

Seizures and Reproductive Function: Insights from Female Rats with Epilepsy

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Objective: Chronic seizures in women can have adverse effects on reproductive function, such as polycystic ovarian syndrome, but it has been difficult to dissociate the effects of epilepsy from the role of antiepileptic drugs. To distinguish the effects of chronic seizures from medication, we used the laboratory rat, because an epileptic condition can be induced without concomitant anticonvulsant drug treatment.

Methods: Adult female rats were administered the chemoconvulsant pilocarpine to initiate status epilepticus, which was decreased in severity by the anticonvulsant diazepam. These rats developed spontaneous seizures in the ensuing weeks, and are therefore termed *epileptic*. Controls were saline-treated rats, or animals that were injected with pilocarpine but did not develop status epilepticus. Ovarian cyclicity and weight gain were evaluated for 2 to 3 months. Serum hormone levels were assayed from trunk blood, which was collected at the time of death. Paraformaldehyde-fixed ovaries were evaluated quantitatively.

Results: Rats that had pilocarpine-induced seizures had an increased incidence of acyclicity by the end of the study, even if status epilepticus did not occur. Ovarian cysts and weight gain were significantly greater in epileptic than control rats, whether rats maintained cyclicity or not. Serum testosterone was increased in epileptic rats, but estradiol, progesterone, and prolactin were not.

Interpretations: The results suggest that an epileptic condition in the rat leads to increased body weight, cystic ovaries, and increased testosterone levels. Although caution is required when comparing female rats with women, the data suggest that recurrent seizures have adverse effects, independent of antiepileptic drugs.

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There have been many reports of impaired endocrine function in women with epilepsy, including loss of the ovarian cycle (amenorrhea) or irregular cycle duration (oligomenorrhea) and anovulatory cycles.^{1,2} In addition, cases of reduced fertility have been documented,^{3–5,8} as well as premature menopause,^{2,5,6} and polycystic ovaries (PCO) or polycystic ovarian syndrome (PCOS^{7,8}), although the frequency is controversial.^{9–12} PCO involves benign cysts that develop in the ovaries and is more common in women with epilepsy than in the general population.⁷ PCOS is a syndrome that has historically been associated with cystic ovaries, weight gain, hirsutism, and insulin resistance.^{10–12} In 1990, a National Institutes of Health consensus definition was established that includes irregular menstrual cycles and hyperandrogenism, and suggests that weight gain and insulin resistance (“metabolic syndrome”) only be considered as symptoms that are associated with PCOS.¹³ In 2003, the European Society of Human Reproduction and Embryology and the American Society for Re-

productive Medicine consortium (the Rotterdam ES-HRE/ASRM consortium) suggested that cystic ovaries be included in the definition.^{12,13}

Based on the clinical data, it is unclear whether chronic seizures or anticonvulsant use is responsible for endocrinological changes in patients with epilepsy. For PCOS, this has been a subject of considerable debate.^{14–20} Valproic acid appears to consistently increase the risk for ovarian cysts in women with epilepsy, and some other anticonvulsants also do so.^{14–20} One way to address the contributions of chronic seizures versus pharmacotherapy is to use an animal model of epilepsy, because the effects of seizures can be examined independent of anticonvulsant treatment. In addition, the effects of age and environment can be controlled, whereas genetic variation is reduced. Although it is difficult to generalize information from the rodent to humans, several parallels in data from past comparisons suggest it is useful, if approached with caution. Importantly, it appears reasonable to evaluate reproductive

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disorders such as cystic ovaries because valproic acid appears to induce cystic ovaries in normal female rats,¹⁹ suggesting that some common ovarian syndromes may develop across mammals.

The goal of this study was to test the hypothesis that chronic seizures would lead to reproductive dysfunction without any history of antiepileptic drug (AED) treatment, using an animal model of epilepsy. We focused on an animal model of limbic seizures because it has been suggested that seizure activity in limbic structures might be particularly likely to influence the reproductive system, because of the interconnectivity of limbic nuclei with the hypothalamus.^{2,7,20}

Testing this hypothesis is not simple, however, because most studies of female rodents with limbic seizures suggest that the experimental procedures to induce an epileptic state are accompanied by a loss of reproductive function. This problem precludes the goal, that is, to study a reproductively normal animal with epilepsy for a long period and determine whether reproductive function ultimately becomes abnormal. For example, kindling the amygdala in the female rat causes cessation of the ovarian cycle (the "estrous cycle" in rodents) before the epileptic state is reached.²¹ Loss of the estrous cycle may be caused by amygdala damage, because experimental lesions to the amygdala can lead to cessation of the estrous cycle in the rodent.^{22,23}

Other methods to induce epilepsy, besides kindling, also are associated with reproductive abnormalities. For example, after intrahippocampal kainic acid injection in the anesthetized female rat, it was reported that the animals developed spontaneous seizures, but it was also mentioned that they had irregular estrous cycles.²⁴ Systemic pilocarpine administration has been used in female Wistar rats to induce status epilepticus (SE), followed by an epileptic state after a delay of a few weeks.^{25,26} These animals also demonstrated irregular estrous cycles,^{25,26} although not universally. Indeed, some animals appeared to sustain many important components of reproductive function, because some could become pregnant.²⁵ Hormonal changes were reported,^{25,26} but all animals were pooled, both those with apparently normal estrous cycles (cyclic) and irregular cycles (acyclic). Therefore, it is currently unclear how a state of epilepsy in the reproductively normal female rodent may ultimately lead to changes in endocrinological measures such as cyclicity, serum hormone levels, or pathology such as PCOS.

Here, we used the pilocarpine model of temporal lobe epilepsy (TLE) to achieve these goals. Intervention with diazepam shortly after the onset of epilepsy was able to modify the procedure enough so that animals with regular estrous cycles were possible to study after SE. We also studied animals that were treated with pilocarpine but did not experience SE. Some of these animals had a few limbic seizures immediately after pilocarpine administration, instead of SE. These animals

are usually not studied at all, but were of interest because they allowed us to compare the effects of a single cluster of seizures in a normal rat relative to an epileptic state.

Materials and Methods

Subjects

Adult Sprague–Dawley female rats were obtained from Taconic farms (Germantown, NY). Rats were housed two to three per cage at 64°F to 75°F and 40 to 75% relative humidity in standard opaque cages with corn cob bedding and a 12-hour light/dark cycle (lights on, 7:00 AM). Food (Purina 5001 chow; WF Fisher & Son, Somerville, NJ) and water were provided ad libitum. Cages with one to two adult male rats were placed between cages of female rats to facilitate regular estrous cycles.²⁷

All procedures met the guidelines of the New York State Department of Health and National Institutes of Health guidelines. Chemicals were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise specified.

Vaginal Cytology and Terminology Associated with Estrous Cyclicity

Starting at 50 days of age, female rats were examined daily between approximately 9:00 AM and 12:00 PM to assess the relative frequency of cell types of the vaginal epithelium in a 30- μ l sample of vaginal fluid.^{21,28} For the purposes of this study, nomenclature for the days of the estrous cycle are as follows: proestrus, estrus, diestrus 1, and diestrus 2. On proestrus, estradiol surges during the midmorning, followed by an increase in progesterone that begins at approximately midday and ovulation in the evening (if the light cycle begins at 7:00 AM). By estrous morning, both estradiol and progesterone values have returned to baseline levels. During diestrus (diestrus 1 and diestrus 2), values of estradiol and progesterone are low relative to their peak values on proestrus day and the ensuing night.²⁹ We have previously shown that vaginal cytological examination is predictive of cycle stage, confirmed by evaluating serum hormone levels of estradiol and progesterone,²⁸ which matched the values of the normal Sprague–Dawley rat.²⁹ Animals that did not show three consecutive 4-day patterns before treatment were excluded.

