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Ictal activity is sustained by the estrogen receptor β during the estrous cycle

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ABSTRACT

Catamenial epilepsy, defined as a periodicity of seizure exacerbation during the menstrual cycle, affects up to 70 % of epileptic women. Seizures in these patients are often non-responsive to medication; however, our understanding of the relation between menstrual cycle and seizure generation (i.e. ictogenesis) remains limited. We employed here field potential recordings in the *in vitro* 4-aminopyridine model of epileptiform synchronization in female mice (P60–P130) and found that: (i) the estrous phase favors ictal activity in the entorhinal cortex; (ii) these ictal discharges display an onset pattern characterised by the presence of chirps that are thought to mirror synchronous interneuron firing; and (iii) blocking estrogen receptor β-mediated signaling reduces ictal discharge duration. Our findings indicate that the duration of 4AP-induced ictal discharges, *in vitro*, increases during the estrous phase, which corresponds to the human peri-ovulatory period. We propose that these effects are caused by the presumptive enhancement of interneuron excitability due to increased estrogen receptor β-mediated signaling.

1. Introduction

The menstrual cycle is associated with changes in estrogen and progesterone blood concentrations, which have profound physiological effects on brain excitability and thus on behavior (McEwen et al., 1995). These hormonal changes also play a role in changes in cognitive function (Sabaliauskas et al., 2015) and neurological disorders such as epilepsy. Accordingly, women presenting with focal epilepsy can report increased seizure occurrence/severity during their menstrual cycle. This condition is termed catamenial epilepsy (Christian et al., 2020; Duncan et al., 1993; Reddy, 2017), affects up to 70 % of epileptic women (Laidlaw, 1956; Rościszewska et al., 1986; Taubøll et al., 1991) and it is observed in all types of focal epileptic disorders but it is more prevalent in temporal lobe epilepsy (Duncan et al., 1993; Herzog and Frye, 2003; Quigg et al., 2009). Catamenial seizures are often unresponsive to medication (Verrotti et al., 2012) and our understanding of the fundamental mechanisms causing ovarian cycle-dependent changes in ictogenesis (and thus the identification of effective treatments) remains unclear.

The human menstrual cycle lasts approximately 28 days and is divided into two phases: (i) the follicular or proliferative phase and (ii) the luteal or secretory phase (Buffet et al., 1998). In rodents, the estrous cycle has a shorter duration than in women (4–5 days) and is divided into four phases identified as proestrus, estrus, metestrus and diestrus (Caligioni, 2009). A relationship between seizure occurrence and estrous cycle has been found in the kainic acid model of temporal lobe epilepsy, in which the seizure burden increases during proestrus and estrus (Li et al., 2020).

Recently, Clemens et al. (2019) have reported that parvalbumin (PV)-positive inhibitory interneurons in rodents become hyperexcitable during estrus. Such increase in PV-positive interneuron excitability is mediated by the estrogen receptor β (ERβ) (Clemens et al., 2019), and it may favor ictogenesis since increasing evidence obtained from epileptic patients (Elahian et al., 2018) and from *in vivo* and *in vitro* animal models (Fujita et al., 2014; Grasse et al., 2013; Lévesque et al., 2016; Scalmani et al., 2023; Toyoda et al., 2015) indicate that interneuron firing rises shortly before the onset of focal seizures that are often characterized by low-voltage fast (LVF) onset (Avoli et al., 2023). Moreover, optogenetic activation of PV- or somatostatin-positive interneurons triggers ictal-like discharges in brain slices maintained *in vitro* (Chang et al., 2018; Shiri et al., 2015a, 2016a; Wang et al., 2022; Yekhlef et al., 2015a) and in

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pilocarpine-treated epileptic mice *in vivo* (Lévesque et al., 2019, 2022). The paradoxical role of interneuron firing in promoting ictogenesis rests on the consequent release of GABA that, binding to post-synaptic $GABA_A$ receptors, overloads neurons with Cl^- thereby activating the cotransporter KCC2, which in turn causes large increases in extracellular $[K^+]$ (Avoli et al., 2023; Weiss, 2023). It is well established that increased extracellular $[K^+]$ causes seizure activity in *in vivo* (Zuckermann and Glaser, 1968) and in *in vitro* (Jensen and Yaari, 1997; Traynelis and Dingledine, 1988) preparations.

To date, few *in vitro* studies have investigated the neuronal mechanisms that underlie the generation of seizures in catamenial epilepsy. Therefore, we employed here the *in vitro* 4-aminopyridine (4AP) model of epileptiform synchronization (Avoli et al., 2023) and found that the estrous phase, which corresponds to the human peri-ovulatory period, favors the maintenance of ictal discharges in the entorhinal cortex (EC), and that blocking estrogen receptor β-mediated activity significantly shortens ictal discharge duration. Moreover, we report that ictal discharges occurring during the estrous phase are often associated to the presence of chirps (Schiff et al., 2000), which mirror high levels of interneuron synchronous firing (Gnatkovsky et al., 2019). Overall, our findings indicate, for the first time, that GABAergic interneuron hyperexcitability, mediated by estrogen receptor β, may play a significant role in ictogenesis during the estrous cycle.

