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Sex differences in the developing brain impact stress-induced epileptogenicity following hyperthermia-induced seizures

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ABSTRACT

Febrile seizures (FS) are common, affecting 2–5% of children between the ages of 3 months and 6 years. Complex FS occur in 10% of patients with FS and are strongly associated with mesial temporal lobe epilepsy. Current research suggests that predisposing factors, such as genetic and anatomic abnormalities, may be necessary for complex FS to translate to mesial temporal lobe epilepsy. Sex hormones are known to influence seizure susceptibility and epileptogenesis, but whether sex-specific effects of early life stress play a role in epileptogenesis is unclear. Here, we investigate sex differences in the activity of the hypothalamic–pituitary–adrenal (HPA) axis following chronic stress and the underlying contributions of gonadal hormones to the susceptibility of hyperthermia-induced seizures (HS) in rat pups. Chronic stress consisted of daily injections of 40 mg/kg of corticosterone (CORT) subcutaneously from postnatal day (P) 1 to P9 in male and female rat pups followed by HS at P10. Body mass, plasma CORT levels, temperature threshold to HS, seizure characteristics, and electroencephalographic *in vivo* recordings were compared between CORT- and vehicle (VEH)-injected littermates during and after HS at P10. In juvenile rats (P18-P22), *in vitro* CA1 pyramidal cell recordings were recorded in males to investigate excitatory and inhibitory neuronal circuits. Results show that daily CORT injections increased basal plasma CORT levels before HS and significantly reduced weight gain and body temperature threshold of HS in both males and females. CORT also significantly lowered the generalized convulsions (GC) latency while increasing recovery time and the number of electrographic seizures (>10s), which had longer duration. Furthermore, sex-specific differences were found in response to chronic CORT injections. Compared to females, male pups had increased basal plasma CORT levels after HS, longer recovery time and a higher number of electrographic seizures (>10s), which also had longer duration. Sex-specific differences were also found at baseline conditions with lower latency to generalized convulsions and longer duration of electrographic seizures in males but not in females. In juvenile male rats, the amplitude of evoked excitatory postsynaptic potentials, as well as the amplitude of inhibitory postsynaptic currents, were significantly greater in CORT rats when compared to VEH littermates. These findings not only validate CORT injections as a stress model, but also show a sex difference in baseline conditions as well as a response to chronic CORT and an impact on seizure susceptibility, supporting a potential link between sustained early-life stress and complex FS. Overall, these effects also indicate a putatively less severe phenotype in female than male pups. Ultimately, studies investigating the biological

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underpinnings of sex differences as a determining factor in mental and neurologic problems are necessary to develop better diagnostic, preventative, and therapeutic approaches for all patients regardless of their sex.

1. Introduction

Epilepsy has a prevalence of 1% and is the most common neurological disorder worldwide (Asadi-Pooya et al., 2017). Mesial temporal lobe epilepsy (mTLE), the most widespread form of drug-resistant epilepsy, results in neuro-cognitive impairment and reduced quality of life (Berg, 2008; Engel, 2001). While mTLE is a heterogeneous disorder that often becomes symptomatic in the second decade of life or later, a unified theory of its pathophysiology resides on limbic epileptogenesis occurring through a multi-stage process that begins early in life from an external insult combined with intrinsic susceptibility (Scharfman, 2007; Spagnoli et al., 2015). While multiple external insults in early life have been identified, such as perinatal anoxia, birth trauma, and infection, by far the most prevalent is the occurrence of FS during childhood (Sanon et al., 2012; Scharfman, 2007). FS are induced by fever and are the most common neurological disorder observed during childhood ("Guidelines for Epidemiologic Studies on Epilepsy: Commission on Epidemiology and Prognosis, International League Against Epilepsy,", 1993). The overall estimated prevalence of FS is between 2 and 5% in North America, occurring primarily in children aged 3 months to 6 years (Berg and Shinnar, 1996; Hauser, 1994). FS are divided into simple or complex FS. Simple FS are brief isolated seizures, generalized at onset, with rapid recovery; further, children affected by simple FS usually do not suffer further neurologic issues beyond the initial seizure, and typically show normal brain development (Berg and Shinnar, 1996). Comparatively, complex FS are prolonged (>15 min), can have multiple episodes within 24 h, are lateralized and are associated with post-ictal neurological deficits. Complex FS are a major risk factor for the subsequent development of mTLE, which develops in 30–50% of patients with a history of FS (Hamati-Haddad and Abou-Khalil, 1998; Nelson and Ellenberg, 1976). However, not all patients with complex FS develop mTLE suggesting that other variables likely play a role in leading to epileptogenesis and mTLE.

Environmental stressors, such as early life stress, are known to harbor persistent changes to the limbic system, including increased vulnerability to epileptogenesis (Huang et al., 2002; Jones et al., 2009; Kumar et al., 2011; Lai et al., 2006; Salzberg et al., 2007). Several animal models have shown that postnatal stress, through maternal separation or exogenous corticosterone administration, promote limbic epileptogenesis, possibly through HPA axis hyper-reactivity in adulthood (Huang et al., 2002; Koe et al., 2014). The HPA axis is activated in response to real or perceived stressors and culminates in the production and secretion of glucocorticoids, such as CORT, by the adrenal glands (Munck et al., 1984). Stress can alter hippocampal function and structure, which may render an individual susceptible to FS, and possibly mTLE (Wood et al., 2004). Prenatal stress and exposure to FS studies exhibited a decrease in hippocampal mass, increase in cell death, as well as advanced stages of seizure resulting in a heightened seizure response (Qulu et al., 2015; Qulu et al., 2012). Furthermore, postnatal stress studies have shown that chronically elevated CORT levels promote hippocampal hyperexcitability and structural changes, decrease seizure threshold, and increase interictal epileptiform activity in adult animals (Castro et al., 2012; Kumar et al., 2007).

While previous experimental studies have demonstrated that patient-related factors, such as cortical malformations, may partially explain certain individuals' predisposition to both complex FS and mTLE (Bocti et al., 2003), which is similarly observed in animal models (Scantlebury et al., 2005; Scantlebury et al., 2004), sex hormones may also play a role (Awad et al., 2016; Desgent et al., 2012). Current studies indicate that sex differences in the developing brain are likely caused by changes in steroid exposure, and that these differences account for changes in neuronal excitability during early development (Kight and McCarthy, 2014). These variations include (among others) the timing of the shift from depolarizing to hyperpolarizing GABA (Galanopoulou and Moshé, 2003; Wolf et al., 2019), differences in neuroimmune activation (Schwarz et al., 2012; Wynne et al., 2011), higher susceptibility to ischemic neuronal death in males (Hill and Fitch, 2012) and differences in epigenetics (Kight and McCarthy, 2014). Sex differences are apparent in patients with FS, which have a male-to-female ratio of approximately 1.6 to 1 (Millar, 2006; Leung and Robson, 2007). Sex hormones have been shown as a predisposing factor to epileptogenesis in a two-hit mTLE model following HS; the experiment used a freeze lesion to mimic focal cortical dysplasia on the first day of birth. This lesion, combined with HS at P10, led to the development of mTLE in male pups and testosterone-treated females, but not in untreated females (Desgent et al., 2012; Seale et al., 2005). Overall, these findings further support that these differences in sex hormones may influence seizure severity and outcome in patients with a history of complex FS.

