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Are patterns of cortical hyper-excitability altered in catamenial epilepsy?

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Abstract

Objective: We used transcranial magnetic stimulation to determine menstrual cycle related changes in cortical excitability in women with and without catamenial epilepsy and investigated whether these changes differed between ovulatory and anovulatory cohorts.

Methods: Healthy non-epilepsy women and women with generalized and focal epilepsy were investigated during ovulatory (n=11, 46 & 43 respectively) and anovulatory (n=9, 42 & 41) cycles. Patients were divided based on seizure pattern into catamenial (C1: peri-menstrual, C2: peri-ovulatory, and C3: luteal seizure exacerbation), non-catamenial and seizure free. Cortical excitability was assessed using motor threshold (MT) and paired pulse stimulation at short (2- 15 ms) and long (100-300 ms) interstimulus intervals twice; 1. late follicular and 2. mid luteal phases of the menstrual cycle.

Results: In controls, cortical excitability was greatest in the follicular study where intracortical facilitation was increased ($P < 0.05$). The opposite was seen in women with epilepsy where intracortical facilitation was greatest and intracortical inhibition was least in the luteal studies ($p < 0.05$). There were no differences between the ovulatory and anovulatory groups in any of the cohorts. No changes were observed in MT.

Interpretation: Non-hormonal factors are involved in the cyclicity of cortical excitability across the menstrual cycle. Normal menstrual cycle variations in cortical excitability are altered in a similar pattern in ovulatory and anovulatory women with epilepsy regardless of seizure patterns. The underlying neural changes associated with epilepsy may alter responses to sex hormones. This may be an important underlying mechanism for catamenial seizure clustering.

Introduction

Seizure clustering in relation to the menstrual cycle is a common phenomena that is found in a significant proportion (~35%) of women with epilepsy^{1,2}. Many mechanisms have been proposed to explain this association. The most established hypothesis is that it is due to neuroactive properties of sex hormones and cyclic variations in their serum levels^{1,2}. In ovulatory cycles, estrogen increases late in the follicular (pre-ovulatory) phase and then gradually declines during the luteal (pre-menstrual) phase while progesterone is low prior to ovulation and increases during the luteal phase. Evidence for this hypothesis comes from numerous clinical reports of changes in seizure patterns in relation to puberty, pregnancy and menopause³⁻⁵ as well as periodical hormonal fluctuations across the menstrual cycle^{1,2}. It also comes from animal data demonstrating a seizure threshold lowering effect with estrogen^{6,7} and depressed neuronal firing as well as decreased epileptiform discharges with progesterone⁷⁻⁹. This evidence is indirect, and while the anticonvulsant effects of progesterone are a common finding, there is contradictory evidence on the effects of estrogen both in clinical reports that do not link this hormone to seizure exacerbation¹⁰ and many animal studies that do not find it to have a pro-convulsant effect¹¹⁻¹³. Furthermore, even if these competing properties were a proven feature, if seizure exacerbation in women with epilepsy was only linked to fluctuations in sex hormone levels then raised seizure risk would be predicted when the estrogen/progesterone ratio increases. This would either be right before or during ovulation when estrogen levels are at their highest or right before and during menstruation when progesterone declines. Indeed these patterns are seen in some women with catamenial epilepsy^{2,14}. However, seizure exacerbation during the entire second half (luteal phase) of the cycle when progesterone levels are expected to be high is also commonly reported^{15,16}. In some, this may be due to an inadequate luteal phase

with insufficient rise in progesterone levels to be protective against seizures in these women^{15,16}. Clinical data indicating that the catamenial pattern of seizure exacerbation is more often observed in women with temporal lobe epilepsy¹⁷ where reproductive endocrine disorders such as anovulatory cycles and oligomenorhea are also commonly present¹⁸⁻²⁰ support this proposition but it is not a universal finding²¹, nor is it applicable to all seizure types experienced by these women¹⁹. Furthermore, while perimenstrual and peri-ovulatory seizure clustering was found to be much more frequent in women with ovulatory than those with anovulatory cycles, luteal phase clustering was evenly distributed among both cycle types¹⁵. It is thus possible that epilepsy related factors influence the response of neuronal circuits to sex hormone. This interaction may be reflected as a disturbance in cortical excitability and could underlie increased seizure susceptibility in women with epilepsy.

Transcranial magnetic stimulation (TMS) is a safe and sensitive measure of both excitatory and inhibitory functions of motor cortical neurons that has a well-established role in studying cortical excitability changes in epilepsy²²⁻²⁶. Variations in cortical excitability were recently reported in relation to the sleep-wake cycle^{27,28}, sleep deprivation²⁹⁻³¹ and seizures^{32,33} in patients with epilepsy. There are also reports of variations across the menstrual cycle in normal ovulatory^{34,35} and anovulatory women³⁶ and in a small group with catamenial epilepsy³⁷.

In the current study we used TMS in a large cohort of women with ovulatory and anovulatory cycles to determine whether there are variations in cortical excitability across the menstrual cycle in women with epilepsy. We asked whether these changes differ from healthy women without epilepsy, and if they are linked to fluctuations in sex hormone levels across the menstrual cycle. We also investigated if this difference relates

to seizure patterns, such that each of the known catamenial patterns of seizure clustering would have specific cortical excitability changes which would be different compared to those observed in other women with refractory seizures and those who are seizure on medication.

Methods

I. Study populations:

The participants (non-epilepsy controls and patients) were consecutively recruited until a minimum of eight women per group completed the study. This was based on power calculations and was done to ensure evenly distributed groups. The patients were recruited through (a) Outpatient Epilepsy Clinic and (b) screening the databases of the Epilepsy Clinic and Epilepsy Surgery Program at St Vincent's Hospital in Melbourne. These are tertiary referral centres; the first provides the management of patients with epilepsy and the latter aims for the characterization and pre-surgical evaluation of patients with refractory focal epilepsy. The diagnoses were made by at least two experienced epileptologists who were unaware of the study based on clinical history, EEG and imaging findings.

The study protocol was approved by the St Vincent's Hospital Human Research Ethics Committee and written informed consent was obtained from each participant.

Participants were grouped according to (Table 1):

Ovulatory/anovulatory cycles: All participants (non-epilepsy controls and patients) were divided based on their menstrual cycle profile into ovulatory and anovulatory. This was verified by asking each participant before being included to maintain menstrual diaries

for three months prior to the onset of the study. Women with regular menstrual cycles and with progesterone levels exceeding 5 ng/ml in the luteal phase, (serum levels were measured 7 ± 1 day before the onset of menstruation¹⁶) were classified as ovulatory.