2. Materials and methods

Animals - All performed experimental procedures were designed according to the guidelines of the Canadian Council on Animal Care and were approved by the McGill University Animal Care Committee. PV-ChR2 females were obtained from crossbreeding PV-Cre [B6; 129P2-pvalbtm1(cre)Arbr/J, The Jackson Laboratory; RRID: IMSR_JAX:008069] with Ai32 mice [R26-lox-stop-lox-ChR2(H134R)-EYFP, The Jackson Laboratory; RRID IMSR_JAX:012569]. All lines were maintained in-house. The study is reported in accordance with ARRIVE guidelines.

Identification of the estrous phase - The estrous phase of female mice (60- to 130-day old) was identified around 10 a.m., 1 h before they were decapitated. Animals in which we could not clearly identify the phase of the estrous cycle were excluded from further experiments. Samples of vaginal smear were collected by placing a saline-filled dropper tip at the opening of the vaginal canal. The bulb of the dropper was gently pressed to expel ~25–50 μl of saline at the opening of the vaginal canal. Then, the bulb of the dropper was slowly released, which withdrew the fluid back into the tip. Stages of the estrous cycle were determined under 100X magnification by observing the presence of leukocytes, cornified epithelial cells, and nucleated epithelial cells in the vaginal smear under microscope (Quignon, 2023). The proestrous phase was characterised by the presence of nucleated epithelial cells (Fig. 1A, blue arrows). The estrous phase was characterised by the presence of cornified epithelial cells (Fig. 1A, red arrows). The metestrous phase was characterised by the presence of cornified epithelial cells (Fig. 1A, red arrows) and leucocytes (Fig. 1A, black arrow). The diestrous phase was characterised by the presence of leucocytes (Fig. 1A, black arrow).

The vaginal proestrous and estrous phases were combined into "estrus", since the sexual receptivity of mice (as well as ovulation) continues from the proestrous phase into the estrous phase (Clemens et al., 2019; Young et al., 1941). Moreover, as reported by (Li et al., 2020), the combination of proestrus and estrus is representative of the late follicular and very early luteal period of the human menstrual cycle, a time of increased seizure burden in women with periovulatory catamenial epilepsy (Li et al., 2020). The vaginal metestrus and diestrus were therefore considered as "non-estrus". Slices collected from mice

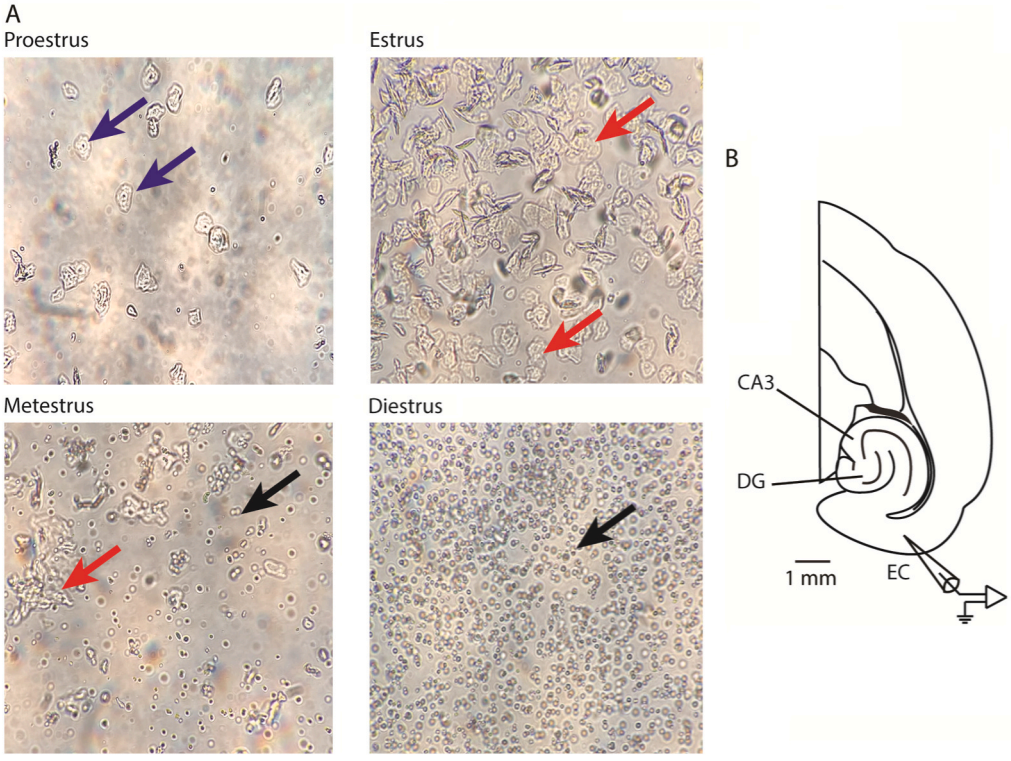

Fig. 1. - Estrous cycle identification and schematic diagram of experimental setup. A: Microscopic images showing the cells compositions of vaginal smears from four estrous phases. The proestrous phase was characterised by the presence of nucleated epithelial cells (blue arrows). The estrous phase was characterised by the presence of cornified epithelial cells (red arrows). The metestrous phase was characterised by the presence of cornified epithelial cells (red arrows) and leucocytes (black arrow). The diestrous phase was characterised by the presence of leucocytes (black arrow). **B**: Schematic diagram showing the location of the recording electrode in the EC of a horizontal brain slice. EC, entorhinal cortex. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

during pro-estrus or estrus were identified as estrous slices, whereas slices collected from mice during metestrus and diestrus were identified as non-estrous slices.