An increasing body of evidence suggests that early-life stress responses may also influence the developing brain's predisposition to seizures and epilepsy, with possible sex-based discrepancies in adulthood (Brummelte and Galea, 2010; Desgent et al., 2012; Gallagher et al., 1984; Kumar et al., 2011; Salzberg et al., 2007). While it is well established that early life stress has long-term consequences, these studies focus mainly on sex differences during adulthood. To date, a relationship between chronic stress and mTLE has not been clearly established in the developing brain; further, despite the HPA axis exhibiting sex-biased activity (Goel et al., 2014; Heck and Handa, 2019), few preclinical studies have reported sex differences in HPA axis functionality during the stress hyporesponsive period (SHRP) (Hary et al., 1986; Shanks et al., 1994). Therefore, the current study seeks to further define the sex-dependent activity of the HPA axis during SHRP following chronic stress and using a HS experimental model.

2. Material and methods

2.1. Animal subjects

Time-pregnant (non-primapara) Sprague-Dawley female rats were obtained from Charles River laboratories (St. Constant, QC, Canada) at gestational day 10. The pregnant dams were left to be accustomed to the animal facility environment for thirteen days (*i.e.*, until parturition), with 12-h light/dark cycle and *ad libitum* food and water availability. All animal-related procedures were conducted in accordance with the Canadian Council on Animal Care regulations and conformed to the guidelines of protocol #617, which was approved by the Comité Institutionnel de Bonnes Pratiques Animales en Recherche (CIBPAR) at Sainte-Justine Hospital Research Centre affiliated to Université de Montréal (Montreal, Quebec, Canada). All efforts were made to minimize the number of animals used and their suffering. The final data are derived from 188 Sprague-Dawley rat pups, 94 females and 94 males sampled from 15 litters. The animals were used for the different endpoints described on the experimental design (Fig. 1). However, 45 females and 32 males were used in a different set of long-term experiments that are not included in the current study.

2.2. Daily postnatal CORT injections

At P1, the newborn male and female rat pups were identified by sex and randomly assigned to one of two treatment groups: 1. VEH: vehicle-injected; 2. CORT: corticosterone-injected. Using a 50:50 ratio approach, the rat pups from each litter were separated in two groups in

which both sexes were equally represented, controlling for litter effects. From P1 to P9, each rat pup received daily subcutaneous CORT (40 mg/kg, Sigma-Aldrich Canada, Oakville, Ontario) injections (10 μl/g of body) suspended in saline containing 0.1% dimethyl sulfoxide (DMSO) or VEH with 0.1% DMSO in saline. The CORT solution was sonicated 30 s before each use to ensure an even suspension of the hormone micelles throughout the solution. The body mass of all pups was measured daily, and the dose adjusted accordingly. The injections were administered at the same time of the day, during the light phase of the light/dark cycle.

Despite endogenous stress hormones being greatly reduced and minimally responsive to increases in stressful stimuli during the SHRP, which occurs between P4 and P14 in rats (Sapolsky and Meaney, 1986; Walker and Scribner, 1991), the decision of injection during this period was made due to study goal of investigating the effects of high CORT levels of a pharmacological range (Claflin et al., 2017) on the well-established HS experimental model at P10 (Dubé et al., 2009; Scantlebury et al., 2005). Consequently, a high CORT dose was chosen in the attempt to investigate any possible sublet sex differences.

2.3. Hyperthermia-induced seizures (HS)

Pups at P10 (*i.e.*, 24 h after the last CORT injection) were exposed to HS as previously described (Scantlebury et al., 2005). To minimize the effects of circadian variation, all experiments started at 1 PM, the dam was withdrawn, and the offspring were weighed. Then, each pup was individually placed in a Plexiglass box equipped with heated airflow to increase body external temperature to 44-46 °C (*i.e.*, corresponding to 40-42 °C internally) (Sanon et al., 2017). Each pup was monitored until the onset of a GC characterized by loss of posture, lying on the side and tonic body flexion lasting 10 consecutive seconds. Seizure induction was coupled with real-time vEEG recordings for each pup rat. At this point, they were immediately removed from the box for further behavioral observations and vEEG monitoring of the pursuing generalized seizure. Between experimental sessions the box was cleaned, dried, and cooled down to maintain a starting temperature at around 23 °C.

2.4. vEEG recordings of HS at P10

To determine the prevalence of HS, animals were implanted at P7 with a stainless-steel bipolar electrode of 125 μm in diameter (Plastics-1 Inc., Roanoke, VA, USA) which was positioned into the right dorsal hippocampus in *cornus ammonis* region one (CA1), at the following coordinates with reference to bregma: AP = −3.0, ML = −2,4, DV = −2.0, under constant general anesthesia with isoflurane. At P10, following implantation of hippocampal electrodes, animals were placed in individual Plexiglas cages surrounded by a Faraday cage to undergo vEEG recordings before, during and after HS, like what was previously described (Desgent et al., 2012). EEGs and behavior were recorded simultaneously with a Stellate Harmonie system linked to a 32-channels Lamont amplifying unit and an infrared analogical video camera positioned 1.5 m in front of the cages (Stellate Systems v 6.2e, Victoria, Montreal, Qc, CAN). Using the Harmonie Software, with EEG data acquired at 200 Hz, 5-min windows containing electrographic activity tracings were collected every 10 min for the first 30 min after GC occurred. These data point windows were imported to MATLAB (MathWorks, MATLAB 9.1) for further analysis.