When estrous cycles became irregular in the course of this study, two patterns were evident. Both patterns reflect acyclicity but suggest differences in the associated endocrine state.^{30,31} One pattern, termed *irregular*, was defined by vaginal cytology that lacked any pattern. From one day to the next, samples demonstrated leukocytes primarily, or the major cell type was an epithelial cell or cornified epithelial cell. There was no predominance of any one cell type for any 2-day period, which occurs during the normal estrous cycle, that is, when leukocytes dominate for 2 days of diestrus, followed by a 2-day period when samples primarily include epithelial and cornified epithelial cells, signaling proestrus, and estrus, respectively. In the acyclic rats that were irregular, ovulation occurred intermittently, indicated by a vaginal sample dominated by cornified epithelial cells, but there was no consistent diestrous period before or afterward.

The second pattern of acyclicity was associated with per-

sistent vaginal cornification, which reflects the endocrine state that is common in the aged rat, "persistent estrus." Persistent estrus is accompanied by increased serum estradiol levels and the predominance of cornified epithelial cells in the daily vaginal sample (ie, an absence of leukocytes).^{21,32,33} Persistent estrus is also associated with cystic follicles.²¹ Because of the differences in a rat that is an intermittent ovulator (irregular) and a rat in persistent estrus (no ovulation, chronic increase in estradiol without diestrus), animals with persistent estrus are referred to differently than animals with intermittent ovulation.

Assays of Steroid Hormones

Trunk blood was collected at the time of death and centrifuged to separate serum. Serum samples were frozen at -20°C until use. Radioimmunoassay was conducted for estradiol as described previously,^{28,34} and enzyme-linked immunosorbent assay (ELISA) for progesterone and testosterone (ELISA kits 582701 and 582901 from Cayman Chemicals, Ann Arbor, MI). Assays were conducted in triplicate, and averaged values used for statistical comparisons. The investigator who conducted the assays was blinded to the history of the animal, that is, saline versus pilocarpine treatment, epileptic or not epileptic.

Histology

After deep anesthesia by urethane (2.5gm/kg intraperitoneally), animals were perfusion fixed through the aorta with 0.9% NaCl for 3 minutes by gravity feed, followed by 4% paraformaldehyde (in 0.1M phosphate buffer, pH 7.4) for 5 minutes. One ovary per animal was transferred to 30% sucrose solution after at least 3 days. Sections (35 μm) were cut using a cryostat (IEC Minotome; Global Medical Instrumentation, Ramsey, MN), and consecutive sections were mounted on subbed slides. After drying overnight, sections were stained using cresyl violet. Slides were transferred through a graded series of alcohol (70%, 90%, 95%, 100%, 100%, 3 minutes each), cleared in xylene (5 minutes), rehydrated, rinsed in distilled water, and then incubated in 1% cresyl violet acetate for approximately 1 minute. Sections were then rinsed in distilled water, dehydrated, cleared in xylene, and coverslipped in Permount (Fisher Scientific, Pittsburgh, PA).

To evaluate ovarian morphology more closely, we embedded the second ovary in paraffin. Eight-micrometer sections from the ovary were cut with a microtome (Ultracut; Leica Microsystems, Bannockburn, IL), deparaffinized, and alternate sections were mounted on subbed slides and stained with hematoxylin and eosin (Hematoxylin counterstain kit; Vector Laboratories, Burlingame, CA). Slides were incubated in hematoxylin solution for 1 minute, rinsed in distilled water, immersed in acid rinse solution, rinsed in distilled water, incubated in bluing solution for 1 minute, rinsed again, and mounted using Permount.

A blinded investigator examined sections at 2 to 100 \times using an Olympus BX51 microscope (Olympus of America, Center Valley, PA) and Insight Spot camera (Spectra Services, Ontario, NY) to quantify the number of cysts per ovary and to obtain photomicrographs. Cysts were defined as large, fluid-filled cavities that differed from normal follicles in having only a thin granulosa cell layer.²¹ Consecutive sections were evaluated be-

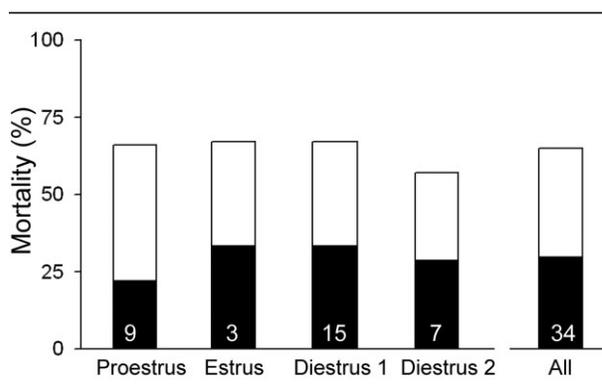


Fig 1. Mortality after pilocarpine-induced status epilepticus (SE) in female rats. Mortality associated with pilocarpine-induced SE is listed as a function of the day of the estrous cycle when pilocarpine was administered. Each animal was injected with pilocarpine in the morning (see Materials and Methods). The mortality in each group was defined either as death during SE (white bars) or within 48 hours of SE (black bars). The total mortality is indicated by the top of each bar (sum of mortality during and after SE). There were no statistically significant differences in total mortality in the four groups (proestrus, estrus, diestrus 1, or diestrus 2; Fisher's Exact test 2^*4 , $p = 0.9502$), suggesting that mortality was not influenced by the cycle stage when pilocarpine was administered. Only three animals were treated with pilocarpine on estrous morning because previous studies showed that SE rarely occurs in animals treated on estrous morning using the dose in this study (350mg/kg³⁵).

cause cysts were large enough that they were present in more than one section. To be sure that each cyst was counted only once, we examined serial sections. There was no evidence of asymmetry in ovarian pathology, so the results report the number of cysts for only one of the ovaries.

Pilocarpine Model

Atropine methylbromide (1mg/kg subcutaneously) was injected, followed 30 minutes later by pilocarpine hydrochloride (350mg/kg subcutaneously). After 1 hour of SE had passed, diazepam (5mg/kg intraperitoneally; Henry Schein, Melville, NY) was injected. Saline control animals received all drugs except pilocarpine, which was replaced by an equivalent volume of 0.9% saline. Diazepam was administered to saline control animals and to pilocarpine-treated rats that did not enter SE. Diazepam was administered to these rats after approximately the same delay after atropine injection as the animals that had SE (approximately 1.5 hours after injection of atropine).

Approximately 4 hours after diazepam injection, dextrose-lactate Ringer's solution (Henry Schein) was injected subcutaneously (2.5ml) at the time when diazepam-induced sedation was maximal. For the subsequent 7 days, an apple that was cut open was left at the base of the cage. Food pellets were also placed at the base of the cage. Procedures for control animals were the same as those animals that had SE.