Women were assumed to have anovulatory cycles if progesterone levels did not rise above 5 ng/ml during that phase (Table 2).

Clinical features: The patients were further categorized based on (Table 1):

Syndrome:

A. “Genetic” (previously named idiopathic) generalized epilepsy syndromes including juvenile myoclonic epilepsy, juvenile absence epilepsy and generalized epilepsy with tonic-clonic seizures alone.

B. Focal epilepsy syndromes including temporal lobe and extra temporal lobe epilepsy.

Seizure frequency:

Our previous studies showed that TMS measures differ depending on whether the patients are studied at onset prior to exposure to anti-epileptic drugs (AEDs), continue to have refractory seizures or become seizure free³⁸. Consequently we divided our patient groups into:

A. PATIENTS WITH REFRACTORY SEIZURES

Patients were considered refractory if they continued to have seizures for at least three years despite trials of at least two different AEDs at therapeutic doses^{39,40}. This included generalized or secondarily generalized tonic-clonic seizures, absences, myoclonic seizures, focal seizures with loss of awareness and unequivocal focal seizures

comprising visual, auditory, motor, sensory or autonomic manifestations with retained awareness. Isolated infrequent non-specific vague feelings, uneasiness or brief déjà vu were not considered seizures. Those patients were divided into:

1. Catamenial epilepsy: Defined as doubling of seizure frequency in relation to the menstrual cycle observed at least for the last 12 months. These patients were further divided based on the known three patterns of catamenial epilepsy¹⁶ into:
 - a. Peri-menstrual (C1): Seizure exacerbation on day -3 to day 3 (day 1 being the first day of menstrual bleeding).
 - b. Peri-ovulatory (C2): day 10 to day -13 (day -14 being mid-cycle or the day of ovulation in ovulatory cycles).
 - c. Luteal (C3): day -12 to day -4.
2. Non-catamenial epilepsy: Women with refractory epilepsy but with no history of seizure exacerbation in relation to the menstrual cycle.

B. SEIZURE FREE PATIENTS

Patients were included in this group if they did not experience any form of the seizures described above for at least 12 months prior to the TMS test.

Inclusion criteria:

- (a) 18-40 years of age.
- (b) Regular menstrual cycle of 25-30 days during the last six cycles.
- (c) Normal neurological examination.
- (d) Generalized epilepsy: generalized epileptiform abnormalities (3.5-5 Hz spike-wave) on at least one EEG recording and a history of generalized tonic-clonic seizures, myoclonic and/ or absence seizures.

(e) Focal epilepsy: Seizure symptomatology (specifically characteristics of the aura when consistently present) and the EEG showed either a left or right-sided lateralization. The EEG was considered localizing only if definite and prominent sharp-slow discharges were seen consistently lateralized to one region. Patients with temporal intermittent rhythmic delta activity (TIRDA) were included only if the activity was consistently recorded over one hemisphere. Non-specific slowing or sharp waves were not considered lateralizing or localizing even if only recorded on one side. Further localizing signs were found on brain MR images. (Imaging was only routinely performed on patients thought to have focal epilepsy. The findings were available for all patients and are summarized in Table 2).

Exclusion criteria (all participants)

- (a) Fluctuation in cycle length of more than a day.
- (b) Menstrual related affective disorders.
- (c) Use of hormonal contraceptive methods throughout the study period or the preceding 6 months.
- (d) Pregnancy or lactation.
- (e) History of ovariectomy.
- (f) Suffering from any medical condition at the time of the study particularly endocrinal abnormalities or hormonal disturbances.
- (g) Previous cortical resections or craniotomies.
- (h) Cardiac pacemaker, vagal nerve stimulator or intracranial metal implants.

Exclusion criteria (non-epilepsy controls)

- (a) History of seizures, migraine or any other neurological condition.
- (b) History of head trauma or skull fractures.
- (c) Previous exposure to AEDs.
- (d) Currently taking any medication of any kind.

Exclusion criteria (patients)

- (a) Suspicion of non-epileptic events (psychogenic non-epileptic seizures, migraine, parasomnias etc).
- (b) Patients with an undetermined epilepsy syndrome (not clear whether generalized or focal epilepsy).
- (c) Seizure foci originating in the vicinity of the motor area (seizure semiology or on imaging).
- (d) Bilateral seizure foci.
- (e) Any change to the AED regimen during the study or at any time in the 6 months preceding it.

II. Timing of the studies:

TMS was performed on all participants (non-epilepsy controls and patients) twice, 12-15 days apart (Figure 1).

In women with ovulatory cycles, one study was in the *late follicular phase* (day 12 ± 2 , 1-2 days pre-ovulation, when estrogen is at its highest level) and the other was in the *mid luteal phase* (day -7 ± 2 , 7 days pre-menstruation, when the progesterone level is at its peak). In women with anovulatory cycles, the follicular and luteal studies were timed to

coincide with approximately the same phases, the first being 1-2 days before mid-cycle and the second 5-10 days prior to the expected time of menstruation.

The timing of the two studies was calculated for each participant based on the duration of her menstrual cycles. Plasma estrogen and progesterone levels were measured at the time of each study to verify the phase of menstrual cycle.

To avoid any hypothetical “order effect”, the follicular phase study was randomly performed before the luteal phase study in half the participants in each group and the order was reversed in the others.

In all groups, care was taken to perform both TMS studies for each participant between 10 am – 3 pm so as to increase the repeatability of the measurements. Care was also taken to avoid clustering of any of the participants in a group to a particular time, and the studies were spread evenly over this time interval in all groups. All participants were asked to maintain regular sleep patterns with seven - nine hours of sleep the night before each study. The results were only analysed after a two day seizure free period was confirmed (based on seizure diaries) on either side of the study. If seizures were reported within this time frame, the study was repeated. No patients were excluded due to seizures.