Slice preparation and maintenance - Mice were deeply anesthetized with isoflurane and transcardially perfused with ~25 ml choline cutting solution containing 132.5 mM choline chloride, 2.5 mM of KCl, 0.7 mM of $CaCl_2$, 3 mM of $MgCO_2$, 1.2 mM of NaH_2PO_4, 25 mM of $NaHCO_3$, and 8 mM of glucose. Mice were then decapitated, and the brain was quickly removed and transferred into ice-cold, oxygenated (95% O_2 and 5% CO_2) choline cutting solution. The brain was separated into two hemispheres and then sliced horizontally with a vibratome (VT1000S; Leica, Wetzlar, Germany); 400-μm thick slices were transferred to a "storage" dish where they were kept under room temperature in artificial cerebrospinal fluid (ACSF) containing 124 mM of NaCl, 2 mM of KCl, 26 mM of $NaHCO_3$, 2 mM of $CaCl_2$, 2 mM $MgSO_4$, 1.25 mM of KH_2PO_4, and 10 mM of D-glucose (pH 7.4, 305 mosmol/kgH_2O) and humidified gas (O_2/CO_2, 95%/5%). For the first set of experiments, only ACSF was used to superfuse brain slices. For the second set of experiments, investigating the role of estrogen receptor β (ERβ), 17 β-estradiol was added into ACSF. Following a recovery period of ~1 h, slices were transferred to an interface chamber where epileptiform activity was induced by continuous bath application of 150 μM 4-aminopyridine (Sigma-Aldrich, Oakville, ON, Canada) at a flow rate of 1–1.5 ml/min.

Electrophysiological recordings - Fig. 1B shows a schematic diagram of a horizontal slice with the location of the recording electrode. Field recordings from the EC were continuously obtained during 4AP application for periods of up to 120 min with glass pipettes (1B150F-4; World Precision Instruments, Sarasota, FL; tip diameter <10 μM, resistance 5–10 MΩ) filled with ACSF. Signals were sampled at 5000 Hz, amplified with a high impedance amplifier and digitized (Digidata 1322 A, Molecular Devices, Palo Alto, CA). PCLAMP software (Molecular Device) was used to visualize and to record the signals on a computer.

Pharmacology – To activate estrogen receptor β, 17 β -estradiol powder (Sigma Aldrich, Qc, Canada) was dissolved in DMSO and added to the ACSF superfusing solution. The final concentration of 17 β-estradiol in ACSF was 100–132 nM and the final DMSO concentration was <0.001%. Prior to recording, slices were incubated in oxygenated 17 β-estradiol containing ACSF for 1 h. Then, slices were transferred to the recording chamber. To block estrogen receptor β, PHTPP powder (Tocris, UK) was dissolved in DMSO and added to the ACSF circulating solution. The final concentration of PHTPP in ACSF was 3 μM and the final DMSO concentration was <0.001%.

Analysis of interictal and ictal discharges - Custom MATLAB (R2022b, MathWorks, MA) scripts were written to analyze data sets. Signals were first filtered with a Gaussian low-pass filter at 40 Hz and down sampled to 1000 Hz. Visual inspection of the signal was first performed to remove artifacts and identify ictal discharges, which were defined as events that were longer than 5 s. Peaks equal to or higher than 4SD in amplitude above the mean of the average signal and lasting less than 5 s were considered as interictal spikes. The deflection from baseline was considered as the onset of the interictal discharge whereas the return to baseline was considered as the end. Onset and end of each interictal discharge were automatically determined by considering the slope and area under the curve of each peak relative to the baseline signal neighboring to the peak.

Analysis of chirps at ictal discharge onset - Chirps were identified by three trained reviewers. The power spectrum analysis was first calculated for every ictal discharge. An ictal discharge was considered as having a chirp when more than two reviewers agreed on its presence in the power spectrum, which was characterised by a monotonic decrease in spectral power (Schiff et al., 2000). Otherwise, the ictal discharge would be counted as having no chirp. Reviewers were blind to the estrous or non-estrous group.

Statistical analysis - Since data were not normally distributed, nonparametric Kruskal–Wallis, Wilcoxon and Chi-square tests were used. Outliers were excluded from statistical comparisons. The level of significance was set at $p < 0.05$.

3. Results

Ictal discharge duration is increased during the estrous phase - Field recordings obtained from the EC under 4AP application revealed the presence of interictal spikes (Fig. 2A, asterisks) and ictal discharges (Fig. 2A, solid lines) during both the non-estrous (n = 37 slices from 8 animals) and estrous phase (n = 43 slices from 9 animals). Fig. 2A (a and b insets) shows representative examples of interictal spikes occurring in a non-estrous and in an estrous slice on a short time scale. The duration (Fig. 2B) and rates (Fig. 2C) of interictal spikes were not significantly different between the non-estrous and estrous phase.

Ictal discharges were observed in a higher proportion of estrous slices compared to non-estrous slices, although this difference did not reach significance ($p = 0.35$) (Fig. 2D). Analysis of the duration, however, revealed that ictal discharges lasted significantly longer in the estrous compared to those occurring during the non-estrous phase ($p < 0.001$) (Fig. 2E). Rates of ictal discharges in non-estrous and estrous phases were similar ($p = 0.48$) (Fig. 2F). These findings indicate that the estrous phase favors the maintenance of 4AP-induced ictal discharges in the EC *in vitro* but does not modulate their rate of occurrence.