Seizure Monitoring

Monitoring was conducted to confirm that animals that had experienced SE developed a state of epilepsy. An epileptic

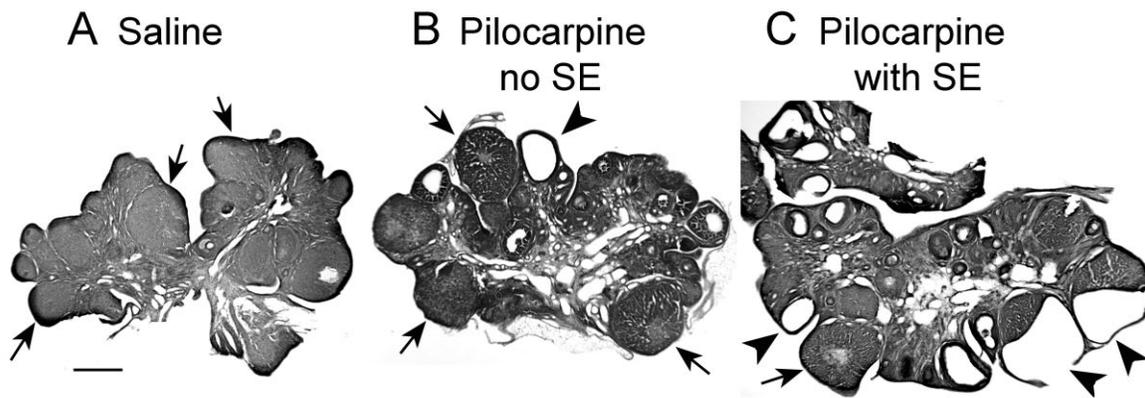


Fig 2. Epileptic rats exhibited greater ovarian pathology than control rats. (A) Micrograph demonstrates normal ovarian morphology in a saline control rat that was killed on first morning of diestrus, 3 months after treatment with saline. The ovary is dominated by corpora lutea (arrows), and there is no evidence of cystic follicles. Calibration bar = 500 μ m. (B) Micrograph of an ovary from a different rat that was treated with pilocarpine but did not experience more than one stage 5 seizure. This rat demonstrated regular estrous cycles and was killed 2.5 months after pilocarpine treatment, on the morning of diestrus 1. There are normal corpora lutea (arrows) and a cyst (arrowhead). (C) Micrograph of an ovary from a rat that had pilocarpine-induced status epilepticus (SE) and maintained regular estrous cycles for 2.5 months thereafter, the length of the study. There are many cystic follicles (arrowheads), and some have a broken wall (arrowheads at bottom right). The ovary exhibits only a few normal corpora lutea (arrows), despite the fact that the animal was killed on the first morning of diestrus, when corpora lutea should be abundant.

state was defined as recurrent stage 3 to 5 seizures⁴³ that were unprovoked and were observed sporadically over the months of the study. Intermittent observations captured multiple spontaneous seizures in all animals that had SE, so the use of intermittent observation did not appear to limit the ability to define an epileptic state. Daily behavioral observations were made for 10- to 30-minute periods, chosen at random between 8:30 AM and 6:30 PM, starting immediately after treatment with pilocarpine. Observations were made of rats that had SE and pilocarpine-treated rats that did not have SE. Behaviors associated with seizures (stages 1–5) were observed only in the animals that had SE. For the epileptic rats in this study, the first observed convulsion (stage 3–5) occurred 5 to 10 days after pilocarpine treatment.

Data Analysis

Statistical comparisons were made using Origin (version 7.0; OriginLabs, Northampton, MA), Simple Interactive Statistical Analysis (SISA; www.quantitativeskills.com), or Microsoft Excel (Microsoft Corporation, Redmond, WA). Means \pm standard errors are reported in the Results. Student's *t* tests assumed unequal variance and were two-tailed. The criterion for statistical significance (*p*) was 0.05. Micrographs were assembled using Adobe Photoshop CS (Adobe Systems, San Jose, CA).

Results

Experimental Groups

Adult female rats were administered saline (*n* = 14) or pilocarpine (*n* = 74) on the morning (10:00–11:00 AM) of proestrus, estrus, diestrus 1, or diestrus 2, at approximately 2 to 3 months of age. In the text below, rats that were injected with saline instead of pilocarpine are referred to as “saline control animals,” and

pilocarpine-treated rats that did not have SE are referred to as “pilocarpine control animals.” Pilocarpine-treated rats that had SE are referred to as “epileptic” because they experienced an epileptic state after SE, as defined earlier (see Materials and Methods). There was no significant difference in the mean age at the time of treatment (saline control animals: 2.65 \pm 0.17 months, *n* = 14; pilocarpine control animals: 3.11 \pm 0.24 months, *n* = 40; epileptic rats: 2.62 \pm 0.36 months, *n* = 9; one-way analysis of variance [ANOVA], *F* = 0.922549; *p* = 0.4029).

An additional group of age-matched, untreated animals (*n* = 14) was pooled with saline control animals for some comparisons. In these instances, the pooled group is referred to as “controls” rather than “saline controls.” The groups were pooled because their serum hormone values, incidence of cysts, acyclicity, and changes in body weight were not significantly different (discussed further below).

Animals were killed at approximately 5 months of age during the midmorning (10:00–11:30 AM). The age at the time of death for all animals was not different (control animals: 5.92 \pm 0.35 months, *n* = 28; pilocarpine control animals: 6.22 \pm 0.30 months, *n* = 40; epileptic rats: 5.69 \pm 0.25 months, *n* = 9; one-way ANOVA, *F* = 0.444369; *p* = 0.6429).

For those animals that had pilocarpine-induced SE (*n* = 34), there was mortality in the 48 hours after SE, despite the use of diazepam to reduce the severity of SE (Fig 1). This was surprising because male rats that were treated with pilocarpine to induce SE, and were similar in age to the female rats (ie, the age range for

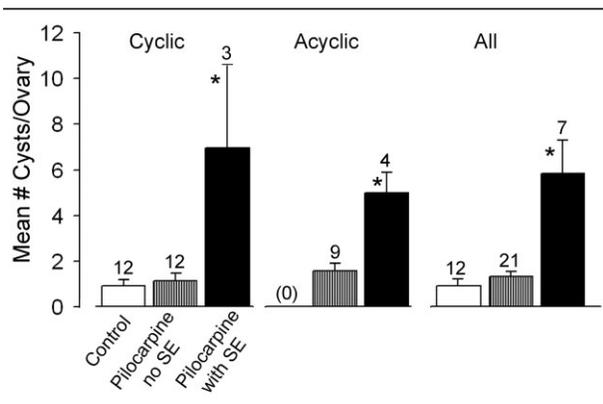


Fig 3. Ovarian cysts in control and epileptic rats. Ovarian morphology was examined in animals that were administered saline or pilocarpine and were killed approximately 2 to 3 months after treatment. There were significantly more ovarian cysts in animals that had status epilepticus (SE), regardless of whether they maintained estrous cyclicity throughout the study. Animals were similar in age at the time of treatment and age at the time of death; therefore, age was unlikely to be a factor. Sample sizes are listed over the bars, and significance is indicated by the asterisks. Means and statistical comparisons are listed in the text.

male rats was 2.2–3.5 months), exhibited significantly lower mortality, even with the greater dose of pilocarpine that is required to initiate SE (380mg/kg instead of 350mg/kg; 4/21 or 19% mortality within 48 hours for male rats compared with 22/34 or 65% for female rats; χ^2 test, $p < 0.05$). Mortality in female rats did not appear to vary with cycle stage (see Fig 1). Note that the lack of variation with cycle stage suggested that animals that received pilocarpine on different days of the estrous cycle could be pooled for further analysis, and this was done for the results described below. Few animals were injected with pilocarpine on the morning of estrus (3/34), because a previous study showed that SE rarely occurs when pilocarpine is injected at this cycle stage, using the pilocarpine dose employed here.³⁵

Estrous Cyclicity

EFFECTS OF STATUS EPILEPTICUS ON ESTROUS CYCLICITY. In epileptic rats, acyclicity was common. Of the nine rats that survived SE, three rats stopped cycling immediately, and the remaining six that demonstrated regular estrous cycles initially after SE did not necessarily continue. Three of the six eventually experienced irregular estrous cycles.