III. Transcranial magnetic stimulation paradigm:

Both hemispheres were studied in each participant (patients and controls). During TMS, the participants sat in a comfortable, reclining chair. Surface electromyographic (EMG) recording was made from the abductor pollicis brevis muscle (APB). Stimuli were delivered to the contralateral cerebral hemisphere by applying the appropriate direction of coil current flow (anticlockwise for left cortical stimulation and clockwise for right

cortical stimulation), using a flat circular 9 cm diameter magnetic coil (14 cm external diameter) with the centre of the coil positioned over the vertex and held in a plane tangential to it using a pair of Magstim 200 magnetic stimulators (Magstim, Whitland, Dyfed, United Kingdom). Paired stimulation at various interstimulus intervals (ISIs) was performed using a Bistim module to connect 2 stimulators to the coil.

The motor evoked potentials (MEPs) were recorded and digitized online via a CED 1401 interface (Cambridge Electronic Design Ltd, Cambridge United Kingdom) and stored on computer for offline analysis. Signal software (Cambridge Electronic Design Ltd, Cambridge United Kingdom) was used for automated acquisition and marking of the recorded MEPs. Filters for the acquisition were set to low frequency of 10 Hz and high frequency of 5 KHz. Sweep speed for threshold determination and paired pulse TMS at short ISIs was 100 ms and the sensitivity was set to 100 μ V/division. For longer ISIs the sweep was adjusted to 500 ms and sensitivity to 2 mV/division. The MEP amplitude was measured from peak to peak.

The experimental session lasted for 60-90 minutes and the following parameters were recorded:

Motor threshold (MT): MT was determined in all tested hemispheres while the participant was at rest, verified by continuous visual and auditory EMG feedback. Stimulation commenced at 30% of maximum output and increased in 5% increments until the MEP was established. 1% changes in intensity were then used to measure the threshold value. Motor threshold was defined as the lowest level of stimulus intensity which produced a MEP in the target muscle of peak-to-peak amplitude $> 100 \mu$ V on 50% or more of 10 trials ⁴¹.

Intracortical inhibition and facilitation: Cortical recovery curves were derived using paired pulse TMS. For the short ISIs of 2, 5, 10, 15 ms, the first stimulus was given at 80% of MT and the second stimulus 20% above MT. Ten stimuli at 20% above MT without a preconditioning stimulus were also given. For longer ISIs, the stimulation intensity was 20% above MT using paired stimuli in 50 ms increments at ISIs of 100 - 300 ms. A minimum interval of 15 seconds was kept between the delivery of each pair of stimuli. Stimuli were given at randomly selected ISIs until a total of 10 at each ISI was achieved.

Recovery curves at short ISIs (2 - 15 ms) were constructed for each hemisphere using the ratio of the mean peak to peak amplitude of the response (termed test response [TR]) at each ISI following the conditioning stimulus given below MT expressed as the percentage of the mean MEP when the test stimulus was given alone without a preconditioning stimulus (TR/MEP%).

Recovery curves at longer ISIs (100 - 300 ms) were constructed for each hemisphere using the ratio of the mean peak-to-peak amplitudes of the response to the second stimulus termed the test response (TR) and the response to the first stimulus termed the conditioning response (CR) at each ISI measured as a percentage (TR/CR%).

Each participant was given a unique alpha numeric code. This was the only identifying feature on the TMS data acquired. The analysis was performed after all participants had been tested. This ensured that the investigator analysing the TMS results was blinded to clinical information during the analysis.

IV. Statistical analysis:

In non-epilepsy controls and generalized epilepsy the results were analysed according to hemisphere dominance assessed according to the Edinburgh Handedness Inventory⁴².

In focal epilepsy the results were analysed according to the ipsilateral and contralateral hemisphere (the ipsilateral hemisphere is defined here as the hemisphere with the presumed seizure focus). This was based on seizure semiology and EEG findings.

Correlation between clinical features (age, age at onset of seizures, seizure type and frequency) and AED (type and serum levels) with change in cortical excitability was performed using Pearson's correlation co-efficient as well as the chi-square test.

For cortical excitability measures (MT and ISIs), a two-way repeated measures analysis of variance (ANOVA) was used. Each test had a within participant factor (timing of the study [the dependent variable was the change; follicular minus luteal value at each measure] and a between-participants factor "group" (menstrual cycle type, type of epilepsy; seizure pattern, non-epilepsy controls). This was performed for each hemisphere in each group.

A one-way analysis of variance test was used to compare plasma estrogen and progesterone levels measured in each group at the time of the follicular and luteal studies.

For all analyses, $p < 0.05$ was chosen as the significance level. Fisher's Protected Least Significant Difference *post hoc* tests for correction of multiple comparisons were performed as appropriate. The analysis was performed on SPSS, 15.0 for windows®.

The effect size was calculated for the significant results (mean MT and mean value at each ISI) using the formula:

Effect size = mean of luteal values – mean of follicular values / (standard deviation of follicular values + standard deviation of luteal values)².

Effect size 0.2 was considered small, 0.5 was considered medium and ≥ 0.8 was considered large⁴³.

Results

There were no significant differences between the groups in the average cycle day of testing in the follicular or luteal phases.

1. Hormonal changes

Table 1 shows the mean plasma estrogen and progesterone levels measured on the day of each TMS test in each group. Other than the expected changes in hormone levels between the two phases in each group, the only significant differences observed were lower estrogen levels during the follicular phase and lower progesterone levels during the luteal phase in all groups with anovulatory cycles compared to those with ovulatory cycles. No other significant inter-group hormone level differences were observed.

2. Cortical excitability measures

Inter-study variability in cortical excitability did not correlate with age, age of seizure onset or frequency or type of seizures in any of the sub-groups. It also did not correlate to the number of AEDs used or their different combinations or serum levels. .

There were no inter-hemispheric differences in any of the groups in any of the studies. Consequently, even though the results from both hemispheres were analysed, only the results from the dominant hemisphere for non-epilepsy controls and generalized epilepsy and the ipsilateral hemisphere for focal epilepsy are presented.

There were no differences in MEP sizes to test stimuli at 120% between the groups.

Motor threshold

There were no differences in mean MT comparing the follicular and luteal values in any of the groups (Table 3).

Intracortical inhibition and facilitation

A. Menstrual cycle variations in cortical excitability

Non-epilepsy controls

In non-epilepsy controls there was increased cortical excitability evidenced by higher intracortical facilitation (10 and 15 ms ISIs) in the *follicular* study compared to the *luteal* study ($F=4.98$, $p<0.05$, effect size 0.4). There were no changes in short (ISIs 2 & 5 ms) or long (100-300 ms) intracortical inhibition comparing the two studies (Figure 2).