Electrographic seizures were defined *via* MATLAB as the occurrence of discrete episodes of uninterrupted high voltage spike and/or poly-spike discharges lasting at least 10 s, with a mean frequency higher than 1 Hz and amplitude higher than a mean voltage threshold that was calculated from a 15 s segment of baseline activity for each case. The categories were: overt electrographic seizures (>10 s), poly-spike bursts (>3 and < 10 s), and interictal spikes (<3 s). The severity of the HS was assessed by measuring the number and total duration of ictal events before return to baseline. Furthermore, to confirm seizure activity, epileptic behaviours corresponding to score levels of 3 to 5 on the modified Racine scale (Racine, 1972) had to be present simultaneous to seizure activity on the EEG. EEG monitoring was pursued until the recovery of baseline activity and exploring behavior. Recovery from seizures was defined as disappearance of both clinical GC and epileptic activity, given that spikes persist after the GC and recovery correlates with the disappearance of the epileptic activity. Animals that did not

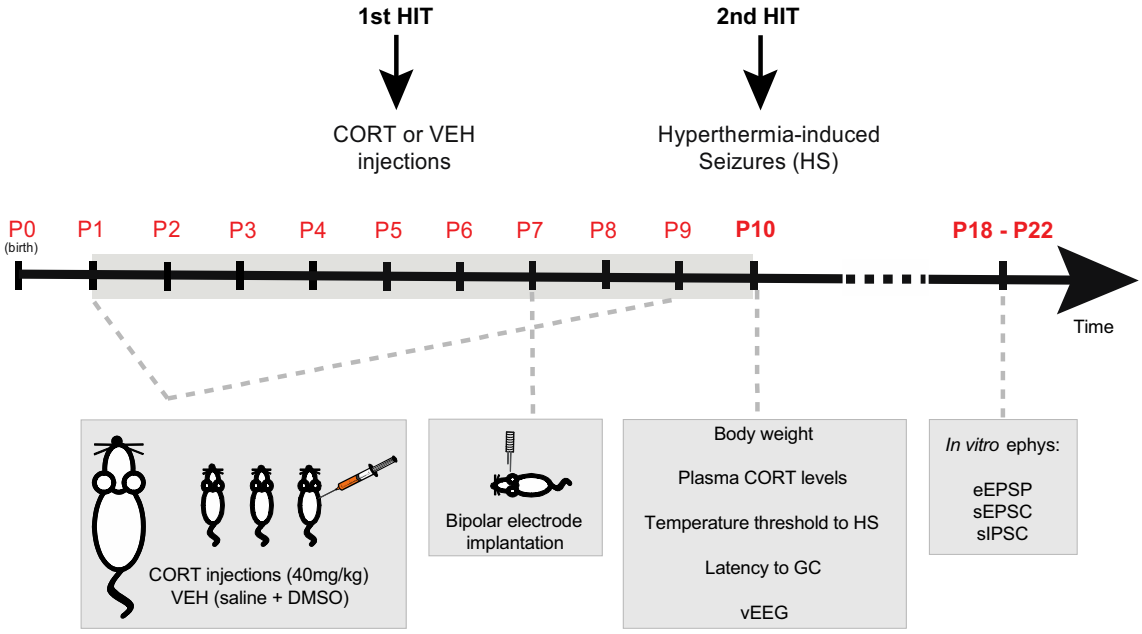

Fig. 1. Experimental design. Starting at P1, subcutaneous CORT injections were given daily until P9. Weight gain was monitored everyday throughout the experiment. At P7, a subset of animals underwent a surgery to implant bipolar electrode used during vEEG recordings at P10. At P10, pups underwent HS. Plasma CORT levels, temperature threshold to HS and latency to GC were measured. *In vitro* recordings were performed around P18-P22. CORT = corticosterone, HS = hyperthermia-induced seizures, GC = generalized convulsions, vEEG = video and EEG recordings, eEPSP = evoked excitatory postsynaptic potentials, sEPSC = spontaneous excitatory postsynaptic currents, sIPSC = spontaneous inhibitory postsynaptic currents.

recover within the first 120 min post GC were attributed this maximal time value as recovery latency. Three separate observers blinded to the treatment groups, reviewed the vEEG recordings to detect epileptiform events and clinically associated behaviours.

2.5. Thermographic measurements during HS

To gather body temperature data non-invasively during HS, we used a ThermoVision A40M (FLIR systems, Inc.) thermographic camera and computer vision Matlab tools, in a subgroup of animals. The camera allowed us to obtain every 1/30th of a second, temperature measurements as 320 × 240 pixels greyscale images, where each pixel corresponded to a temperature measurement in the camera field of view. The thermographic camera lens was placed through a hole pierced in the box cover, giving an overhead view of the pup. The camera was set with a linear measurement range between 20 and 53 °C. Temperature data was analyzed by taking measurements every 7 s (Bilodeau et al., 2015; Sanon et al., 2017).

2.6. Plasma CORT level measurements

Plasma CORT levels were sampled in P10 rats for each treatment and sex groups. Baseline values were obtained 30 min before HS *via* a blood sample (between 20 and 30 μl) collected through rapid saphenous vein puncture with a capillary. Two hours after the first blood sample and the HS, the pups were quickly beheaded, and a second sample was taken. Whole blood samples were collected into commercially available anticoagulant-treated tubes (EDTA-treated) and centrifuged immediately (10 min at 1000–2000 x*g* using a refrigerated centrifuge). Plasma was collected and kept in an Eppendorf tube at -80 °C until further processing. CORT levels were measured directly in plasma with a commercially available radioimmunoassay kit (Corticosterone ^{125}I RIA Kit, Medicorp inc., Montreal, Canada). This assay enabled us to reliably measure CORT levels superior to 0.022 nmol/ml.

2.7. Electrophysiological recordings

Patch-clamp recordings has been described previously (Sanon et al., 2010). Briefly, hippocampal slices were prepared from P18-P22 rats anesthetized with isoflurane. Brain tissue was quickly removed and placed in cold artificial cerebrospinal fluid (ACSF) containing in mM: 126 NaCl, 3 KCl, 2 $MgSO_4$-H_2O, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 10 D-Glucose, bubbled with 95% O_2 / 5% CO_2, with pH and osmolarity adjusted to 7.3–7.4 and 300–310 mOsm respectively. The 300 μm thick hippocampal slices were cut using a vibratome (Leica Microsystems VT1000S, Concord, On, Canada) and transferred to a room temperature chamber with oxygenated ACSF. After one-hour incubation period, individual slices were placed into a recording chamber and continuously superfused with oxygenated and heated ACSF at ~32 °C using a temperature controller (Warner CL-100 Bipolar Temperature Controller, Hamden, CT, USA).

Hippocampal CA1 pyramidal cells were visualized using an upright microscope (Olympus BX50WI, Richmond Hill, ON, Canada.) fitted with differential interference contrast (DIC) optics and infrared (IR) video camera (Hitachi Kokusai Electric Canada, St-Laurent, QC, Canada). Recording patch pipettes were pulled from borosilicate glass tubing with filament (World Precision Instruments, Sarasota, FL, USA), with resistance ranging from 5 to 7 MΩ when filled with intracellular solutions containing in mM: 140 K-gluconate or CsCl, 5 NaCl, 2 $MgCl_2$, 10 Hepes buffer, 0.5 EGTA, 10 phosphocreatine, 2 ATP-Tris, 0.4 GTP-Li. CsCl based solution also included the Na^+ channel blocker N-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide (QX314, 2 mM) to internally block Na^+ channels (Sigma, St-Louis, MO, U.S.A.). The pH and osmolarity were adjusted to 7.2–7.3 with KOH or CsOH, and 280-290 mOsm respectively. After tight seals (> 1 GΩ) on pyramidal cell bodies and whole-cell configuration was attained, recorded signals were amplified using an Axopatch 200B, low-pass filtered at 1 kHz, digitized with a Digidata 1440A A/D converter and acquired at a sampling rate of 10 kHz using the pClamp software (versions 8 and 10; Molecular Devices, Sunnyvale, CA, USA).