ESTROUS CYCLICITY IN CONTROL RATS.

Even in some control animals, estrous cycles became irregular during the course of the study, which is typical of laboratory rats.^{30,31} In our control group, 1 of 14 saline control and 1 of 14 untreated control animals

(2/28; 7.1%) experienced irregular cycles. In the pilocarpine control group (pilocarpine treatment but no SE), 15 of 29 rats (51.7%) became irregular in their estrous cycles before death. This difference in the incidence of acyclicity was significantly different (Fisher's Exact test, $p = 0.000203$). The pilocarpine control rats that experienced irregular estrous cycles were not statistically different in age from the control rats that ultimately exhibited acyclicity; therefore, age was unlikely to be a contributing factor (control animals were followed until 5.93 ± 0.35 months of age, $n = 28$; pilocarpine control animals were monitored until they were 5.44 ± 0.20 months old, $n = 29$; Student's t test, t statistic = 1.1892, $p = 0.2409$).

The acute effects of pilocarpine appeared unlikely to be a factor because animals only experienced acyclicity several weeks after pilocarpine was administered (>20 days after pilocarpine administration). Therefore, even an acute period of stage 3 to 5 seizures that developed immediately after pilocarpine treatment, without SE, appears to ultimately influence estrous cycles adversely.

INCIDENCE OF PERSISTENT ESTRUS.

The acyclic rats discussed above demonstrated a pattern of vaginal cytology described as irregular. A minority of animals exhibited another pattern, which was reflected by the consistent cornified epithelial cell cytology associated with "persistent estrus," a common condition in the aged rat (see Materials and Methods^{36,37}).

Interestingly, no animal that experienced SE had persistent estrus. The incidence was 0 of 9 (0%) animals that experienced SE compared with 4 of 28 (14.3%) control animals and 11 of 40 (27.5%) pilocarpine control animals. The three groups were significantly different (Fisher's Exact test 2×3 , $p = 0.01342$). Therefore, although brief stage 3 to 5 seizures may increase the risk for persistent estrus, SE appears to decrease it.

The greater incidence of persistent estrus in the pilocarpine-treated group that did not have SE was surprising and prompted us examine this group more closely. Pilocarpine control rats that developed persistent estrus typically were the ones that exhibited stage 3 to 5 seizures immediately after pilocarpine treatment. Thus, 10 of 11 (90.9%) pilocarpine control animals that developed persistent estrus had stage 3 to 5 seizures. Only 1 of 12 pilocarpine control rats without stage 3 to 5 seizures developed persistent estrus (10/11 or 90.9% vs 1/12 or 8.3%; Fisher's Exact test, $p = 0.000098$). Therefore, although pilocarpine itself may not induce persistent estrus, if robust seizures follow pilocarpine administration, there appears to be an increase in the incidence of persistent estrus.

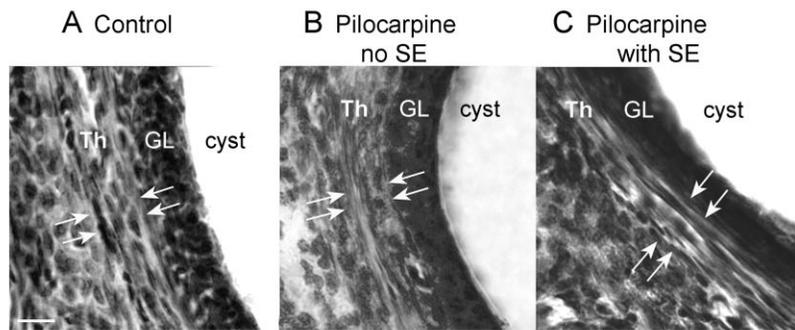


Fig 4. Lack of thecal cell hyperplasia in ovarian cysts of pilocarpine-treated rats. Cystic follicles from a saline control (A), pilocarpine control (B), and epileptic rat (C). Hyperplasia of the thecal cell layer (Th) surrounding the granulosa cell layer (GL) was not detectable. Arrows demarcate the borders of the thecal cell layer. Calibration bar = 50 μ m.

Ovarian Pathology

Rats that maintained estrous cyclicity through the study were killed on the morning of the first day of diestrus. At this cycle stage, approximately 36 hours after ovulation, the normal ovary is dominated by corpora lutea. Both saline control and pilocarpine control rats demonstrated ovarian morphology consistent with the normal ovary: a predominance of corpora lutea with few mature follicles and almost no evidence of cystic follicles (Figs 2 and 3). If cysts were present, they were small (<300 μ m at their widest extent; see Fig 2). In contrast, epileptic rats had many cystic follicles, and cysts were often large (>500 μ m; see Fig 2). The thin cyst wall was often severed during the sectioning procedure (see Fig 2), precluding quantitative comparisons of cyst size across groups.

The gross morphology of cystic follicles was similar in each group. Thus, the wall was composed of a dense granulosa cell layer (see Figs 2 and 4), which tended to be thin and fragile in the epileptic animals, as mentioned above. There was no evidence of thecal cell hyperplasia around the cystic follicles in pilocarpine-treated as compared with control rats (see Fig 4), in contrast with previous reports in kindled rats.²³ However, there was a characteristic of the ovary of epileptic rats that appeared distinct from saline control rats. The ovary of the cyclic epileptic rats was not dominated by corpora lutea, despite the fact that animals were killed during diestrus (see Fig 2). Instead, numerous follicles at various stages of maturity were present, in addition to some corpora lutea. This mixture of cystic, maturing, and degenerating follicles, as well as corpora lutea, contrasted with the ovaries of cyclic control animals, which were dominated by corpora lutea, as would be expected for this stage of the estrous cycle (see Fig 2).

Only cyclic animals are discussed above. However, even when acyclic rats were evaluated, epileptic rats developed statistically greater numbers of cysts per ovary than pilocarpine control rats (see Fig 3). Therefore, independent of the regularity of the estrous cycle, epilep-

tic rats demonstrated significantly more cystic follicles per ovary.

Weight Gain

As shown in Figure 5, epileptic rats that maintained estrous cycles gained more weight than control rats. For cyclic rats, epileptic rats gained 2.77 ± 0.36 gm/day ($n = 3$), whereas control and pilocarpine control rats gained 0.64 ± 0.11 gm/day ($n = 5$) and 0.57 ± 0.056 gm/day ($n = 12$), respectively (note that weight was not followed in every animal, so the sample size is smaller than in other parts of the Results section). These differences were significant (one-way ANOVA, $F = 72.44715$; $p < 0.0000001$). Tukey-Kramer post hoc analysis demonstrated no differences between control and pilocarpine control rats ($p > 0.05$), but they were each statistically different from epileptic rats ($p < 0.05$).