Women with epilepsy

1. Catamenial refractory seizures

a. Peri-menstrual (C1)

There was no difference between the follicular and luteal studies at any of the ISIs in in

either group with generalized or focal epilepsy (Figure 3a,b and 4a,b). There was also no difference between the ovulatory and anovulatory groups in either study.

b. Peri-ovulatory (C2)

c. Similar to the groups with C1 catamenial patterns, no difference was found between the follicular and luteal studies at any of the ISIs in the groups with generalized or focal epilepsy (Figure 3c,d and 4c,d). There was also no difference between the ovulatory and anovulatory groups in either study. Luteal (C3)

A pattern opposite to that seen in controls was found. Cortical excitability was higher in the *luteal study* compared to the follicular one.

This was present in both groups with generalized (Figure 3e,f) and focal (Figure 4e,f) epilepsy and was significant at the short ISIs of 10 and 15 ms ($F=4.56-5.92$, $p < 0.05$, effect sizes ranging 0.3-0.5) and all the long ISIs ($F=5.31-16.73$, $p < 0.05$, effect sizes ranging from 0.4 – 0.9), being maximum at the 300 ms ISI and greater in generalized epilepsy.

Cortical excitability was higher in the luteal study in a similar fashion in both the ovulatory and anovulatory groups with no difference between them.

2. Non-catamenial refractory seizures

A pattern identical to that observed in patients with the C3 catamenial pattern was found, but with smaller effect sizes. Cortical excitability was higher ($F=4.01-5.64$, $p < 0.05$, effect sizes 0.3-0.4 at the 10 and 15 ms ISIs, and $F= 3.97-10.31$, $p < 0.05$, effect sizes ranging from 0.3-0.7 at the long ISIs) in the *luteal study* compared to the follicular one

in both patients with generalized and focal epilepsy (Figure 3g,h and 4g,h). Again the changes were similar in both the ovulatory and anovulatory groups.

3. Seizure free

Cortical excitability was also higher in both groups with generalized and focal epilepsy in the *luteal study* compared to the follicular one (Figure 3i,j and 4i,j). The differences were significant at the short ISIs of 10 and 15 ms ($F=3.90-4.13$, $p < 0.05$, effect sizes ranging 0.2-0.3) and the long ISIs 250 and 300 ms ISIs only ($F=4.06-6.48$, $p < 0.05$, effect sizes ranging from 0.3 – 0.5), being maximum at the 300 ms ISI. This was greater in generalized epilepsy, where a significant difference was also observed at the 150 ms ISI ($F=3.59$, $p < 0.05$, effect size 0.3). Here too the changes were the same in both the ovulatory and anovulatory groups.

B. Comparisons with non-epilepsy controls

Inter-group comparisons confirmed our previously reported increase in cortical excitability in patients with epilepsy compared to controls which relied on whether they were refractory or seizure free³⁸. The magnitude of this depended on the phase of menstruation studied. Comparison of *follicular studies* between patients and controls showed significantly higher cortical excitability in both ovulatory and anovulatory patients with generalized and focal epilepsy at the short ISIs of 2 and 5 ms ($F=5.49-6.08$, $p < 0.05$, effect sizes 0.5 and 0.6) and all the long ISIs ($F=12.21-19.91$, $p < 0.05$, effect sizes ranging between 0.9-1.4) in patients with refractory seizures and only at the 250 and 300 ms ISIs ($F=5.57-7.26$, $p < 0.05$, effect sizes ranging between 0.4-0.6) in seizure free cohorts.

Effect sizes were larger at these same ISIs when the same comparison was performed for

the *luteal studies* (ranging from 0.5-1.7). In addition, cortical excitability was also significantly higher in both ovulatory and anovulatory generalized and focal epilepsy with refractory seizures at the 10 and 15 ms ISIs ($F=4.52-8.14$, $p < 0.05$, effect sizes ranging from 0.3 - 0.7) compared to controls in the *luteal studies*. These changes were observed in all the refractory groups (catamenial and non-catamenial) with no differences between them.

Discussion

The current study shows that normal variations in cortical excitability during the menstrual cycle are altered in women with epilepsy regardless of clinical presentation, or type of cycle (ovulatory or anovulatory). Cortical excitability is highest in healthy, non-epileptic women during the *follicular* phase. In women with epilepsy, the pattern is reversed and cortical excitability is highest during the *luteal* phase.

Changes in cortical excitability in non-epileptic women: Increased intracortical facilitation (10 and 15 ms ISIs) was seen during the *follicular* phase in normal ovulating women compared with the luteal phase. This finding replicates that published in two previous TMS studies performed in groups of healthy ovulating women across the menstrual cycle^{34, 35}. Intracortical facilitation is not clearly a pure intracortical phenomenon with a suggestion of both cortical and segmental contributions⁴⁴. Despite the complex nature of its underlying substrate, increased intracortical facilitation may be taken to imply increased glutamate mediated neuronal excitability⁴⁵ and possibly also decreased GABA_A mediated inhibition⁴⁶ during the follicular phase in normal non-epileptic women. A lowering of GABA_A mediated inhibition during the follicular phase is further supported by the reported reduction in intracortical inhibition at the short (< 5 ms) ISIs^{47, 48} in two previous studies^{34, 35}, although we did not find this in the current

study. We also found no evidence of change in long intracortical inhibition. Long intracortical inhibition is mainly thought to reflect GABA_B mediated inhibition⁴⁹⁻⁵¹, though this has only been pharmacologically confirmed at the 100 ms ISI. If hormonal fluctuations were an important factor underlying variations observed in cortical excitability then higher excitability during the follicular phase of ovulatory cycles could be explained by increased levels of estrogen associated with decreased levels of progesterone during the follicular phase, or it could be due to a reduction in cortical excitability in the luteal phase resulting from increased levels of progesterone. The cyclical variability could also reflect the interplay of both factors at each phase of the cycle. Hormonal fluctuations however do not explain why cortical excitability was higher during the follicular phase in women with anovulatory cycles as well. It is known that anovulatory cycles do not show the same variations in hormonal levels¹⁶. In addition, there is a report of increased intracortical inhibition in the luteal phase in women with anovulatory cycles compared to those with ovulatory cycles despite the high levels of progesterone in the latter group³⁶. Our direct human data clearly shows a lack of difference between the women with ovulatory cycles and those with anovulatory cycles. While this contrasts with conclusions drawn from indirect evidence based on pharmacological studies mostly performed on ovariectomized animals⁶⁻⁹, it is supported by others¹¹⁻¹³, particularly the report that hormonal fluctuations during rats estrous cycle do not correspond at all to increased excitability measured as progression into pilocarpine-induced status epilepticus¹³. This underscores the need for more studies to clarify the physiological nature of the neuronal-hormonal interaction. It also suggests that non-hormonal factors may be involved in the cyclicity of cortical excitability changes across the menstrual cycle.