The estrous phase may favor interneuron excitability - Ictal discharges that are characterised by an LVF onset pattern are thought to mirror the preponderant involvement of inhibitory cells that lead to synchronous GABAergic-mediated events (Avoli et al., 2016, 2023). In line with this view, analysis of spectral power during LVF seizures revealed the presence of a monotonically decreasing spectral power band called chirp (Schiff et al., 2000), which is believed to mirror synchronous interneuron activity (Gnatkovsky et al., 2019). Therefore, we analysed the presence of chirps at the onset of ictal discharges occurring in non-estrous and estrous slices.

Fig. 3 shows representative ictal discharges with their corresponding spectrogram. In both non-estrous and estrous slices, ictal discharges could be associated with no chirp (Fig. 3A) or with the presence of a chirp (Fig. 3B, white arrow). When comparing the proportion of ictal discharges with chirps, we found that a significantly higher proportion of ictal discharges recorded from estrous slices (n = 5 animals, 15 slices, and 69 ictal discharges) were characterised by the presence of chirps compared to ictal discharges recorded from non-estrous slices (n = 5 animals, 11 slices, and 124 ictal discharges) ($p < 0.05$) (Fig. 3C). This is further illustrated in Fig. 3D, in which the frequency (in Hz) with maximum power at seizure onset is plotted over time. Ictal discharges during the estrous phase were mostly associated at onset with the presence of a monotonically decreasing spectral power band (or chirp).

Blocking estrogen receptor β modifies the duration of ictal discharges – Having observed that 4AP-induced ictal discharges recorded from estrous slices have longer duration compared to those recorded from non-estrous slices (Fig. 2E), we tested whether applying the estrogen receptor β blocker PHTPP (3 μM dissolved in $3x10^{-4}$% DMSO) to estrous slices would decrease ictal duration. Ictal discharges were observed after the application of 4AP to estrous slices (Fig. 4Aa) but contrary to what expected, ictal discharge duration significantly increased under PHTPP ($p < 0.05$) (n = 4 animals, 15 slices, 127 ictal events under 4AP, and 130 events under 4AP + PHTPP) (Fig. 4B).

Since PHTPP was dissolved in DMSO (final concentration $3x10^{-4}$%), we speculated that this effect could be caused by DMSO. Therefore, we performed further experiments in which only DMSO was used, thus recording field activity under 4AP only and under 4AP + DMSO in estrous slices (Fig. 4Ab). Again, longer ictal discharges were observed after the application of 4AP + DMSO compared to 4AP only ($p < 0.05$) (n = 4 animals, 15 slices, 127 ictal events under 4AP, and 15 events under 4AP + DMSO) (Fig. 4B). These findings indicate that the increased duration of ictal discharges under PHTPP may indeed be caused by DMSO application. No significant difference in rates of occurrence were observed in estrous slices under 4AP only, 4AP + PHTPP, or 4AP +

