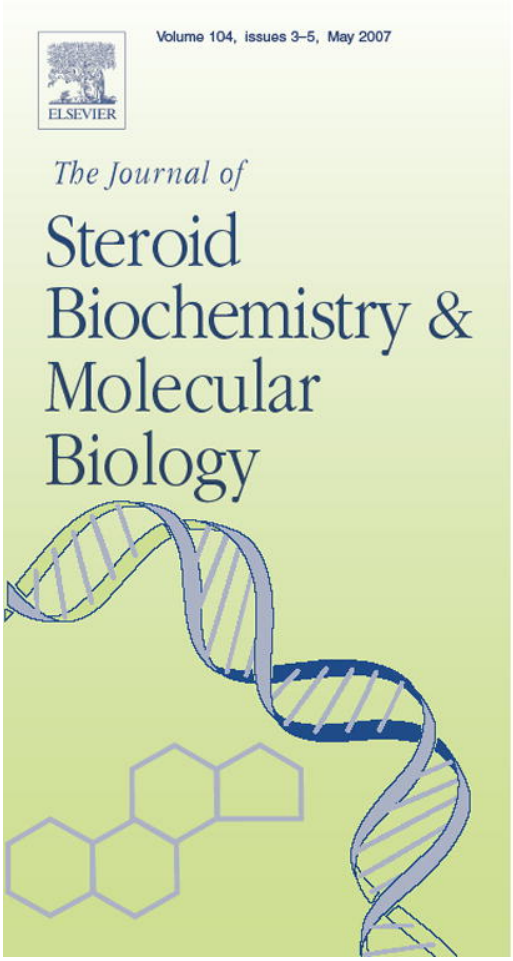

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The role of ovarian steroid hormones in the regulation of basal and stress induced absence seizures☆

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Abstract

Ovarian hormones play an important role in the regulation of absence seizures in patients as well as in animal models. The present study examined whether chronic progesterone exposure would induce tolerance for the occurrence of absence seizures and whether reduction in gonadal steroids (*via* ovariectomy) would alter the number of basal and stress induced absence seizures in WAG/Rij rats, a genetic model for absence epilepsy.

Methods: 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 3 days while a third group received CD injections on Days 1 and 2 and P on Day 3. 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) over 3 days. The EEG was recorded 1 h before and 2 h after each FS series.

Results: 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.

Conclusions: 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.

Keywords: 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 [5–7]. It was also shown that this effect is mediated by the neuroactive derivative allopregnanolone known to facili-

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tate $GABA_A$ 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 their 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 this 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. First, we 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 has also been shown 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 [12–14]. 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 interaction between stress (or HPA axis functioning) and convulsive seizures is well-known [2]. 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 may reduce adaptation to stress in OVX females by enhancing the number of SWDs after repeated foot-shock stress.

2. 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 1 week prior to the experiment and placed in the recording cages 1 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 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 jewellers 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, 2 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 (25 × 25 × 40), which has an electrified grid on the floor, through which shock could be delivered. Scrambled electrical shocks (1.5 mA, 1 s) were administered with random (range 1–10 s) inter-shock intervals.

2.6. Experimental design

2.6.1. Experiment 1

Rats were randomly assigned to one of three groups. 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 3 days. The third group (Group CD-P, n = 7) received CD on Days 1 and 2 and progesterone on Day 3. The experimental deign is also presented in Table 1. The EEG was recorded on Day 0 (base-line level) and than on

Table 1
Experimental design (Experiment 1): the first group was given three injections of progesterone (P), the second group was given three injections of cyclodextrin (CD), and the third group was given two injections of CD followed by an injection of P

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

The dose of P was 20 mg/kg, all injections were i.p.

Days 1–3 immediately after injections, for 2 h between 12.00 and 14.00.

2.6.2. Experiment 2a

EEG recordings were conducted 1 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 4 h and were carried out between 10.00 and 14.00 in the home cages.

2.6.3. 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 2nd day. On the next 3 days, rats were placed into the Perspex boxes again and after 1 h they received a series of 10 FS; the EEG was recorded 1 h before and 2 h 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).

2.6.4. EEG analysis

The EEGs 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, or 4 h episodes (Experiment 2a).

2.6.5. 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 (5 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 Fig. 1, there was a significant main effect of day ($F_{day}=18.81$, d.f. = 3.72, $p<0.001$) and a significant interaction between day and group ($F_{day\times group}=6.39$, d.f. = 6.72, $p<0.001$) in the hourly number of SWDs. This interaction was further analyzed 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 ($F_{group}=3.27$, d.f. = 2.26, $p=0.05$) and Day 3 ($F_{group}=4.50$, d.f. = 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 analyzed. 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 ($F_{day}=9.78$, d.f. = 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