Changes in cortical excitability in women with epilepsy: Paradoxically, cortical excitability was highest (increased intracortical facilitation and decreased long intracortical inhibition) during the *luteal* phase of the cycle in our cohort of ovulatory and anovulatory women with both generalized and focal epilepsy. The fact that this occurs in ovulatory women as well implies that C3 patterns are not solely explained by hormonal disturbances in anovulatory cycles as suggested by clinical observations ^{1,2}.

Anovulatory cycles are characterized by an abnormal absence of a substantial increase in progesterone secretion during the luteal phase despite the late follicular estrogen surge which results in a high ratio of neuroexcitatory estrogen to neuroinhibitory progesterone throughout the luteal phase ^{15,16}. This disturbance does not apply to ovulatory women.

Furthermore, luteal phase seizure exacerbation cannot be fully attributed to the rapid withdrawal of the anti-seizure effects of progesterone, because cortical excitability was found to be high at a time when progesterone levels were very high (confirmed by the measurements obtained on the day of testing in our cohorts). Another intriguing finding is that increased cortical excitability in the luteal phase was not limited to women with C3 patterns. It was found in all women with epilepsy whether they suffered from catamenial epilepsy or not and even in those who were seizure free, though it was not statistically significant in the cohorts with the C1 and C2 patterns. Again there was a lack of difference between ovulatory and anovulatory women. Normal sex hormone levels were also reported despite increased cortical excitability during the luteal phase in a TMS study of ovulatory women with known catamenial epilepsy ³⁷. This occurred in women with all three patterns of seizure exacerbation (C1, C2 and C3), though the numbers were very small. Furthermore, we also found luteal phase hyper-excitability in a different cohort of women with new onset epilepsy who did not have a definite history of catamenial seizure clustering ⁵². None of those patients were taking AEDs. All these

findings show a striking reversal of the normal pattern of cortical excitability changes over the menstrual cycle in women with epilepsy which seems to occur soon after the onset of epilepsy and is present regardless of clinical presentation and use of AEDs.

The results of the present study provide novel direct human evidence that the changes in cortical excitability cannot be solely explained by hormonal fluctuations across the menstrual cycle. This is in contrast to previous observational and clinical studies on this topic, which just associated and linked periodical clustering of seizures to the hormonal changes. The findings also underscore the complexity of the effects of sex hormones on neuronal excitability, seizures and seizure induced damage. This is likely to be due to the multifaceted action of steroid hormones which include modulation of multiple genes by their up or down regulation and activation of membrane orphan G-protein coupled receptors, specific membrane hormone receptors or by direct binding to neurotransmitter receptors⁵³. Alterations in GABA_A (decreased short intracortical inhibition^{47,48}) and GABA_B (decreased long intracortical inhibition⁴⁹⁻⁵¹) circuits have been suggested as a mechanism for the increased cortical excitability observed in numerous TMS studies of epilepsy²²⁻²⁶. These changes may alter the response of intracortical circuits to all the factors involved in cyclicity of cortical excitability across the menstrual cycle (hormonal and non-hormonal) and cause neurons to interact in an atypical way to circulating sex hormones. There is evidence that the effects of estrogen can be influenced by the region or neurotransmitter system involved, the seizure type or animal model used and the specific expression of individual estrogen receptor types⁵⁴. There are also distinct modulatory effects of estrogens on the neurotransmitter system involved in seizure genesis⁵⁴. It is also known that progesterone metabolites function as potent positive modulators of the GABA_A receptor^{8,9}, which in the setting of chronic illnesses such as epilepsy can cause changes in GABA_A receptor subunit composition to compensate for

sustained levels of inhibition due to periods of prolonged exposure and withdrawal ⁵⁵. The changes in subunit composition have been shown to profoundly alter GABA_A receptor structure and function ⁵⁶, and subsequently the response to hormones. While these conclusions are predominantly based on pharmacological studies on animal models and involve non-cortical structures which may not be applicable to the cortical excitability changes we observed here, they nevertheless suggest there may be disturbance in the way neurons in general react. This interaction is likely to also be affected by the use of AEDs and seizure control. It would therefore appear that the final result of the cascade of events initiated by sex hormones on cortical excitability and seizure risk is influenced by many factors, but this may express itself at the whole brain level as increased excitability at a time that corresponds to the timing of seizure worsening. Clearly, more work is needed to delineate the nature of all factors involved in this interaction. Further clarification of the effects of sex hormones on healthy neurotransmitter systems and how they are altered in epilepsy is also important as this is a significant factor in seizure control unique to women with epilepsy.

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Table 1: Demographics of participants included in each group.

Group	Sub-group	Number	Mean Age in years (range 18- 40 in all)	Age of Onset in years (range)	Mean Cycle Length in days (range 25- 30 in all)	Seizure Frequency; all types (range)	AEDs	Lesions on MRI (number)	
Non-Epilepsy Controls									
	Ovulatory	11	23	-	29	-	-	-	
	Anovulatory	9	24	-	29	-	-	-	
Generalized Epilepsy									
Ovulatory	Catamenial Epilepsy	C1	9	26	20 (14-23)	28	6/month (2- 12)	VPA, LEV, LTG, TPM	-
		C2	9	25	18 (12-24)	29	6/month (3- 11)	VPA, LEV, LTG, TPM	-
		C3	9	27	18 (12-24)	27	8/month (2-	VPA, LEV, LTG,	-