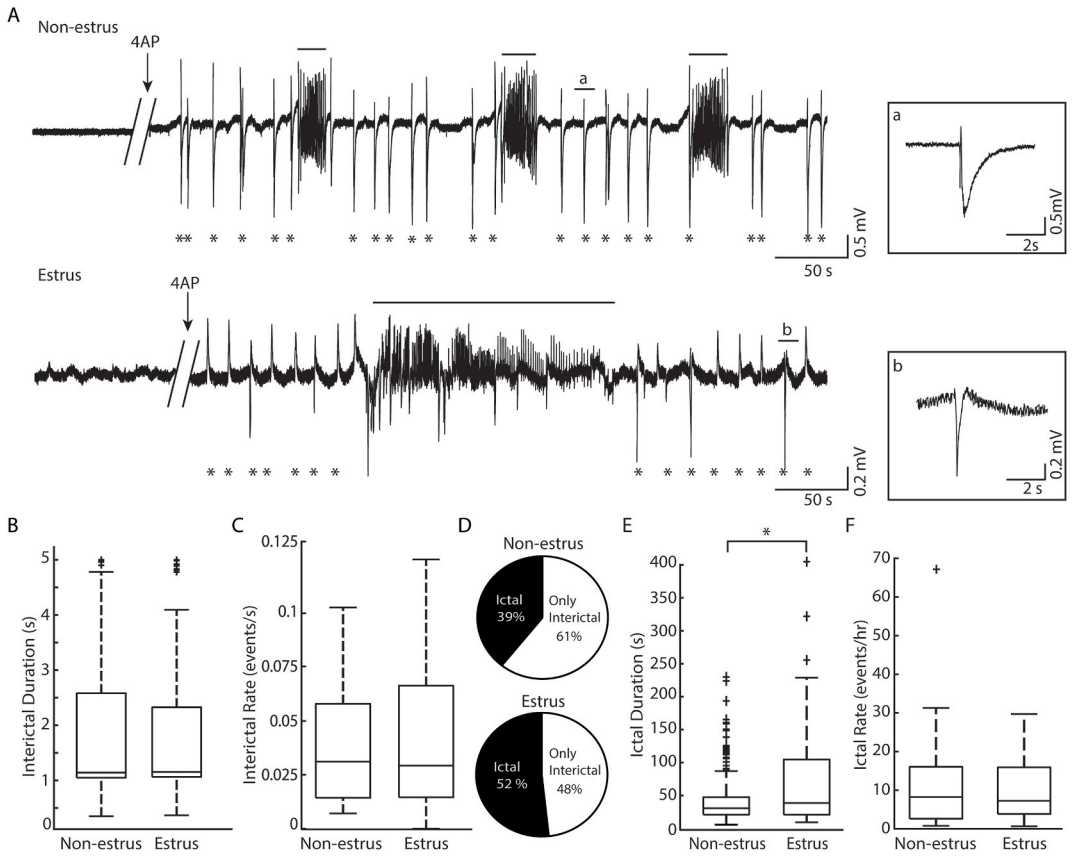

Fig. 2. – Duration and rates of interictal spikes and ictal discharges. A: Field recordings obtained from the EC under 4AP application. Interictal spikes and ictal discharges are marked with asterisks or a solid line, respectively. Interictal spikes on a shorter time scale are shown in panels **a** and **b**. **B–C**: Duration and rates of interictal spikes. No significant differences were found between groups. **D**: Proportion of slices having both interictal spikes and ictal discharges (black) or having interictal spikes only (white). A higher proportion of estrous slices showed ictal discharges compared to non-estrous slices, but this difference did not reach statistical significance. **E-F**: Duration and rates of ictal discharges. No significant difference in rates were found between groups, but ictal discharges lasted longer in the estrous phases compared to the non-estrous phases ($p < 0.001$). In all box plots, the central mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points not considered outliers. Outliers are indicated as "+".

DMSO (n = 4 animals, 15 slices, 127 ictal events under 4AP, 130 events under 4AP + PHTPP, and 15 events under 4AP + DMSO) (Fig. 4C). No significant differences in proportion of ictal discharges with chirp at ictal onset recorded from estrous slices were identified under 4AP only, 4AP + PHTPP, or 4AP + DMSO (n = 4 animals, 15 slices, 127 ictal events under 4AP, 130 events under 4AP + PHTPP, and 15 events under 4AP + DMSO) (Fig. 4D).

Next, we tested the effects of PHTPP in the presence of 17 β-estradiol. We superfused slices with ACSF containing 17 β-estradiol for at least 1 h (100–132 nM dissolved in $1.5x10^{-6}$ % DMSO) followed by the application of the estrogen receptor β blocker PHTPP (3 μM dissolved in $3x10^{-4}$ % DMSO). 4AP-induced ictal discharges were observed under 17 β-estradiol in non-estrous (Fig. 5A) and estrous slices (Fig. 5D). Application of PHTPP in ACSF containing 17 β-estradiol induced a significant decrease in the duration of ictal discharges in both non-estrous ($p < 0.001$) (n = 5 animals, 8 slices, 116 ictal events under 17 β-estradiol + 4AP only, and 127 ictal events under 17 β-estradiol+ 4AP + PHTPP) (Fig. 5B) and estrous slices ($p < 0.001$) (n = 6 animals, 10 slices, 110 ictal events under 17 β-estradiol + 4AP only and 134 ictal events under 17 β-estradiol + 4AP + PHTPP) (Fig. 5E). Rates of ictal discharges were however not different after the application of PHTPP in slices obtained from the non-estrous (Fig. 5C) or estrous phase (Fig. 5F).

4. Discussion

The main findings of our study can be summarised as follows. First, ictal discharges induced by bath application of 4AP were significantly longer in the estrous compared to the non-estrous phase. Second, a high proportion of ictal discharges during the estrous phase were characterised at onset by the presence of chirps, which are believed to mirror synchronous interneuron activity (Gnatkovsky et al., 2019; Lévesque et al., 2016). Finally, the duration of ictal discharges in slices pretreated with 17 β-estradiol was significantly decreased by the application of the estrogen receptor β antagonist PHTPP.

The first two findings suggest that during the estrous cycle, high blood levels of estrogen during the estrous phase induces changes in interneuron excitability that favor the generation of epileptiform discharges. This effect persisted for hours in brain slices that were obtained after euthanasia. Accordingly, we have observed that estrous slices maintained *in vitro*, and no longer exposed to circulating systemic estrogen, show ictal discharges that are significantly longer in duration compared to those occurring during the non-estrous phases. Moreover, a high proportion of ictal discharges during the estrous phase were characterised at onset by the presence of chirps, which presumably mirror synchronous interneuron firing (Gnatkovsky et al., 2019; Lévesque et al., 2016). Chirps have been observed during seizures in epileptic patients (Gnatkovsky et al., 2019; Schiff et al., 2000) and were reported to occur *in vitro* during LVF ictal discharges induced with 4AP (Lévesque et al., 2016). In line with this view, optogenetic activation of PV- or SOM-positive interneurons in the *in vitro* 4AP model triggers ictal discharges with an LVF pattern (Shiri et al., 2015, 2016; Yekhlef et al., 2015). Single-unit recordings have also revealed that interneurons fire at high rates and show strong phase-locking relationships with field oscillations during chirps (Lévesque et al., 2016). These findings therefore