Evoked excitatory postsynaptic potentials (eEPSP) were elicited at -60 mV by electrical stimulation (50% of the maximal stimulation intensity 0.1–0.5 mA; 0.2 ms), using a bipolar electrode placed in the *stratum radiatum* layer, in current-clamp mode in ACSF. Spontaneous excitatory and inhibitory currents (sEPSC and sIPSC) were recorded using a CsCl based intracellular solution in voltage-clamp mode in the presence of bicuculline methiodide, a $GABA_A$ receptor antagonist (1μM) for sEPSC, and with *N*-methyl-D-aspartate (NMDA) receptor antagonist d-(−)-2-amino-5-phosphonovaleric acid (D-AP5; 50 mM) and the non-NMDA glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 mM) for sIPSC. All drugs were purchased from Sigma-Aldrich (Oakville, ON, Canada).

2.8. Statistical analysis

Data was log-transformed to fit a normal distribution and subsequently analyzed using a two-way analysis of variance (ANOVA) with Tukey's *post hoc* test to assess how continuous outcomes varied across multiple experimental groups. An independent unpaired two-tailed Student's *t*-test was used to identify significant differences between two groups. Non-linear regression curves (Gaussian fit) were traced to establish differences between data sets. Finally, the Kolmogorov-Smirnov test was used to establish differences between cumulative probability curves. All statistical analyses were carried out with RStudio 1.2.1335 with a two-tailed *p*-value <0.05 used as the threshold for concluding statistical significance. All data are presented as mean ± standard error of the mean (SEM) except when specified otherwise.

3. Results

3.1. Validation of CORT-treatment as a chronic stress in male and female rat pups

The progression of weight gain in male and female rat pups was monitored during CORT injection experiments from P1 to P9. Both VEH and CORT-treated male and female pups gained weight over the course of the 9 injection days (Fig. 2 A, B). No mortalities were observed. On P1, there were no significant differences in mean body weight for male pups ($p = 0.998$). However, starting at P4, male CORT pups gained weight at a slower rate than their VEH littermates. (two-way ANOVA with Tukey's post hoc analysis, $p = 0.002$), the differential weight gain being sustained until P9 (two-way ANOVA with Tukey's post hoc analysis, $p < 0.001$) (Fig. 2 A). Data suggest that at young ages, male pup weight gain is altered by chronic CORT treatment.

Female pup development followed a similar trajectory, with no significant differences between VEH and CORT-treated rats at P1 ($p = 0.999$). Differences in weight were again first noticed at P4 (two-way ANOVA with Tukey's post hoc analysis, $p = 0.002$) and sustained until P9 (two-way ANOVA with Tukey's post hoc analysis, $p < 0.001$) (Fig. 2B). On P9, there were no significant sex differences in body weight within the VEH ($p = 0.973$) and the CORT (two-way ANOVA with Tukey's post hoc analysis, $p = 0.999$) groups. Overall, data suggest that chronic CORT treatment significantly affects weight gain in both males and females, as seen by the significant main effect of CORT treatment in both male ($F[1767] = 262.8$, $p < 0.001$) and female cohorts ($F[1758] = 259.53$, $p < 0.001$).

At P10, 24 h after the last injections of CORT and before the HS, basal plasma CORT levels in the CORT group were expectedly significantly greater than the VEH group in both male (two-way ANOVA with Tukey's post hoc analysis, $p = 0.001$) and female (two-way ANOVA with Tukey's post hoc analysis, $p = 0.019$) cohorts (Fig. 2C). However, only CORT-injected males had significantly greater plasma CORT levels than their