							16)	TPM	
	Non-catamenial	9	24	18 (11-22)	29	6/month (2-14)	VPA, LEV, LTG, TPM	-	
	Seizure free	10	25	16 (11-21)	30	-	VPA, LEV, LTG, TPM	-	
Anovulatory	Catamenial Epilepsy	C1	8	24	17 (11-23)	28	8/month (3-10)	VPA, LEV, LTG, TPM	-
		C2	8	22	18 (14-22)	29	7/month (2-12)	VPA, LEV, LTG, TPM	-
		C3	9	25	16 (12-24)	29	6/month (4-11)	VPA, LEV, LTG, TPM	-
	Non-catamenial	8	25	18 (13-24)	27	8/month (2-15)	VPA, LEV, LTG, TPM	-	
	Seizure free	9	23	16 (11-21)	27	-	VPA, LEV, LTG, TPM	-	
Focal Epilepsy									
Ovulatory	Catamenial Epilepsy	C1	8	25	23 (11-29)	30	5/month (3-11)	CBZ, GBP, LAC, LEV, LTG, OXC,	1 cortical dysplasia, 1

								TPM, VPA	hippocampal sclerosis
		C2	8	27	20 (13-27)	27	6/month (2-10)	CBZ, GBP, LAC, LEV, LTG, OXC, TPM, VPA	1 hippocampal sclerosis
		C3	9	26	22 (11-30)	28	6/month (2-11)	CBZ, GBP, LEV, LTG, OXC, TPM, VPA	1 cortical dysplasia
	Non-catamenial		9	28	24 (13-32)	27	5/month (3-12)	CBZ, , LEV, LTG, OXC, TPM, VPA	1 hippocampal sclerosis
	Seizure free		9	23	22 (14-29)	29	-	CBZ, LAC, LEV, LTG, OXC, TPM, VPA	-
	Anovulatory	Catamenial Epilepsy	C1	8	23	23 (11-26)	30	5/month (2-10)	CBZ, GBP, LAC, LEV, OXC, TPM, VPA
C2			8	26	20 (17-30)	29	6/month (4-13)	CBZ, LEV, LTG, OXC, TPM, VPA	1 occipital gliosis

	C3	9	24	24 (11-31)	27	4/month (2-10)	CBZ, GBP, LAC, LEV, LTG, OXC, TPM, VPA	1 cortical dysplasia
Non-catamenial		8	26	25 (14-32)	28	5/month (2-11)	CBZ, GBP, LAC, LEV, LTG, OXC, TPM	1 hippocampal sclerosis
Seizure free		8	24	22 (13-29)	28	-	CBZ, LEV, LAC, LTG, OXC, TPM, VPA	1 frontal cyst

C1: peri-menstrual pattern of seizure exacerbation, C2: peri-ovulatory pattern of seizure exacerbation, C3: luteal pattern of seizure exacerbation, CBZ: carbamazepine, GBP: gabapentin, LAC: lacosamide, LEV: levetiracetam, LTG: lamotrigine, OXC: oxcarbazepine, TPM: topiramate, VPA: sodium valproate.

Table 2: Plasma hormone levels in each group measured on the day of the TMS test (mean \pm SD). C1: peri-menstrual pattern of seizure exacerbation, C2: peri-ovulatory pattern of seizure exacerbation, C3: luteal pattern of seizure exacerbation.

Group	Subgroup	Follicular		Luteal		
		Estrogen (pg/ml)	Progesterone (ng/ml)	Estrogen (pg/ml)	Progesterone (ng/ml)	
Non-epilepsy controls						
	Ovulatory	199.7 \pm 60.4	0.7 \pm 0.4	77.8 \pm 17.2	8.2 \pm 3.9	
	Anovulatory	54.8 \pm 15.7	0.6 \pm 0.4	49.4 \pm 12.4	1.1 \pm 0.3	
Generalized Epilepsy						
Ovulatory	Catamenial Epilepsy	C1	193.4 \pm 52.7	1.1 \pm 0.3	79.1 \pm 12.7	12.0 \pm 3.9
		C2	186.1 \pm 68.1	0.7 \pm 0.5	89.4 \pm 15.4	9.4 \pm 4.1
		C3	190.0 \pm 59.1	0.9 \pm 0.4	84.5 \pm 19.6	8.7 \pm 4.8
	Non-Catamenial Epilepsy	180.9 \pm 66.4	1.2 \pm 0.4	79.5 \pm 19.1	11.7 \pm 5.3	
	Seizure Free	197.1 \pm 39.4	0.9 \pm 0.3	85.3 \pm 20.3	9.4 \pm 5.0	
Anovulatory	Catamenial Epilepsy	C1	55.7 \pm 14.9	0.9 \pm 0.2	44.2 \pm 11.8	1.2 \pm 0.5
		C2	64.1 \pm 14.6	0.8 \pm 0.5	55.8 \pm 12.9	0.9 \pm 0.2
		C3	49.5 \pm 19.3	0.8 \pm 0.3	41.3 \pm 10.9	0.8 \pm 0.2
	Non-Catamenial Epilepsy	38.9 \pm 17.9	1.0 \pm 0.5	32.7 \pm 11.2	0.9 \pm 0.5	
	Seizure Free	50.6 \pm 12.7	0.8 \pm 0.3	46.8 \pm 9.4	0.8 \pm 0.3	
Focal Epilepsy						
Ovulatory	Catamenial	C1	204.1 \pm 44.2	0.7 \pm 0.5	79.7 \pm 24.2	8.9 \pm 5.0

	Epilepsy	C2	188.9±39.2	1.2±0.2	86.7±16.8	9.9±6.2
		C3	189.5±57.1	1.0±0.2	89.2±14.6	12.4±4.8
	Non-Catamenial Epilepsy		200.2±45.1	0.8±0.6	84.6±22.1	10.2±4.9
	Seizure Free		185.2±67.3	0.8±0.5	79.9±16.9	11.2±5.6
	Anovulatory	Catamenial Epilepsy	C1	57.4±18.4	1.0±0.3	50.1±10.9
C2			42.7±17.2	0.9±0.4	35.3±9.1	0.7±0.5
C3			54.3±19.3	0.7±0.5	39.2±11.8	0.7±0.3
Non-Catamenial Epilepsy		61.3±11.1	0.9±0.6	44.2±15.1	0.8±0.2	
Seizure Free		44.3±10.0	1.0±0.4	35.3±13.1	0.8±0.5	

Table 3: Menstrual related variation in MT in the dominant hemisphere in non-epilepsy controls and generalized epilepsy and the ipsilateral hemisphere in focal epilepsy (mean \pm SD). C1: peri-menstrual pattern of seizure exacerbation, C2: peri-ovulatory pattern of seizure exacerbation, C3: luteal pattern of seizure exacerbation.