For all rats (pooled, ie, both cyclic and acyclic), epileptic animals gained more weight (1.75 ± 0.36 gm/day; $n = 8$) than control (0.55 ± 0.098 gm/day; $n = 7$) and pilocarpine control animals (0.71 ± 0.094 gm/day; $n = 26$; one-way ANOVA, $F = 10.72397$; $p = 0.000203$). Therefore, weight gain was greater in epileptic rats regardless of estrous cyclicity.

Serum Hormone Levels

CYCLIC ANIMALS

Comparisons were made between cycling animals that were control ($n = 21$), pilocarpine control ($n = 14$), or epileptic animals ($n = 3$). Values for estradiol, progesterone, and testosterone in saline ($n = 9$) versus untreated control animals ($n = 12$) were not statistically different (Student's t tests, estradiol: t statistic = 1.1882, $p = 0.2545$; progesterone: t statistic = 1.2480, $p = 0.2340$; testosterone: t statistic = 1.293, $p = 0.2435$).

Prolactin is known to increase after seizures in patients and in laboratory animals,³⁶⁻³⁸ but prolactin lev-

els in cyclic epileptic rats were not different from non-epileptic control animals (saline control group: 25.5 ± 7.5 ng/ml, $n = 6$; pilocarpine control group: 39.5 ± 16.5 ng/ml, $n = 4$; epileptic group: 30.0 ± 7.0 ng/ml, $n = 3$; one-way ANOVA, $F = 0.04721$; $p = 0.9540$). Therefore, based on serum values for prolactin that were measured in a subset of the cohort, it is unlikely that a recent seizure occurred immediately before death, which could potentially be confounding.

Serum values for estradiol were not statistically different among the three groups (control group: 7.17 ± 0.94 pg/ml, $n = 17$; pilocarpine control group: 6.00 ± 1.12 pg/ml, $n = 14$; epileptic group: 12.33 ± 5.21 pg/ml, $n = 3$; Fig 6; one-way ANOVA, $F = 2.4232$; $p = 0.1052$), neither were the values for serum progesterone (control group: 10.12 ± 0.78 ng/ml, $n = 18$; pilocarpine control group: 8.05 ± 1.09 ng/ml, $n = 13$; epileptic group: 13.30 ± 3.51 ng/ml, $n = 3$; see Fig 6; one-way ANOVA, $F = 2.6988$; $p = 0.08308$). However, testosterone values were increased in the epileptic rats (control group: 49.92 ± 7.36 pg/ml, $n = 13$; pilocarpine control group: 40.17 ± 6.14 pg/ml, $n = 12$; epileptic group: 101.00 ± 19.22 pg/ml, $n = 3$; see Fig 6; one-way ANOVA, $F = 7.1242$; $p = 0.00356$). Post hoc tests demonstrated that there was no significant difference between control and pilocarpine control animals (Tukey post hoc test, $p < 0.05$), but they were significantly different from the epileptic group (Tukey post hoc tests, $p < 0.05$).

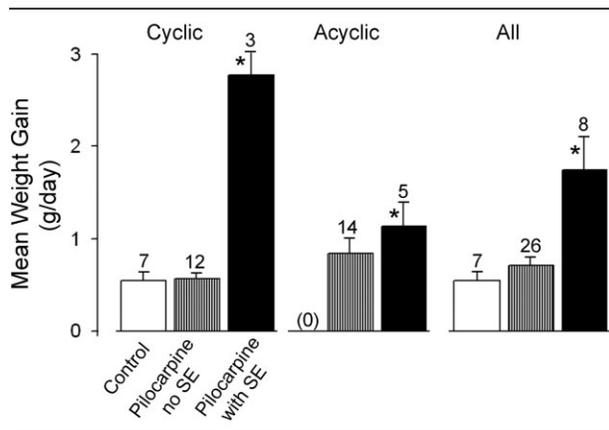


Fig 5. Weight gain in control and epileptic rats. In animals that were treated with saline or pilocarpine, the body weight change in grams per day was calculated from the time of treatment to the time of death. Animals that experienced status epilepticus (SE) gained more weight per day than control animals, regardless of whether they maintained estrous cycles throughout the study. Animals that experienced development of persistent estrus were excluded because this condition is accompanied by chronic increase of estradiol, which has an anorectic effect in the rodent. Asterisks denote statistical significance. Mean values and statistics are provided in the text.

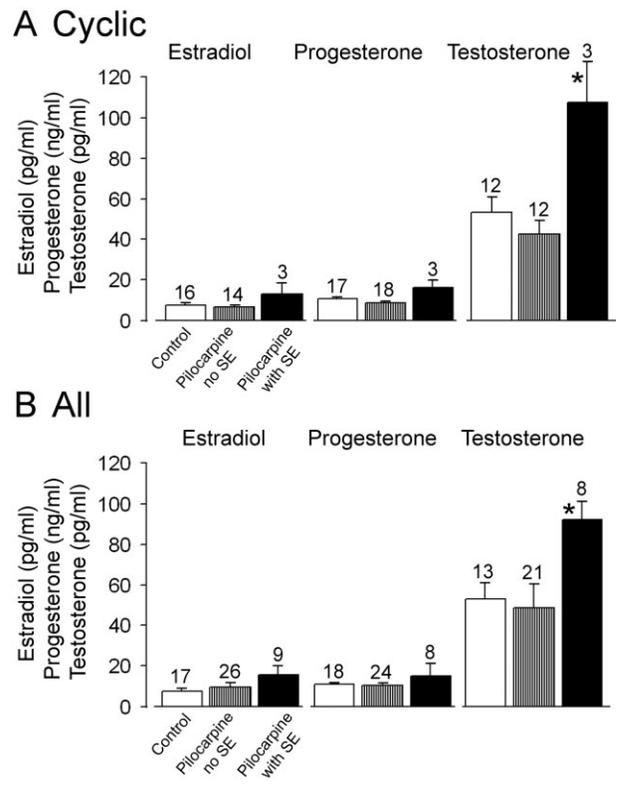


Fig 6. Increased serum testosterone levels in epileptic rats. Comparison of serum from control rats and rats that were treated with pilocarpine demonstrates increased testosterone in rats that had pilocarpine-induced status epilepticus (SE), regardless of whether they maintained estrous cyclicity throughout the study. Animals were killed at the same time of day and time of the estrous cycle (diestrus 1 for cyclic rats, 10:00–11:30 AM; diestrus for acyclic rats, 10:00–11:30 AM). Mean values and statistics are provided in the text.

Acyclic Animals

For animals that developed an irregular pattern in their daily vaginal cytology, death occurred on a day when the vaginal sample was dominated by leukocytes. This choice was made so that the time would approximate diestrus, which would provide a similar endocrine state at the time of death across animals and maximize the ability to compare data with the cyclic group (killed on the first day of diestrus). Control animals were not included because only two control animals experienced irregular estrous cycles, precluding statistical comparisons. Serum estradiol levels were not statistically different between pilocarpine control rats with irregular cycles (estradiol: 11.96 ± 3.84 pg/ml, $n = 12$) or epileptic rats with irregular cycles (15.83 ± 6.28 pg/ml, $n = 6$; Student's t test, t statistic = -0.5263 ; $p = 0.6113$), and progesterone values also were not different (pilocarpine control group: 12.20 ± 2.26 ng/ml, $n = 11$; epileptic group: 13.23 ± 9.41 ng/ml, $n = 5$; Student's t test, t statistic = -0.1056 ; $p = 0.9201$),

but testosterone levels were greater in the epileptic rats (pilocarpine control group: 53.11 ± 23.81 pg/ml, $n = 9$; epileptic group: 77.6 ± 6.96 pg/ml, $n = 5$; Student's t test, t statistic = -0.9874 ; $p = 0.3493$; see Fig 6). Therefore, whether rats were cyclic or irregular, testosterone was increased, but estradiol and progesterone were not.