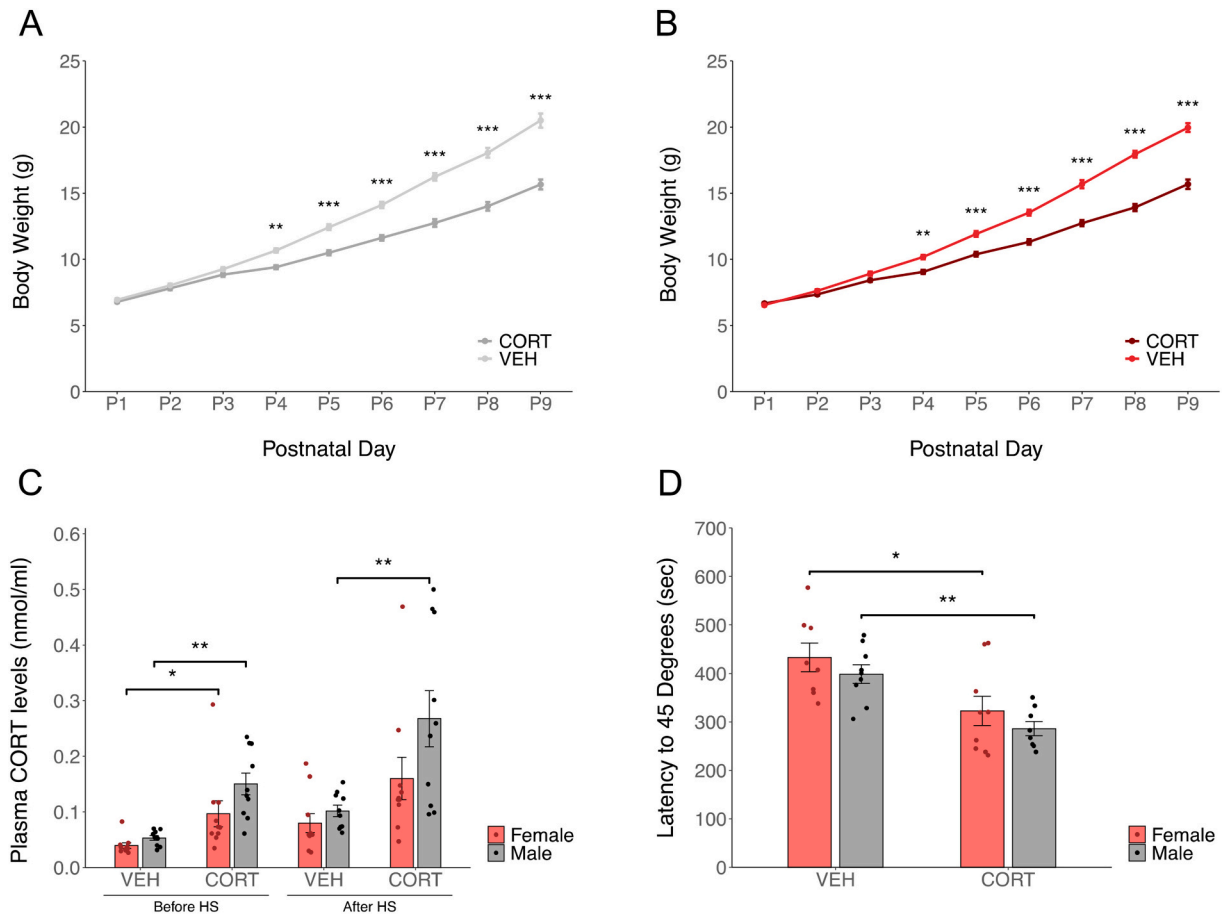

Fig. 2. Temporal effects of postnatal CORT administration on weight gain, plasma CORT levels and temperature threshold of HS in male and female rat pups. A. Body weight curve from P1 to P9 during daily CORT injections shows a significant effect in male CORT-injected (n = 48) relative to VEH-injected animals (n = 46) (P1: VEH, 6.9 ± 0.1 g vs. CORT, 6.8 ± 0.1 g; P4: VEH, 10.7 ± 0.2 g vs. CORT, 9.4 ± 0.1 g; P9: VEH, 20.5 ± 0.5 g vs. CORT, 15.7 ± 0.4 g). B. Body weight curve of female pups (VEH n = 51 and CORT n = 43) during the daily CORT injections shows a significant decrease in weight like males (P1: VEH, 6.6 ± 0.1 g vs. CORT, 6.7 ± 0.1 g; P4: VEH, 10.2 ± 0.2 g vs. CORT, 9.1 ± 0.1 g; P9: VEH, 20.0 ± 0.3 g vs. CORT, 15.7 ± 0.4 g, $p < 0.001$. On P9, there were no significant sex differences in body mass within the VEH (male, 20.5 ± 0.5 g vs. female, 20.0 ± 0.3 g) and the CORT (male, 15.7 ± 0.4 g vs. female, 15.7 ± 0.4 g) groups. C. Bar chart of basal plasma CORT levels in the CORT group (n = 10) were significantly greater than the VEH group (n = 10) in both male (VEH 0.05 ± 0.01 nmol/ml vs. CORT, 0.15 ± 0.02 nmol/ml) and female (VEH, 0.04 ± 0.02 nmol/ml vs. CORT, 0.10 ± 0.02 nmol/ml) cohorts before HS. However, after HS, only CORT-injected males had significantly greater plasma CORT levels than their VEH counterparts (VEH, 0.10 ± 0.01 nmol/ml vs. CORT, 0.27 ± 0.105 nmol/ml). Plasma CORT levels in CORT-injected females after HS did not significantly differ from VEH-injected females (VEH, 0.08 ± 0.02 nmol/ml vs. CORT, 0.16 ± 0.04 nmol/ml). D. Bar chart of latency to reach the critical external temperature of 45 °C shows that CORT males (n = 8) were significantly quicker to arrive at that point than VEH injected males (n = 9) (VEH, 399 ± 19 s vs. CORT, 286 ± 15 s). This time discrepancy was also observed in female pups (VEH, 433 ± 29 s, n = 8 vs. CORT, 322 ± 30s, n = 9). Dots represent individual data points. Graphs represent mean ± SEM. Significant differences are represented by * - $p < 0.05$; ** - $p < 0.01$, *** - $p < 0.001$.

VEH counterparts after HS (two-way ANOVA with Tukey's post hoc analysis, $p = 0.009$). Plasma CORT levels in CORT-injected females after HS did not significantly differ from VEH-injected females ($p = 0.059$) (Fig. 2C). These results indicate a sex-dependent impact of postnatal chronic stress treatment on plasma CORT levels between males and females.

During hyperthermia in the heating apparatus at P10, CORT-injected male pups reached the critical body surface temperature of 45 °C (corresponding to a core temperature of 40.2–41.2 °C) significantly faster than their VEH littermates (two-way ANOVA with Tukey's post hoc analysis, $p = 0.008$). This time a discrepancy was also observed in female pups (two-way ANOVA with Tukey's post hoc analysis, $p = 0.013$) (Fig. 2D, E). Therefore, the chronic CORT treatment lowers the temperature threshold to generalized convulsions (GC) allowing these animals to reach the critical body temperature faster as compared to the VEH-treated counterparts during hyperthermia.

3.2. Sex-specific effects of CORT-treatment on clinical seizures

Regarding the clinical manifestation of GC during hyperthermia, representative EEG traces of VEH and in CORT-injected male and female rats show basal activity, epileptiform events, and full-blown seizures after HS. The clinical manifestations in animals typically included freezing or head bobbing, myoclonic hind limb jerks and abrupt loss of posture (Fig. 3A, B). The latency to GC was significantly shorter in CORT males relative to VEH-treated control males (two-way ANOVA with Tukey's post hoc analysis, $p < 0.001$) (Fig. 3C). This was also the case for the female population, with CORT female rats seizing faster than VEH female rats (two-way ANOVA with Tukey's post hoc analysis, $p < 0.001$). VEH-treated male rats also seized more rapidly than their VEH-treated female littermates (two-way ANOVA with Tukey's post hoc analysis, $p = 0.019$). However, latency to GC was not significantly different between CORT males and CORT females ($p = 0.245$) (Fig. 3C). Overall, chronic CORT treatment had a significant main effect on the mean latency for GC in both sexes ($F[1119] = 54.622$, $p < 0.001$).