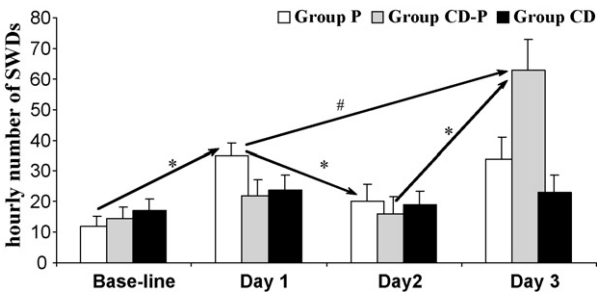

Fig. 1. The number of SWDs (mean ± S.E.M.) in the base-line and experimental days (Day1–Day3) in the three groups during the first hour after injection of progesteron (P) and cyclodextrin (CD). Asterisk (*) shows significant difference between days ($p<0.05$) in Group P and CD-P according to *post hoc*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

2) was significantly lower than after the first injection (Day 1) ($t = 2.9$, d.f. 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 ($F_{day} = 11.06$, d.f. = 3.18, $p < 0.001$): there were more SWDs after progesterone on Day 3 than after CD on Days 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 ($t = 2.05$, d.f. 16, $p < 0.05$) (Fig. 1).

The ANOVA for the number of SWDs during the second hour after injection revealed only a significant main effect of day ($F_{day} = 4.56$, d.f. = 3.72, $p < 0.01$). The number of SWDs was larger on Days 1 and 3 than on Days 0 and 2.

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

The two-way ANOVA revealed only a significant main effect of day ($F_{day} = 5.55$, d.f. = 8.80, $p < 0.01$) for the duration of SWDs. Orthogonal trend analysis showed significant linear ($F_{lin} = 11.64$, d.f. = 1.10, $p < 0.01$) and cubic ($F_{cub} = 5.68$, d.f. = 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

3.3.1. SWDs in the base-line preceding FS exposure

The ANOVA revealed a significant main effect of day ($F_{day} = 15.13$, d.f. = 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 ($F_{lin} = 62.77$, d.f. = 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 ($F_{lin} = 13.61$, d.f. = 1.8, $p < 0.01$). In both groups the increase was characterized by a significant linear orthogonal trend (OVX: $F_{lin} = 127.95$, d.f. = 1.6, $p < 0.000$; sham: $F_{lin} = 18.63$, d.f. = 1.6, $p < 0.01$), *post hoc* comparisons revealed an increase in the number of SWDs in the base-line hour over the 4 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 Days 3 and 4 compared to Day 0 (base-line). The difference between the OVX and sham group was significant at Days 3 and 4 (Fig. 2).

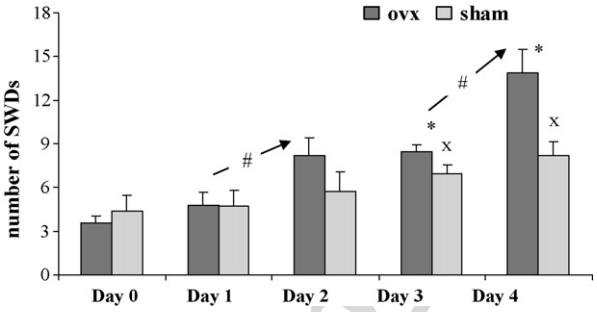

Fig. 2. The hourly number (mean and S.E.M.) of SWDs in base-line (Day 0) and before the first (Day 1), second (Day 2), third FS exposure (Day 3) and anticipated fourth (Day 4) FS exposure. Asterisk (*) shows significant difference between the groups ($p < 0.05$ according to *post hoc t*-test); symbol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

3.3.2. SWDs in the first hour after FS

As shown in Fig. 3, there was a main effect of day ($F_{day} = 10.55$, d.f. = 3.36, $p < 0.001$) for the number of SWDs. Changes over days were characterized by significant linear and quadratic orthogonal trends ($F_{lin} = 13.72$, d.f. = 1.12, $p < 0.01$; $F_{quad} = 7.02$, d.f. = 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 ($F_{time} = 44.23$, d.f. = 3.36, $p < 0.001$) characterized by a significant linear trend ($F_{lin} = 85.74$, d.f. = 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 ($F_{operation} = 11.27$, d.f. = 1.12, $p < 0.01$): OVX rats had more SWDs than sham-operated animals. The significant interaction between day and time ($F_{day \times time} = 9.50$, d.f. = 9.108, $p < 0.001$) was characterized by significant linear and quadratic orthogonal trends ($F_{lin} = 74.06$, d.f. = 1.12, $p < 0.001$; $F_{quad} = 11.19$, d.f. = 1.12, $p < 0.01$) indicating that the trends over the four 15 min episodes were changing over the 4 experimental days. Initially, at the 1st 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 2 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 ($F_{time \times operation} = 3.54$, d.f. = 3.36, $p < 0.05$) with a linear orthogonal trend ($F_{lin} = 6.34$, d.f. = 1.12, $p < 0.05$) and a significant linear trend in the day × time × operation interaction