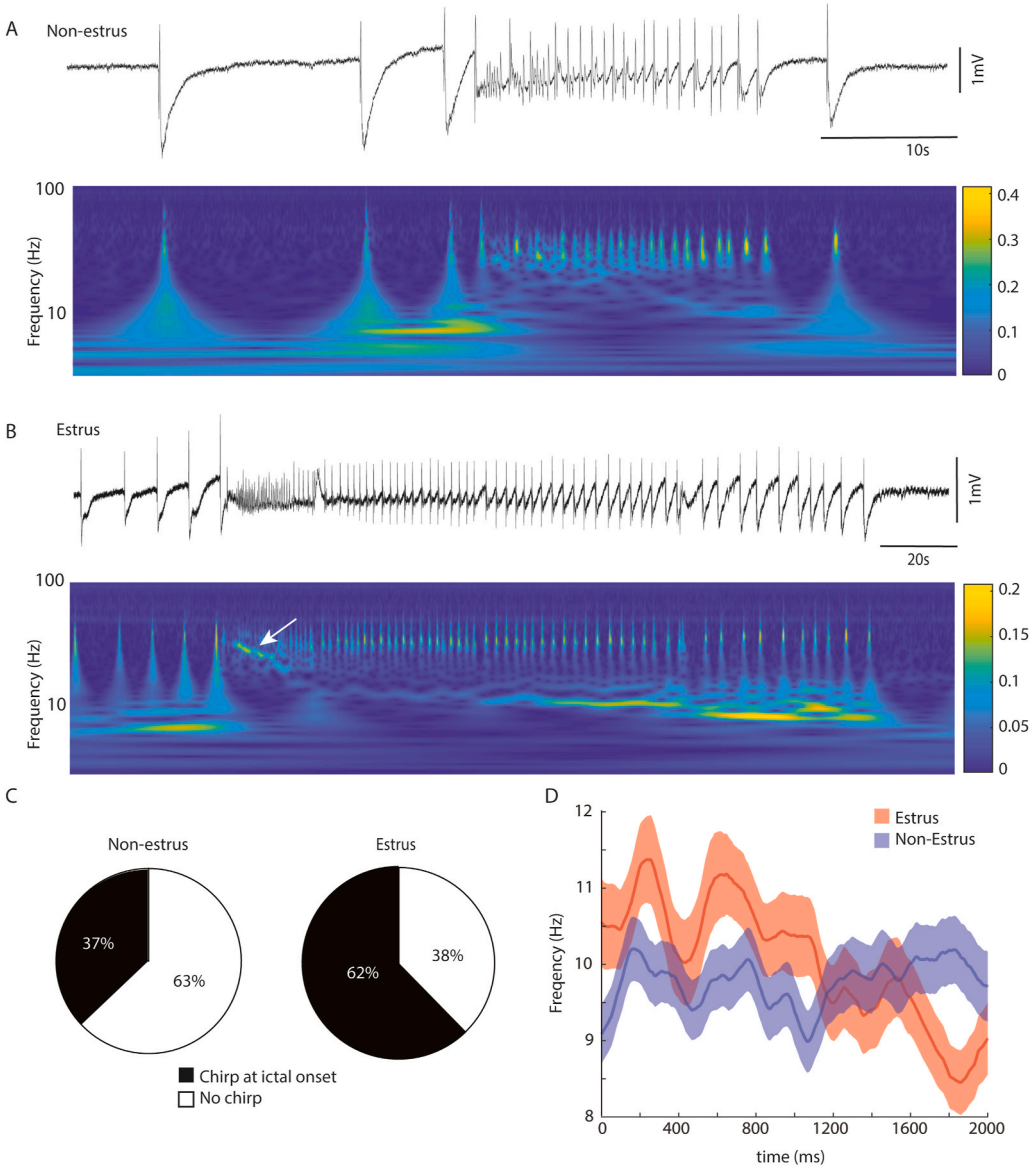

Fig. 3. - Chirps at the onset of ictal discharges in both non-estrous and estrous slices. A: Ictal discharge recorded from a non-estrous slice without a chirp and its corresponding spectrogram. **B**: Ictal discharge recorded from an estrous slice with a chirp at onset (white arrow) and its corresponding spectrogram. **C**: Proportion of ictal discharges with chirps (black) or not showing chirps (white) at onset. Note that a higher proportion of ictal discharges recorded from estrous slices showed chirps at ictal onset ($p < 0.05$). **D**: Average frequency of field activity with the highest power over time in estrous (red) ($n = 15$ slices) and non-estrous (blue) slices ($n = 11$ slices). The solid lines indicate the mean and the shaded areas indicate standard deviation. Note that the estrous phase (red) trace displays a downward trend, while the non-estrous (blue) trace is flat over time, indicating the presence of a chirp at ictal onset in estrous slices. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

indicate that the high blood levels of estrogen that occur *in vivo* during estrus induce persistent changes in interneuron excitability that outlast the slicing procedure and favor the generation of epileptiform discharges that are associated to interneuron firing and thus to the occurrence of chirps. It is also known that estrogen modulates dendritic spine formation and neurogenesis in female rodents (Sheppard et al., 2019).

The role of estrogen receptor β-mediated activity in the maintenance of ictal discharges was further established by pre-treating slices with 17 β-estradiol and then applying the estrogen receptor β antagonist PHTPP. In these experiments, we have observed that ictal discharge duration was significantly reduced in both non-estrous and estrous slices under PHTPP. This finding is in line with previous evidence suggesting that the estrous phase could favor ictal maintenance by increasing interneuron (presumably PV-positive) excitability through estrogen receptor β-mediated activity (Clemens et al., 2019).

We also found that 4AP-induced ictal discharges recorded from both estrous and non-estrous slices were of similar duration under 17 β-estradiol and that these ictal discharges were shorter in duration than the ictal discharges recorded in estrous slices with no circulating 17 β-estradiol. These results suggest that application of 17 β-estradiol may acutely diminish inhibitory synaptic transmission (Huang and Woolley, 2012); it is indeed well established that $GABA_A$ signaling contributes to ictogenesis, and even more so in the *in vitro* 4AP model of synchronization (Avoli et al., 2016, 2023).