After GC, VEH-treated rats recovered significantly faster than their

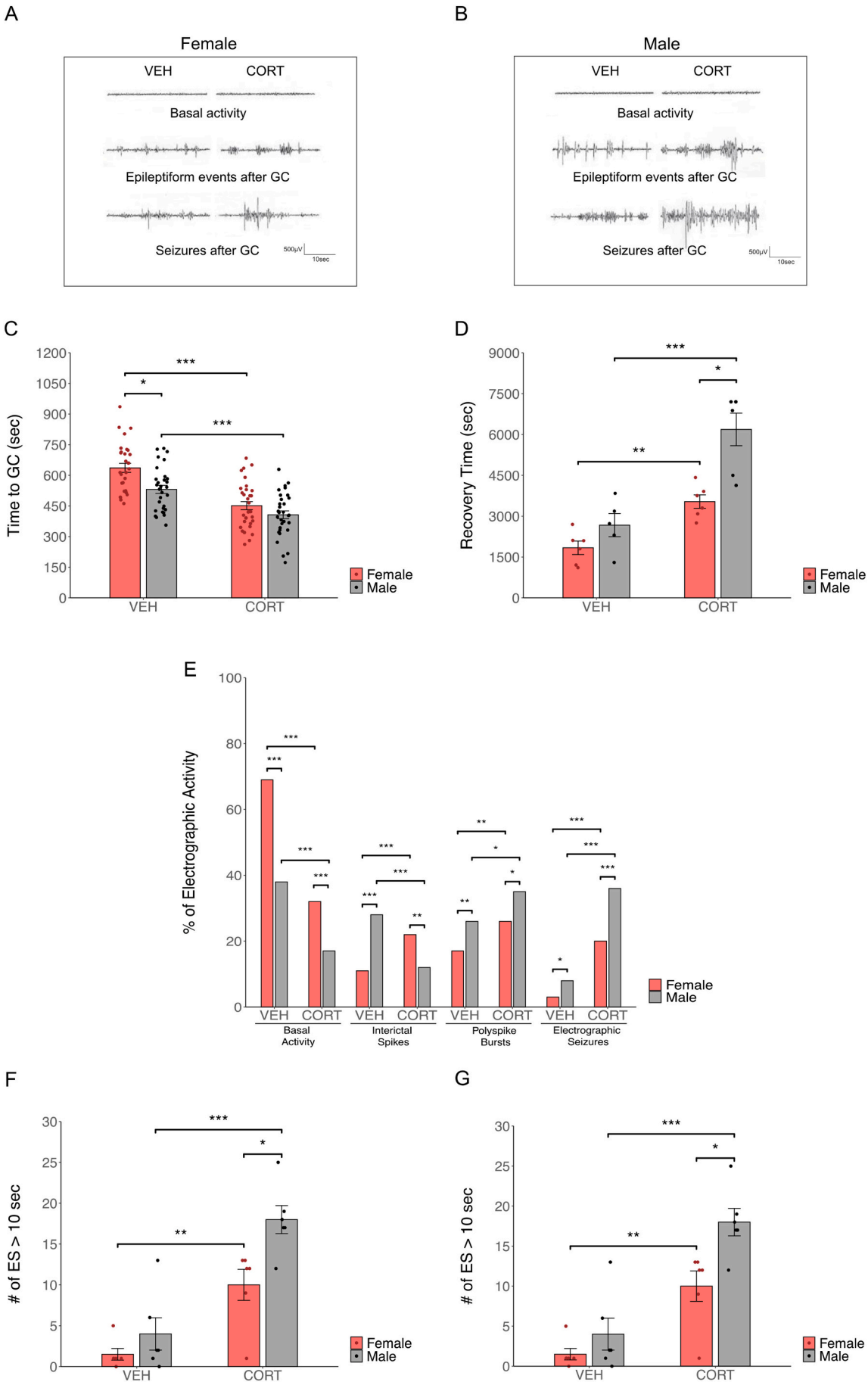

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Fig. 3. Sex-dependent electrographic modifications of CORT-treatment during HS. A. Representative EEG traces in VEH and in CORT-injected female rats showing basal activity and the following epileptiform events: interictal spiking (top), mix of interictal epileptic spike and polyspikes (middle) and complex polyspike bursts (bottom). B. Representative EEG traces in VEH and in CORT-injected male rats Horizontal bar =10 s; vertical bar = 500 μV. C. Bar chart of latency to generalized convulsion (GC) during hyperthermia shows that CORT-treatment significantly shortens the latency to GC during hyperthermia in males (VEH, 531 ± 20s, n = 31 vs. CORT, 407 ± 19 s, n = 31) and in female (VEH, 636 ± 22 s, n = 30 vs. CORT, 452 ± 20s n = 32), but that the latency is already shorter in males than in females with VEH treatment (531 ± 20s vs. 636 ± 22 s). D. Bar chart of recovery time that after GC, VEH-treated rats recovered significantly faster than their CORT treated counterparts in both male (VEH 2670 ± 427 s, n = 5 vs. CORT, 6185 ± 595 s, n = 6) and female (VEH, 1838 ± 246 s n = 6 vs. CORT, 3537 ± 248 s, n = 6) cohorts. However, CORT-injected males took significantly longer to recover than CORT-injected females (6185 ± 595 s vs. 3537 ± 248 s). E. Pie chart shows that CORT-injected male animals had significantly more frequent epileptic discharges than their VEH littermates. Electrographic seizures and complex polyspike bursts constituted 36% and 35% of the electrophysiological activity in CORT-injected males (n = 6), relative to 8% and 26% of activity in VEH males (n = 6). F. Similarly, pie chart shows that CORT-injected female also had significantly more frequent epileptic discharges than their VEH counterparts with electrographic seizures and polyspike bursts making up 20% and 26% of the electrophysiological activity in CORT-injected females (n = 6) and 3% and 17% in VEH females (n = 6), respectively. When comparing the frequency of electrographic seizures and polyspike bursts between different sexes of the same treatment, males were significantly more affected than females. G. Bar chart shows that the CORT male rat group experienced significantly more seizures than VEH males (VEH, 4 ± 2, n = 6 vs. CORT, 18 ± 2, n = 6). The CORT female group was also significantly different from VEH female group (VEH, 1 ± 1, n = 6 vs. CORT 10 ± 3, n = 6). Sex differences were found in between CORT groups (CORT male, 18 ± 2 vs. CORT female 10 ± 3). H. Bar charts shows that seizure duration of electrographic seizures was longer in CORT males compared to VEH males (VEH, 15 ± 1 s, n = 6 vs. CORT, 25 ± 3 s, n = 6). The CORT female group was also significantly different from VEH female group (VEH, 10 ± 0.2 s, n = 6 vs. CORT, 17 ± 2 s, n = 6). Sex differences were found in between VEH (VEH male, 15 ± 1 s vs. VEH female 10 ± 0.2 s) and CORT groups (CORT male, 25 ± 3 s vs. CORT female 17 ± 2 s). Dots represent individual data points. Graphs represent mean ± SEM. Significant differences are represented by Significant differences are represented by * - $p < 0.05$; ** - $p < 0.01$, *** - $p < 0.001$.

CORT treated counterparts in both male (two-way ANOVA with Tukey's post hoc analysis, p = 0.001) and female (two-way ANOVA with Tukey's post hoc analysis, p = 0.005) cohorts (Fig. 3D). The VEH female group recovered particularly quickly, with some individuals taking less than 30 min. Recovery time did not significantly differ between sexes for the VEH cohort (p = 0.244). However, CORT-injected males took significantly longer to recover than CORT-injected females (two-way ANOVA with Tukey's post hoc analysis, p = 0.029) (Fig. 3D).