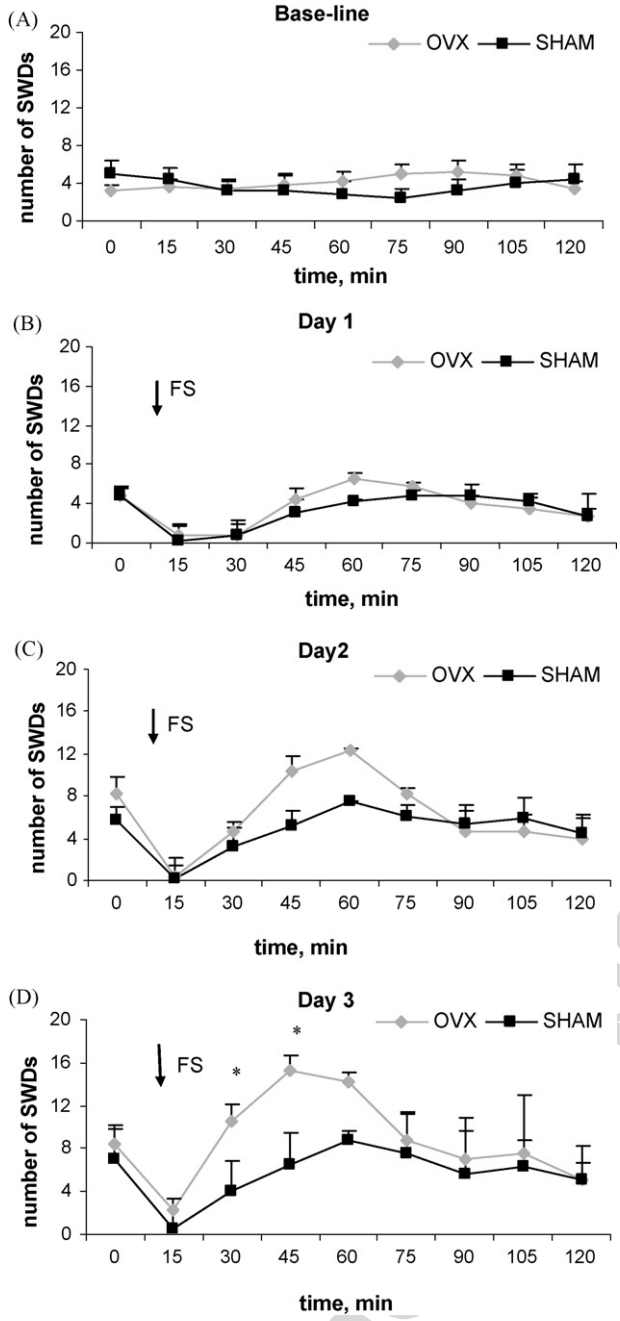

Fig. 3. The number of SWDs in the base-line (A, 3 h) and before (1 h) and after (2 h) the administration of 10 FS repeated for 3 subsequent days (B–D). Asterisk (*) shows a significant difference between the groups at Day 3 ($p < 0.05$ according to *post hoc t*-tests).

($F_{lin} = 10.12$, d.f. = 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. 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 and 60 min after FS administration. At the third session the difference became significant: OVX rats demonstrated a higher number of SWDs between 15 and 30 and 30 and 45 min ($t = 2.38$, d.f. = 12, $p < 0.05$; $t = 2.61$, d.f. = 12, $p < 0.05$) after FS administration compared to sham-operated animals.

3.3.3. SWDs in the second hour after FS

There was a significant main effect of day ($F_{day} = 7.18$, d.f. = 3.36, $p < 0.001$) and a significant main effect of time ($F_{time} = 4.68$, d.f. = 3.36, $p < 0.01$) in SWDs in the second hour after FS. An orthogonal trend analysis revealed a significant linear trend ($F_{lin} = 20.31$, d.f. = 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 ($F_{lin} = 9.55$, d.f. = 1.12, $p < 0.01$) for the main effect of time, indicating a decrease in the number of SWDs.

3.3.4. 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 ($F_{day} = 6.72$, d.f. = 2.24, $p < 0.01$) and time ($F_{time} = 162.87$, d.f. = 1.12, $p < 0.001$) and a significant interaction between day and time ($F_{day \times time} = 3.59$, d.f. = 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 and 60 min in the second FS session and a significantly higher number of SWDs between 30 and 45 and 45 and 60 min in the third FS session ($t = 2.5$, $t = 4.5$, d.f. 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 both in a situation of stress anticipation and as reaction to FS stress 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 h 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 co-workers showed that tolerance to allopregnanolone developed already after 60–90 of continuous exposure [18]. 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 [18–20], 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 et al. [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 [5–8].

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, the 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 after re-exposure to the stressor in the same environment OVX rats display an increased number of c-Fos–positive nuclei in a number of limbic structures the hypothalamus, dentate gyrus, medial prefrontal cortex and central and medial amygdala [29]. Considering that the hippocampus as well as some other limbic structures, such as nucleus accumbens and prefrontal cortex are also involved in the modulation 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 stress-induced activation of brainstem catecholamine-ergic systems [31], which may also mediate the alterations in the incidence of SWDs. Additional experiments are 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.

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