To note that we found an increase in ictal discharge duration in estrous slices under 4AP + DMSO treatment. To date, no study has reported that DMSO increases the duration of epileptiform activity *in vitro,* but some *in vivo* evidence suggest that it could alter network excitability and favor seizure generation. For instance, it was shown that DMSO can enhance the proconvulsant properties of drugs dissolved in this medium

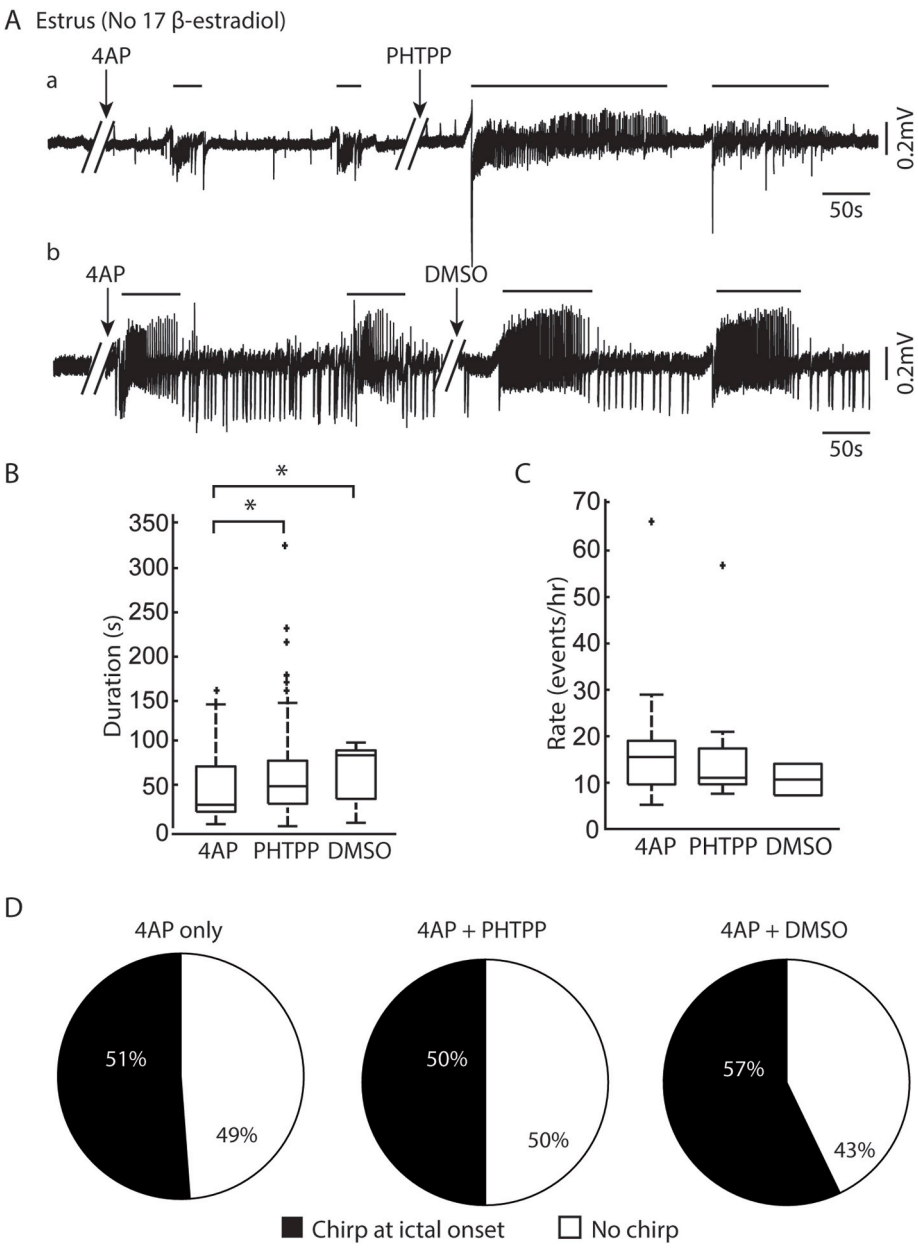

Fig. 4. - Ictal discharges before and after PHTPP application. A: Field potential recordings obtained from an estrous slice (no 17 β-estradiol in ACSF) under 4AP + PHTPP (**a**) or 4AP + DMSO (**b**). **B–C**: Box plot showing the duration and rate of ictal discharges. Ictal discharge duration (**B**) was significantly increased after either PHTPP or DMSO application ($p < 0.001$), while rates of ictal discharges (**C**) were not significantly different. **D:** Proportion of ictal discharges recorded from slices during estrus under 4AP treatment only, 4AP + PHTPP, and 4AP + DMSO showing chirps at onset (black) or not showing chirps (white). No significant differences were found between the three groups.

by lowering seizure threshold (Bauwens et al., 2005; Kovács et al., 2011; Wong et al., 1988). The mechanisms through which DMSO exerts these proconvulsant effects are currently unclear but some findings suggest that it could shift the excitation/inhibition balance by acting on Na^+, Ka^+ and Ca^{2+} channels as well as on AMPA and NDMA channels (Kovács et al., 2011). As a result, in our study, the effects of 17 β-estradiol and PHTPP on ictal discharge duration could be mingled with those induced by DMSO.