Discussion

In previous studies of women with epilepsy, it has been difficult to clarify the effects of epilepsy from medications, as well as other factors that are difficult to control in a clinical setting. In laboratory animals, such as rats, the possible confounding contributions of medication, age, environment, and genetic variation can be minimized. However, few studies have been conducted because the female rats that have been evaluated, after convulsant procedures that are used to initiate epileptogenesis, rarely exhibit normal estrous cycles.^{21,24-26} For these reasons, there is no information available on the ways that reproductive function in a normal cycling female epileptic rat could be influenced by an extended duration of epilepsy. For example, would a few months of chronic seizures in an epileptic female rat (a relatively long time in the context of the rat lifespan) alter estrous cycles? Would serum hormone levels change? Importantly, there are studies of serum hormone levels in epileptic female rats,^{25,26} but animals were not clearly distinguished between those that were cyclic and those that were not; as a result, animals with distinct endocrine states were likely to have been pooled. For cyclic animals, it is important to control for estrous cycle stage, given the rapid fluctuations in hormone levels that occur even in 8 hours in the laboratory rat.^{29,39}

Altered Cyclicity in Pilocarpine-Treated Female Rats and Women with Epilepsy

The epileptic rats that maintained estrous cycles after SE usually became acyclic at some point thereafter. It is difficult to ignore the similarity to women with epilepsy, because women with epilepsy often experience menstrual cycle disturbances (amenorrhea, oligomenorrhea, anovulatory cycles^{1,2,14,20}). Based on the data provided here, we suggest that a contribution to menstrual cycle changes in women with epilepsy could be the seizures.

There is an aspect of this study that is noteworthy in the context of a discussion concerning women with epilepsy: age. Women who are younger appear more vulnerable to menstrual cycle disturbances than older women.^{4,8} Therefore, it is relevant that the cohort of female rats used in this study were relatively young; in the female rat, puberty is reached at approximately 1.5 months,⁴⁰ and our animals were treated with pilocarpine at approximately 2 to 3 months of age. A greater incidence of acyclicity may have developed in

this study than would have been found if older female rats had been used.

This study may be limited in how much it can tell us about women with epilepsy for several reasons. One that is obvious is that rodents were used. Another issue is that the seizures were limbic. The type of epilepsy may be important to reproductive dysfunction, although this is currently unclear. In analyses of patients with distinct types of epilepsy, no evidence was provided that the type of epilepsy was a factor in fertility⁹ or menstrual disorders.⁴¹ However, it has been suggested that limbic seizures are most likely to influence the reproductive system, and laterality plays a role, as well as the seizure focus.^{2,20} In one study that compared patients with TLE and primary generalized epilepsy, it was reported that anovulation was more frequent in the patients with TLE.⁴² It makes intuitive sense that limbic seizures would be likely to influence reproductive function, given the robust interconnections between limbic nuclei and the hypothalamus, as discussed elsewhere.^{2,3,7,17,20} In the pilocarpine model, seizures are considered limbic because the convulsions are consistent with the limbic seizures that Racine⁴³ described. The pilocarpine model in the rat is usually considered analogous to TLE,⁴⁴⁻⁴⁷ although this association might be best viewed with caution given that brain damage is typically greater in the pilocarpine model than in patients with TLE,⁴⁴⁻⁴⁷ and electrographic seizures in pilocarpine-treated rats may show evidence of extralimbic origin. For example, seizures in pilocarpine-treated epileptic rats include spike and wave discharges (presumably thalamic in origin),⁴⁸ which could be caused by thalamic pathology in animals with SE.⁴⁹

Use of the pilocarpine model in this study is therefore appropriate, but not without caveats. One problem is that there was substantial mortality after SE, and this could have led to a sampling bias. Another caveat is that an animal with limbic seizures may have an increased likelihood of reproductive endocrine dysfunction, for reasons discussed earlier. Comparisons with other animal models would be useful. However, information is limited. Studies of the kindling model have already shown that evoked stage 5 seizures readily produce reproductive dysfunction, as mentioned earlier (see the introduction to this article). Therefore, use of kindling as an animal model has its limitations if one is to test the hypothesis that an epileptic state can lead to reproductive dysfunction. In the kindling model, it appears that simply the process of epileptogenesis causes reproductive endocrine abnormalities. Other models that have used kainic acid or pilocarpine in Wistar rats, a strain that is distinct from the one used in this study, did not investigate ovarian pathology but did provide interesting data about pregnancy and hormone levels. These studies also show that acyclicity develops, but

the results are difficult to compare with this study because the animals were not segregated in the same ways.^{24–26} To our knowledge, reproductive function in female rodents using other animal models have not yet been examined, but they could help distinguish results specific to models that involve kindling or SE from other types of epilepsy.

Acute Seizures Influence the Estrous Cycle Adversely

Remarkably, rats that did not have SE, and were not epileptic, but exhibited robust (stages 3–5) seizures immediately after pilocarpine administration still tended to exhibit reproductive abnormalities eventually. This is surprising because most laboratories consider rats treated with pilocarpine to require SE before significant changes develop that have long-term consequences. The data suggest that a brief period of seizures, particularly if they involve limbic structures, will impact the endocrine system. This could be because of changes within the hypothalamus after a brief cluster of seizures, immediately after convulsant administration (in this case, pilocarpine), possibly a consequence glutamate release from neurons projecting to hypothalamus that are involved in seizure activity. Pilocarpine itself is unlikely to have played a role because only pilocarpine-treated rats with convulsions demonstrated long-term effects.

Polycystic Ovaries in Female Epileptic Rats

In this study, female rats with chronic seizures had more ovarian cysts, greater weight gain, and increased serum testosterone levels relative to control rats. The most parsimonious explanation is that a condition similar to polycystic ovarian disease developed in the female rat. Inasmuch as a comparison can be made between the rat model and the clinical condition of PCOS, the results suggest that seizures *per se* can lead to a PCOS-like condition and other associated symptoms that are common in women with epilepsy. One cannot conclude that AEDs do not play a role in women with PCOS, but the data suggest that seizures themselves may contribute. This is significant because it supports the idea that chronic disturbances in reproductive function develop after a history of seizures, independent of AED use.