To study whether postnatal CORT injections altered the pattern and occurrence of epileptiform events following hyperthermia-induced GC, three main types of activity based on duration were quantified: interictal spikes, polyspike bursts and electrographic seizures. CORT-injected male animals had significantly more frequent epileptic discharges than their VEH littermates (two-way ANOVA with Tukey's post hoc analysis, p < 0.001) (Fig. 3E). Similarly, CORT-injected female also had significantly more frequent epileptic discharges than their VEH counterparts (two-way ANOVA with Tukey's post hoc analysis, p < 0.001) (Fig. 3F). When comparing the frequency of electrographic seizures and polyspike bursts during the first 30 min following the HS between different sexes of the same treatment, males were significantly more affected than females. VEH males and CORT males both had a greater proportion of electrographic seizures than VEH females (two-way ANOVA with Tukey's post hoc analysis, p < 0.001) and CORT females (two-way ANOVA with Tukey's post hoc analysis, p < 0.001), respectively. Overall, the CORT male group was the most affected.

Looking only at seizure frequency (>10 s), seizures occurred more frequently in the CORT male group compared to the others. The CORT male rat group experienced significantly more seizures than VEH males (two-way ANOVA with Tukey's post hoc analysis, p<0.001) and CORT females (two-way ANOVA with Tukey's post hoc analysis, p = 0.014). The CORT female group was also significantly different from VEH female group (two-way ANOVA with Tukey's post hoc analysis, p = 0.011) (Fig. 3G). Sexual dimorphism in seizure frequency was only detected between the CORT-treated groups, with no significant sex differences in baseline conditions (p = 0.691). Furthermore, the CORT-treated male and female groups experienced significantly longer seizures than their VEH counterparts (two-way ANOVA with Tukey's post hoc analysis; male, p = 0.004 and female, p = 0.003), and a sex-dependent difference in seizure duration was present in both VEH-treated (two-way ANOVA with Tukey's post hoc analysis, p = 0.029) and CORT-treated groups (two-way ANOVA with Tukey's post hoc analysis, p = 0.043) (Fig. 3H).

3.3. Neuronal circuit alterations in male juvenile rats following chronic CORT-treatment in an in vivo model of limbic epileptogenesis

After identifying stronger effects in males relative to females, we investigated the effects of CORT on hippocampal hyperexcitability specifically in the more susceptible male juvenile rats. Even though spontaneous recurrent seizures do not develop until the third postnatal month (Scantlebury et al., 2005), we have previously shown that neural circuit abnormalities are already evident at P20 (Ouardouz et al., 2010). At the cellular level, evoked excitatory postsynaptic potentials (eEPSPs) in CA1 pyramidal cells yielded significantly greater amplitude responses in CORT-treated rats relative to VEH *(unpaired Student's t-test, p = 0.043)* (Fig. 4A, B). Furthermore, these greater responses were obtained at most stimulation intensities (Non-linear regression, $p = 0.0006$) (Fig. 4C). Despite an increase in the synaptic efficacy, intrinsic properties including input resistance ($p = 0.745$) (Fig. 4D), resting membrane potential (RMP) ($p = 0.573$,) (Fig. 4E), and action potential (AP) threshold ($p = 0.495$) (Fig. 4F) were not different in the CORT group relative to VEH animals.

Studies indicate that stress can lead to epilepsy which may converge to perturb the excitation/inhibition (E/I) balance, due to the dysfunction of excitatory and inhibitory circuits in different brain regions. Therefore, to examine the impact of CORT treatment, spontaneous excitatory/inhibitory postsynaptic currents (sEPSC/sIPSC) from CA1 pyramidal neurons were recorded. Representative traces of sEPSC (Fig. 4G) showed that the mean amplitude ($p = 0.274$) (Fig. 4H) and frequency ($p = 0.997$) (Fig. 4I) were not significantly different between VEH and CORT groups. Cumulative amplitude ($p = 0.985$) (Fig. 4J) and inter-event interval (IEI) ($p = 0.876$) (Fig. 4K) distribution plots for sEPSCs recorded *in CORT* slices overlapped the distribution plots for events recorded in VEH slices, confirming there was no significant difference between CORT and VEH slices in that respect. However, looking at the inhibitory activity onto CA1 pyramidal cells, representative traces of sIPSC (Fig. 4L) in pyramidal cells showed that the mean amplitude of sIPSCs was significantly greater in cells from CORT relative to VEH animals (*unpaired Student's t-test, p = 0.018) (*Fig. 4M*),* while the mean frequency of these currents was unchanged ($p = 0.785$*) (*Fig. 4N*)*. Cumulative amplitude distribution plots for sIPSCs recorded in CORT slices exhibited a rightward shift relative to VEH (Kolmogorov-Smirnov test, $p = 0.005$*) (*Fig. 4O) indicating an increase in the proportion of large amplitude events. Cumulative IEI distribution plots for sIPSCs showed no significant difference between CORT and VEH slices (*p = 0.569)* (Fig. P). All events were blocked with $GABA_A$ antagonist bicuculline. In summary, our findings revealed an increase in evoked potential responses, as well as an increase in the inhibitory drive in the CA1 pyramidal cells.

4. Discussion

In this series of experiments, we examined the effect of chronically

Evoked activity (eEPSP)

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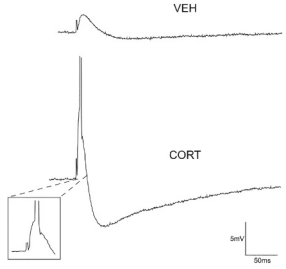

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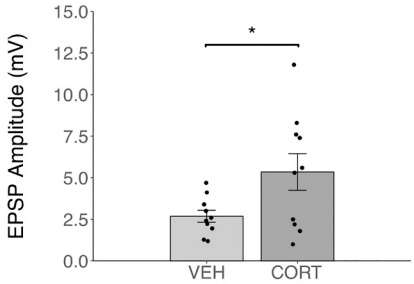

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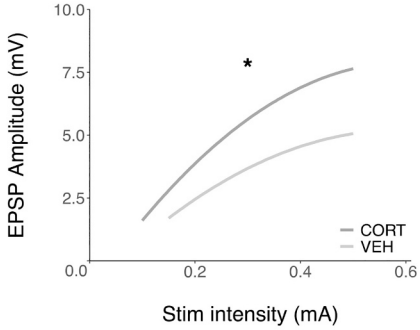

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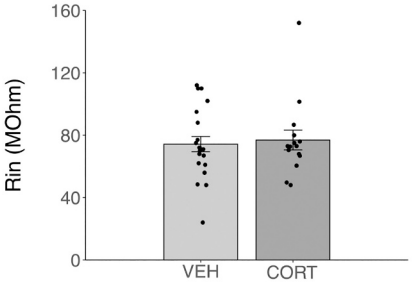