Group	Subgroup		Follicular MT (stimulus intensity %)	Luteal MT (stimulus intensity %)
Non-epilepsy controls				
	Ovulatory		53.9 \pm 5.4	55.1 \pm 4.9
	Anovulatory		54.1 \pm 4.9	53.4 \pm 5.2
Generalized Epilepsy				
Ovulatory	Catamenial Epilepsy	C1	54.2 \pm 5.3	55.2 \pm 5.2
		C2	56.2 \pm 4.8	55.6 \pm 6.3
		C3	51.8 \pm 7.9	53.2 \pm 5.3
	Non-Catamenial Epilepsy		53.2 \pm 5.2	54.6 \pm 6.1
	Seizure Free		56.3 \pm 4.7	56.3 \pm 4.8
Anovulatory	Catamenial Epilepsy	C1	55.7 \pm 4.5	53.9 \pm 6.4
		C2	54.2 \pm 5.4	55.1 \pm 4.5
		C3	53.9 \pm 7.2	55.3 \pm 6.1
	Non-Catamenial Epilepsy		54.1 \pm 5.7	53.4 \pm 6.3
	Seizure Free		56.1 \pm 4.9	55.1 \pm 6.2
Focal Epilepsy				
Ovulatory	Catamenial Epilepsy	C1	58.4 \pm 4.2	59.0 \pm 3.2
		C2	57.1 \pm 3.4	57.6 \pm 6.2

		C3	55.3±6.9	55.9±6.5
	Non-Catamenial Epilepsy		57.3±6.5	56.8±6.9
	Seizure Free		59.2±6.3	59.9±6.7
Anovulatory	Catamenial Epilepsy	C1	54.9±8.4	55.2±3.8
		C2	56.3±5.4	56.1±5.4
		C3	56.8±5.6	55.6±6.3
	Non-Catamenial Epilepsy		55.9±6.2	55.2±6.5
	Seizure Free		57.1±5.1	57.9±6.2

Figure 1: Timing of TMS studies in relation to the menstrual cycle.

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Figure 2: Short and long ISI recovery curves for the dominant hemisphere in non-epilepsy controls at each of the studied phases of the menstrual cycle. Ratios less than 100% indicate inhibition and ratios greater than 100% indicate facilitation. The bars represent standard error.

Figure 3: Short and long ISI recovery curves for the dominant hemisphere in each group with generalized epilepsy at each of the studied phases of the menstrual cycle.. C1: peri-menstrual pattern of seizure exacerbation, C2: peri-ovulatory pattern of seizure exacerbation, C3: luteal pattern of seizure exacerbation. Ratios less than 100% indicate inhibition and ratios greater than 100% indicate facilitation. The bars represent standard error. The upper boundary of the grey shaded area represents the mean of non-epilepsy controls during each phase.

Figure 4: Short and long ISI recovery curves for the ipsilateral hemisphere in each group with focal epilepsy at each of the studied phases of the menstrual cycle. C1: peri-menstrual pattern of seizure exacerbation, C2: peri-ovulatory pattern of seizure exacerbation, C3: luteal pattern of seizure exacerbation. Ratios less than 100% indicate inhibition and ratios greater than 100% indicate facilitation. The bars represent standard error. The upper boundary of the grey shaded area represents the mean of non-epilepsy controls during each phase.

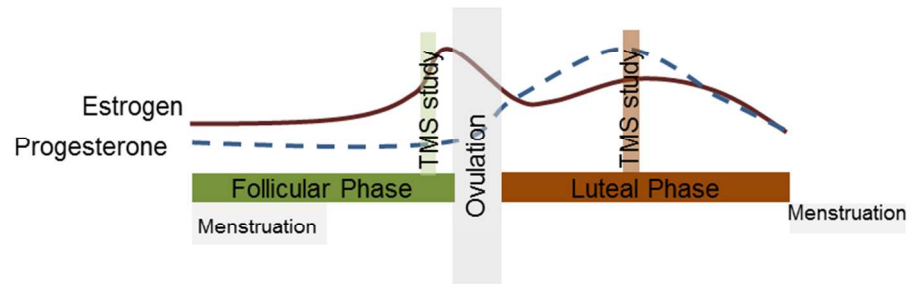


Figure 1: Timing of TMS studies in relation to the menstrual cycle.
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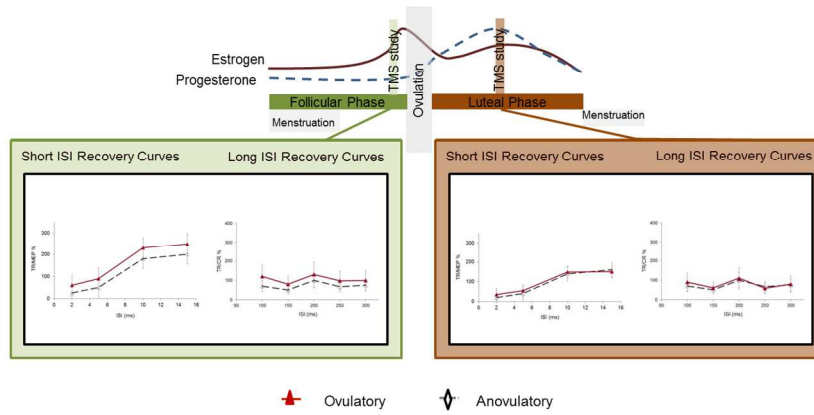


Figure 2: Short and long ISI recovery curves for the dominant hemisphere in non-epilepsy controls at each of the studied phases of the menstrual cycle. Ratios less than 100% indicate inhibition and ratios greater than 100% indicate facilitation. The bars represent standard error.
604x264mm (72 x 72 DPI)