Although there are many hypothetical mechanisms to explain cyst formation, one that may be particularly relevant to women with epilepsy is irregular leuteinizing hormone (LH) secretion, because it has been documented in women with epilepsy.^{50,51} The hypothesis that abnormal LH contributes to cyst formation was originally suggested from studies of women without epilepsy who had irregular LH secretion.⁵² The cysts in these women can be explained by lack of ovulation caused by disrupted neuroendocrine signaling. After ovulation fails, ovarian follicles eventually become

cysts. Because LH normally stimulates the ovarian thecal interstitial cells to secrete androgen, hyperandrogenism would result as well. With consequent disruption of gonadal feedback to the hypothalamus, the basis for a chronic condition associated with cystic ovaries could be explained. This hypothesis remains attractive, although there are many alternative explanations for PCO and PCOS.⁵³

Increased body weight is an important issue in itself because epilepsy is often associated with weight gain in both men and women. Interestingly, this may be more common in female individuals.⁵⁴ In a review of the clinical literature, it was concluded that AED use can explain weight gain⁵⁵ and potentially explain insulin resistance that, in turn, leads to weight gain.¹⁸ However, data from untreated patients with epilepsy are unavailable. In light of the lack of data from an unmedicated population, it is unclear whether an epileptic condition is associated with changes in satiety, energy metabolism, and other factors that could lead to increased body weight. Interestingly, there are data to support an influence of seizures on body weight in rodents. In female rats, seizure induction using lithium and pilocarpine was followed by extreme weight gain.⁵⁶ However, this may have developed because reproductive dysfunction occurred, leading to reduced estrogen levels and a loss of the normal anorectic effect of estrogen.⁵⁷ Consistent with this hypothesis, male rats that were treated in the same way did not gain weight.⁵⁷ Other studies of epileptic male rats have reported loss of weight.^{58,59} Importantly, the present study would support the idea that female rats gain weight after pilocarpine simply because of their seizures, because estrogen levels were examined and were not significantly altered. More experiments will be required to prove that recurrent seizures increase the risk for weight gain and metabolic syndrome, and whether female individuals are at greater risk than male individuals, regardless of AED use.

Vulnerability of the Female Rat to Status Epilepticus

It is clear from the data that address mortality immediately after SE that there is an adverse effect of SE in female rats. The independence of mortality on cycle stage was surprising given the general concept that estrogen and progesterone (or its metabolites) are protective.^{60,61} The lack of a relation between estrogen or progesterone levels and mortality after SE may be because of the fact that SE is an extreme insult, and the protective effects of reproductive steroids are unable to mitigate such a severe challenge. An important consideration is that the chemoconvulsant used was a muscarinic agonist, and it is possible that another method to induce SE, one that would act by a different mechanism, may be more sensitive to the relative concentra-

tions of serum gonadal hormones at the time of convulsant administration.

It is also interesting that there was a sex difference in vulnerability to pilocarpine-induced SE. In male animals, mortality was relatively low compared with female animals. This result could be caused by the difference in body weight in the male and female animals that were tested. Male rats are heavier than female rats after approximately 35 days of age and, therefore, at the ages that were used here. However, it is not clear, to our knowledge, that heavier animals have increased mortality after SE. Therefore, it is possible that there is an inherent sex difference in the risk for death after SE. To our knowledge, this is a novel concept, although studies of seizure severity provide some support. Convulsants appear to elicit more severe responses in female rodents,^{62–65} although this is not always the case.⁶⁶ Whether there is increased risk for mortality after SE in women is not supported by available data,⁶⁷ although few studies appear to have been conducted. Therefore, additional research will be required to evaluate the possibility of sex differences related to mortality after severe seizures, such as SE.

Significance

The major implication of this study is that the female rodent can be used to examine issues that are relevant to endocrine dysfunction in women with epilepsy. Although epileptic female rats have been studied before, it has not been clear that the cycling female epileptic rat develops similar endocrine changes as women with epilepsy. We observed consequences of seizures that, at least superficially, resemble the symptoms reported in women with epilepsy. Although caution clearly has to be exercised in extrapolating from any pharmacologically manipulated animal model to humans, the data are encouraging because they suggest that it may be possible to use the rat model to dissect the underlying mechanisms that lead to reproductive problems in women with this disease. As a corollary of this hypothesis, one might be able to use the rat model as a basis for developing treatments that will enable woman with epilepsy to better maintain normal reproductive function.

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References

1. Svalheim S, Tauboll E, Bjornenak T, et al. Do women with epilepsy have increased frequency of menstrual disturbances? *Seizure* 2003;12:529–533.
2. Herzog AG. Menstrual disorders in women with epilepsy. *Neurology* 2006;66(6 S3):S23–S28.
3. Nappi C, Meo R, Di Carlo C, et al. Reduced fertility and neuroendocrine dysfunction in women with epilepsy. *Gynecol Endocrinol* 1994;8:133–145.
4. Schupf N, Ottman R. Reproduction among individuals with idiopathic/cryptogenic epilepsy: risk factors for reduced fertility in marriage. *Epilepsia* 1996;37:833–840.
5. Morrell MJ, Montouris GD. Reproductive disturbances in patients with epilepsy. *Cleve Clin J Med* 2004;71(suppl 2): S19–24.
6. Klein P, Serje A, Pezzullo JC. Premature ovarian failure in women with epilepsy. *Epilepsia* 2001;42:1584–1589.
7. Harden CL. Polycystic ovaries and polycystic ovary syndrome in epilepsy: evidence for neurogonadal disease. *Epilepsy Curr* 2005;5:142–146.
8. Isojärvi J. Disorders of reproduction in patients with epilepsy: antiepileptic drug related mechanisms. *Seizure* 2008;17: 111–119.
9. Olafsson E, Hauser WA, Gudmundsson G. Fertility in patients with epilepsy: a population-based study. *Neurology* 1998;51: 71–73.
10. Polson DW. Polycystic ovary syndrome and epilepsy—a gynaecological perspective. *Seizure* 2003;12:397–402.
11. Zawadzki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR, eds. *Polycystic ovary syndrome*. Boston: Blackwell Scientific, 1992:377–384.
12. The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;81:19–25.
13. The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004;19:41–47.
14. Duncan S. Polycystic ovarian syndrome in women with epilepsy: a review. *Epilepsia* 2001;42:60–65.
15. Bilo L, Meo R. Epilepsy and polycystic ovary syndrome: where is the link? *Neurol Sci* 2006;27:221–230.
16. Genton P, Bauer J, Duncan S, et al. On the association between valproate and polycystic ovary syndrome. *Epilepsia* 2001; 42:295–304.
17. Herzog AG, Schachter SC. Valproate and the polycystic ovarian syndrome: final thoughts. *Epilepsia* 2001;42:311–315.
18. Isojärvi JI, Taubøll E, Tapanainen JS, et al. On the association between valproate and polycystic ovary syndrome: a response and an alternative view. *Epilepsia* 2001;42:305–310.
19. Taubøll E, Isojärvi JI, Harbo HF, et al. Long-term valproate treatment induces changes in ovarian morphology and serum sex steroid hormone levels in female Wistar rats. *Seizure* 1999; 8:490–493.
20. Herzog AG. Disorders of reproduction in patients with epilepsy: primary neurological mechanisms. *Seizure* 2008;17: 101–110.
21. Edwards HE, Burnham WM, Mendonca A, et al. Steroid hormones affect limbic afterdischarge thresholds and kindling rates in adult female rats. *Brain Res* 1999;838:136–150.
22. Singh KB. Persistent estrus rat models of polycystic ovary disease: an update. *Fertil Steril* 2005;84(suppl 2):1228–1234.
23. Mahajan DK. Polycystic ovarian disease: animal models. *Endocrinol Metab Clin North Am* 1988;17:705–732.
24. Amado D, Verreschi IT, Berzaghi MP, Cavalheiro EA. Effects of intrahippocampal injection of kainic acid on estrous cycle in rats. *Braz J Med Biol Res* 1987;20:829–832.
25. Amado D, Cavalheiro EA. Hormonal and gestational parameters in female rats submitted to the pilocarpine model of epilepsy. *Epilepsy Res* 1998;266–274.