Overall, our findings suggest that on going changes in estrogen during the estrous cycle alter network excitability in many ways. First, systemic circulating estrogen would increase interneuron excitability, which would prolong the duration of ictal discharges and induce the presence of chirps at the onset of ictal discharges. Several studies have indeed reported that interneuron firing rises shortly before the start of focal seizures in epileptic patients (Elahian et al., 2018) and in animal models of focal epilepsy (Fujita et al., 2014; Grasse et al., 2013; Karunakaran et al., 2016; Lévesque et al., 2016; Scalmani et al., 2023; Toyoda et al., 2015), and that these seizure-like events are often characterized by a LVF onset pattern (see for review Avoli et al., 2016, 2023; de Curtis and Avoli, 2016; Weiss et al., 2019). Second, blocking estrogen receptor β-mediated activity with PHTPP would reduce ictal discharge duration in both estrous and non-estrous slices; this finding is similar to what reported by Clemens et al. (2019) who identified decreased interneuron excitability in ovariectomized mice due to PHTPP application. Last, 17 β-estradiol application may acutely decrease inhibitory synaptic transmission (Huang and Woolley, 2012) and shorten ictal discharges.

Three patterns of catamenial epilepsy have been identified: (i) perimenstrual and (ii) periovulatory in normal cycles, as well as (iii) luteal in inadequate luteal phase cycles (Herzog, 2015). Our findings indicate

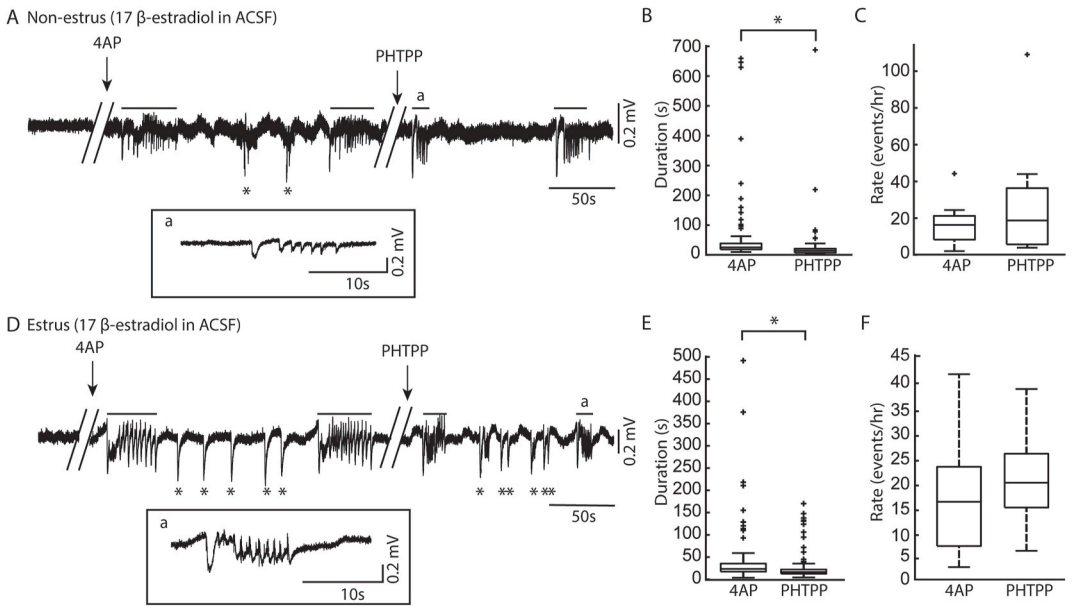

Fig. 5. - Ictal discharges before and after PHTPP application. A: Field potential recordings obtained from a non-estrous slice under 4AP, 17 β-estradiol and PHTPP. Solid lines indicate ictal discharges. Asterisks indicate interictal spikes. One of the ictal discharges after PHTPP application is enlarged and shown in **a**. **B:** Box plot showing the duration of ictal discharges recorded from slices during the non-estrous phase. The application of PHTPP significantly reduced ictal discharge duration ($p < 0.001$). **C**: Box plot showing the rate of ictal discharges recorded from slices during the non-estrous phase. Rates of ictal discharges were not significantly different before and after the application of PHTPP. **D:** Field potential recordings obtained from an estrous slice under 4AP, 17 β-estradiol and PHTPP. One of the ictal discharges after PHTPP application is enlarged and shown in **a**. **E**: Box plot showing the duration of ictal discharges recorded from slices during the estrous phase. The application of PHTPP also induced a significant decrease of ictal discharges ($p < 0.001$). **F**: Box plot showing the rate of ictal discharges recorded from slices during the estrous phase. Rates of ictal discharges were not significantly different before and after the application of PHTPP. To note that these slices were pretreated with 17 β-estradiol.

that the rodent estrous phase, which corresponds to the human peri-ovulatory period, favors the maintenance of ictal activity in the EC in an *in vitro* brain slice preparation, and that increased excitability of GABAergic interneurons may play a significant role. Such pro-ictogenic mechanisms could characterise the periovulatory pattern in catamenial epilepsy, in which blood levels of estrogen increase during the late proliferative phase when seizures become more frequent (Herzog, 2015).

CRediT authorship contribution statement

Fei Ran Li: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Maxime Lévesque:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Siyan Wang:** Software, Formal analysis, Writing – review & editing. **Maria-Isabel Carreño-Muñoz:** Methodology, Writing – review & editing. **Graziella Di Cristo:** Writing – review & editing. **Massimo Avoli:** Conceptualization, Methodology, Validation, Resources, Supervision, Project administration, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crneur.2024.100131.

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