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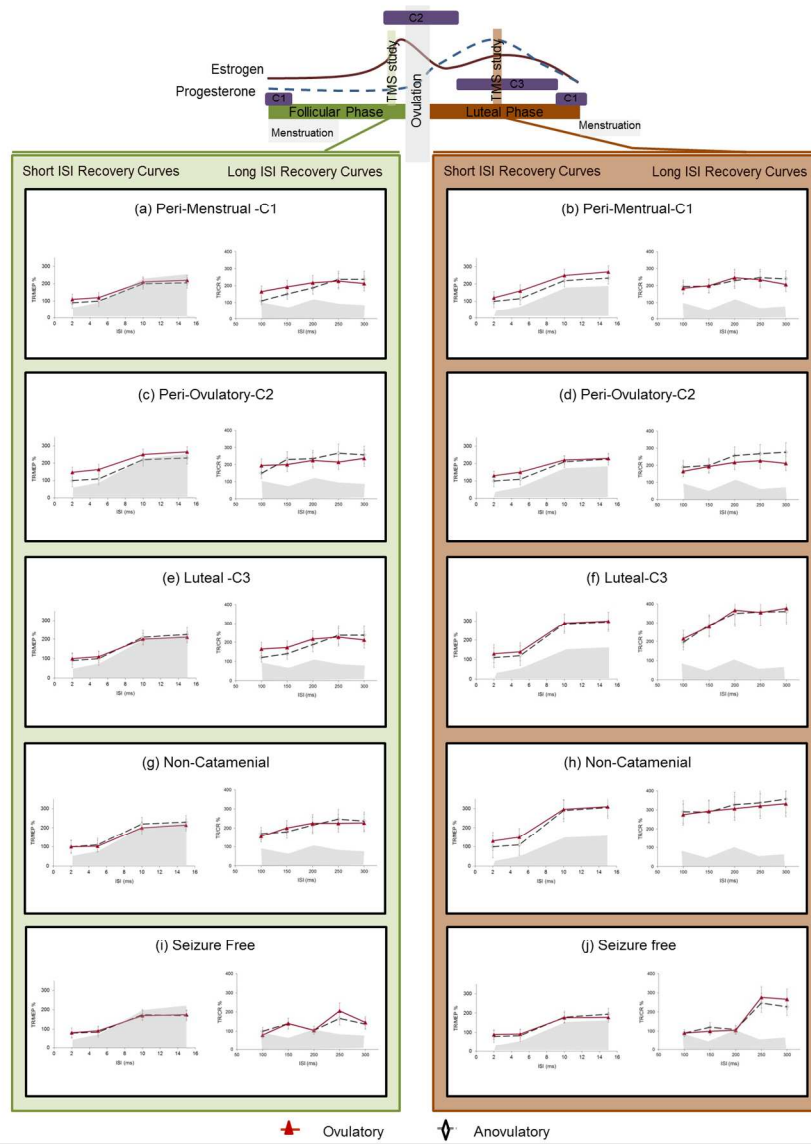


Figure 3: Short and long ISI recovery curves for the dominant hemisphere in each group with generalized epilepsy at at each of the studied phases of the menstrual cycle.. C1: peri-menstrual pattern of seizure exacerbation, C2: peri-ovulatory pattern of seizure exacerbation, C3: luteal pattern of seizure exacerbation.

Ratios less than 100% indicate inhibition and ratios greater than 100% indicate facilitation. The bars represent standard error. The upper boundary of the grey shaded area represents the mean of non-epilepsy controls during each phase.

608x702mm (72 x 72 DPI)

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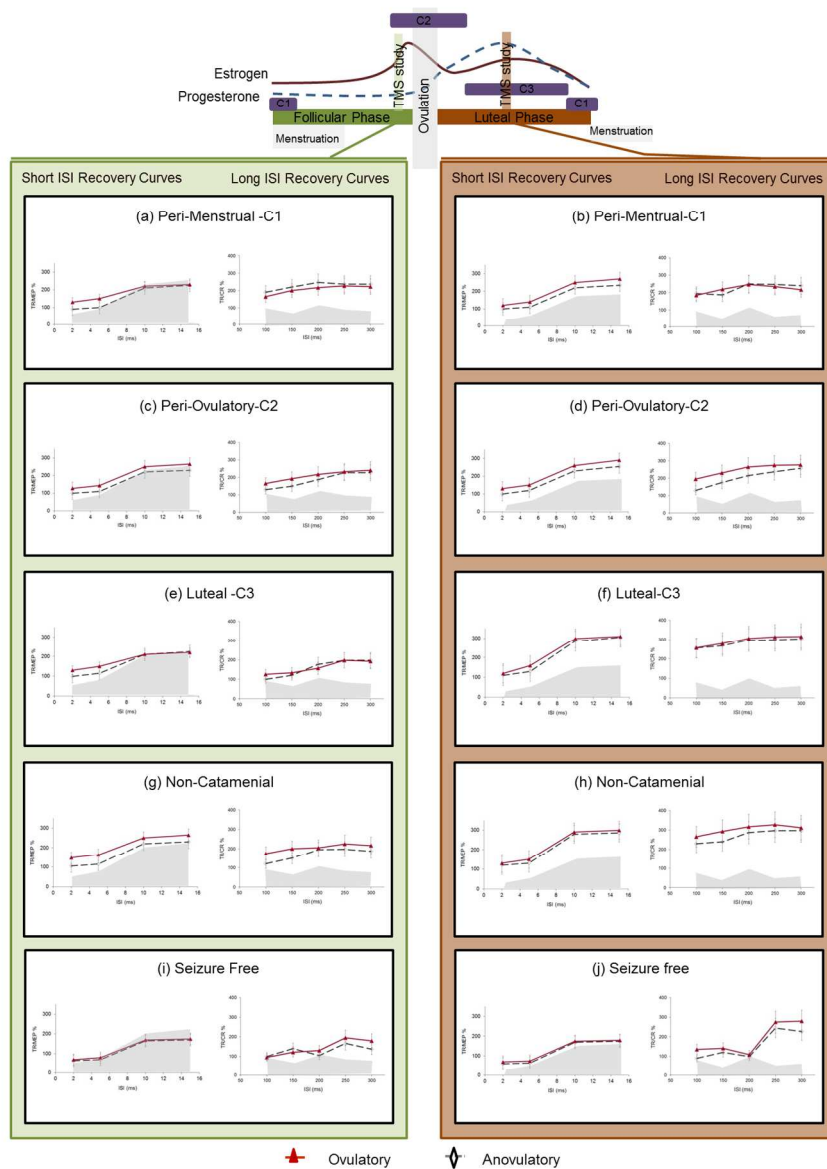


Figure 4: Short and long ISI recovery curves for the ipsilateral hemisphere in each group with focal epilepsy at each of the studied phases of the menstrual cycle. C1: peri-menstrual pattern of seizure exacerbation, C2: peri-ovulatory pattern of seizure exacerbation, C3: luteal pattern of seizure exacerbation. Ratios less than 100% indicate inhibition and ratios greater than 100% indicate facilitation. The bars represent standard error. The upper boundary of the grey shaded area represents the mean of non-epilepsy controls during each phase.

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