26. Amado D, Cavalheiro EA, Bentivoglio M. Epilepsy and hormonal regulation: the patterns of GnRH and galanin immunoreactivity in the hypothalamus of epileptic female rats. *Epilepsy Res* 1993;14:149–159.
27. Whitten W. Modification of the oestrus cycle of the mouse by external stimuli associated with the male. *J Endocrinol* 1956; 13:299–303.
28. Scharfman HE, Mercurio TC, Goodman JH, et al. Hippocampal excitability increases during the estrous cycle in the rat: a potential role for brain-derived neurotrophic factor. *J Neurosci* 2003;23:11641–11652.
29. Smith MS, Freeman ME, Neill JD. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology* 1975;96:219–226.
30. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. *Braz J Biol* 2002;62:609–614.
31. Mora OA, Cabrera MM. Pheromonal male-induced diestrus and cyclicity in aging intact and young estrogenized female rats. *Biol Reprod* 1994;50:603–606.
32. LeFevre J, McClintock MK. Reproductive senescence in female rats: a longitudinal study of individual differences in estrous cycles and behavior. *Biol Reprod* 1988;38:780–789.
33. vom Saal FS, Finch CE, Nelson JF. Natural history and mechanisms of reproductive aging in humans, laboratory rodents, and other selected vertebrates. In: Knobil E, Neill JD, eds. *The physiology of reproduction*. New York: Raven Press, 1994: 1213–1314.
34. Scharfman HE, Hintz TM, Gomez J, et al. Changes in hippocampal function of ovariectomized rats in response to estradiol replacement that simulates the preovulatory estrogen surge. *Eur J Neurosci* 2007;26:2595–2612.
35. Scharfman HE, Goodman JH, Rigoulot M-A, et al. Seizure susceptibility in intact and ovariectomized female rats treated with the convulsant pilocarpine. *Exp Neurol* 2005;196:73–86.
36. Bauer J. Epilepsy and prolactin in adults: a clinical review. *Epilepsy Res* 1996;24:1–7.
37. Lin YY, Yen SH, Pan JT, et al. Transient elevation in plasma prolactin level in rats with temporal lobe status epilepticus. *Neurology* 1999;53:885–887.
38. Swartz CM, Dunbar E. Postictal prolactin elevations in rats. *Neuropsychobiology* 1983;10:1–6.
39. Freeman ME. The ovarian cycle of the rat. In: Knobil E, Neill JD, eds. *The physiology of reproduction*. New York: Raven Press, 1988:1893–1928.
40. Sharp P, La Regina MC. *The laboratory rat*. New York: CRC Press, 1998.
41. Murialdo G, Galimberti CA, Magri F, et al. Menstrual cycle and ovary alterations in women with epilepsy on antiepileptic therapy. *J Endocrinol Invest* 1997;20:519–526.
42. Cummings LN, Giudice L, Morrell MJ. Ovulatory function in epilepsy. *Epilepsia* 1995;36:355–359.
43. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 1972;32:281–294.
44. Cavalheiro EA. The pilocarpine model of epilepsy. *Ital J Neurol Sci* 1995;16:33–37.
45. Coulter DA, McIntyre DC, Löscher W. Animal models of epilepsy: what can they tell us? *Brain Pathol* 2002;12:240–256.
46. Turski L, Cavalehrio DA, Czuczwar SJ, et al. The seizures induced by pilocarpine: behavioral, electroencephalographic and neuropathological studies in rodents. *Pol J Pharmacol Pharm* 1987;39:545–555.
47. Clifford DB, Olney JW, Maniotis A, et al. The functional anatomy and pathology of lithium-pilocarpine and high dose pilocarpine seizures. *Neuroscience* 1987;23:953–968.
48. Nehlig A, Dube C, Koning E. Status epilepticus induced by lithium-pilocarpine in the immature rat does not change the long-term susceptibility to seizures. *Epilepsy Res* 2002;51: 189–197.
49. Bertram EH, Zhang D, Williamson JM. Multiple roles of midline dorsal thalamic nuclei in induction and spread of limbic seizures. *Epilepsia* 2008;49:256–268.
50. Bilo L, Meo R, Valentino R, et al. Abnormal patterns of luteinizing hormone pulsatility in women with epilepsy. *Fertil Steril* 1991;55:705–711.
51. Drislane FW, Coleman AE, Schomer DL, et al. Altered pulsatile secretion of luteinizing hormone in women with epilepsy. *Neurology* 1994;44:306–310.
52. Blank SK, McCartney CR, Marshall JC. The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. *Hum Reprod Update* 2006;12:351–361.
53. Matalliotakis I, Kourtis A, Koukoura O, Panidis D. Polycystic ovary syndrome: etiology and pathogenesis. *Arch Gynecol Obstet* 2006;274:187–197.
54. Hamed SA, Fida NM, Hamed EA. States of serum leptin and insulin in children with epilepsy. Risk predictors of weight gain. *Eur J Paediatr Neurol* (in press).
55. Ben-Menachem E. Weight issues for people with epilepsy—a review. *Epilepsia*. 2007;48(suppl 9):42–45.
56. St-Pierre LS, Persinger MA. Extreme obesity in female rats following prepuberal induction of lithium-pilocarpine seizures and a single injection of acepromazine. *Epilepsy Behav* 2005;7: 411–418.
57. Asarian L, Geary N. Modulation of appetite by gonadal steroid hormones. *Philos Trans R Soc Lond B Biol Sci* 2006;361: 1251–1263.
58. Carl GF, Critchfield JW, Thompson JL, et al. Genetically epilepsy-prone rats are characterized by altered tissue trace element concentrations. *Epilepsia* 1990;31:247–252.
59. Klitgaard H, Matagne A, Vanneste-Goemaere J, Margineanu DG. Pilocarpine-induced epileptogenesis in the rat: impact of initial duration of status epilepticus on electrophysiological and neuropathological alterations. *Epilepsy Res* 2002;51:93–107.
60. Suzuki S BC, Wise PM. Mechanisms of neuroprotection by estrogen. *Endocrine* 2006;29:209–215.
61. Singh M. Progesterone-induced neuroprotection. *Endocrine* 2006;29:271–274.
62. Uzüm G, Akgün-Dar K, Bahçekapılı N, et al. Nitric oxide involvement in seizures elicited by pentylentetrazol and sex dependence. *Int J Neurosci* 2005;115:1503–1514.
63. Thomas J, Yang YC. Allylglycine-induced seizures in male and female rats. *Physiol Behav* 1991;49:1181–1183.
64. Perićić D, Manev H, Bujas M. Gonadal hormones and picrotoxin-induced convulsions in male and female rats. *Brain Res* 1996;736:174–179.
65. Tan M, Tan U. Sex difference in susceptibility to epileptic seizures in rats: importance of estrous cycle. *Int J Neurosci* 2001; 108:175–191.
66. Bujas M, Perićić D, Jazvinsćak MI. Influence of gender and gonadectomy on bicuculline-induced convulsions and on GABA_A receptors. *Brain Res Bull* 1997;43:411–416.
67. Rossetti AO, Hurwitz S, Logroscino G, Bromfield EB. Prognosis of status epilepticus: role of aetiology, age, and consciousness impairment at presentation. *J Neurol Neurosurg Psychiatry* 2006;77:611–615.