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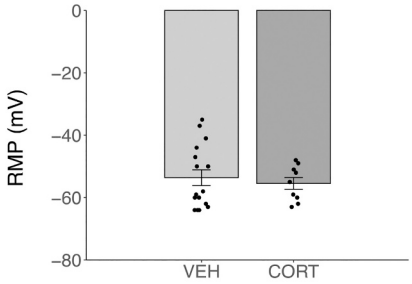

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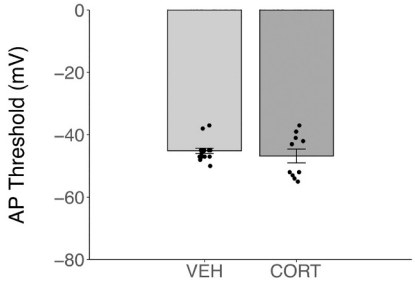

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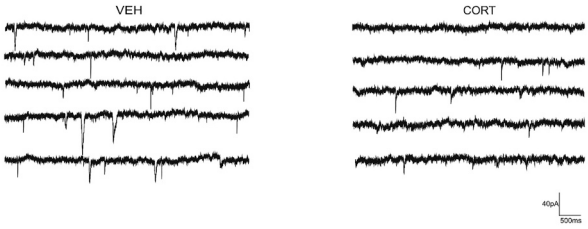

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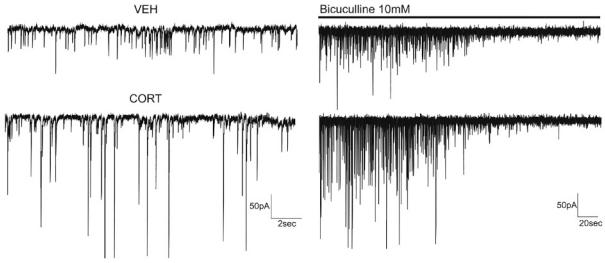

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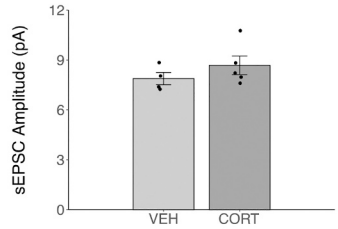

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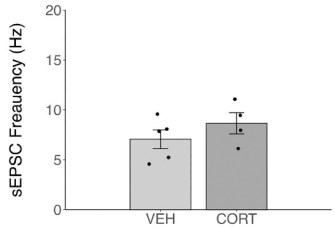

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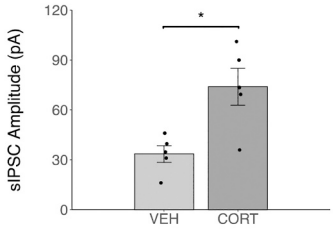

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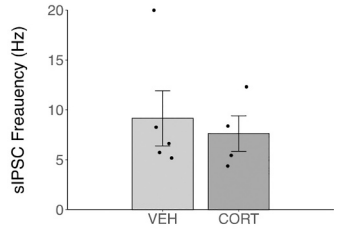

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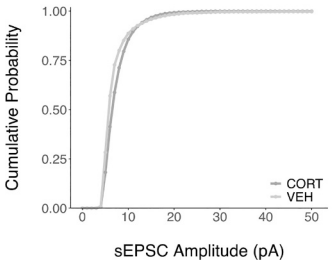

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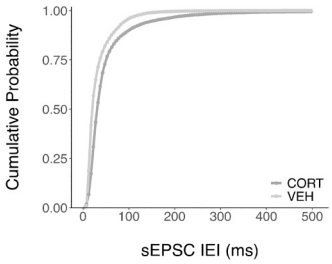

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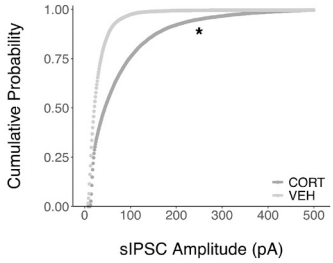

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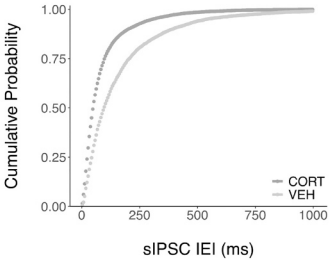

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Fig. 4. Intrinsic and synaptic properties of pyramidal cells in male rats submitted to CORT injections and HS. A. Representative traces of excitatory post-synaptic potentials (EPSPs) evoked on CA1 pyramidal cells upon Schaffer collateral stimulation in ACSF. Inset shows an action potential (AP) as present mostly in the CORT group. B. Bar graph including all recorded cells from VEH and CORT showing a notable increase in EPSP amplitude in the CORT group relative to VEH (VEH 2.7 ± 1.1 mV, n = 10 vs. CORT, 5.4 ± 1.1 mV, n = 10). C. Gaussian fits of stimulus-response curves of EPSPs showing significantly greater amplitudes at all stimulation intensities in the CORT rats (n = 10) relative to the VEH group (n = 14). D. Bar graphs shows no differences between CORT and VEH groups in input resistance (VEH 74.26 ±, 22.42 MΩ, n = 21 vs. CORT, 76.89 ± 24.60 MΩ, n = 15), E. resting membrane potential (RMP) (VEH, −53.63 ± −10.18 mV, n = 16 vs. CORT, −55.44 ± −5.73 mV, n = 9) and F. action potential (AP) threshold (VEH -45.14 ± −3.30 mV, n = 16 vs. CORT, −46.80 ± −6.99 mV, n = 10). G. Representative traces of sEPSC in pyramidal cells. H. Bar graphs of mean amplitude (VEH 7.88 ± 0.73pA, n = 4 vs. CORT, 8.67 ± 1.25pA, n = 5) and I. mean frequency (VEH 8.65 ± 2.11 Hz, n = 4 vs. CORT, 7.06 ± 2.09 Hz, n = 5) show similar sEPSC in cells from VEH and in CORT groups. J. Cumulative probability distribution plots of sEPSC amplitude and K. IEI confirming previous findings. L. Representative traces of sIPSC in pyramidal cells. M. Bar graph of mean amplitude shows greater amplitudes in CORT group relative to VEH (VEH, 33.46 ± 11.22pA, n = 5 vs. CORT, 73.92 ± 24.81pA, n = 5). N. Bar graph shows no significant change in mean frequency (VEH, 7.62 ± 3.56 Hz, n = 4 vs. CORT, 9.15 ± 6.17 Hz, n = 5). All events were blocked with $GABA_A$ antagonist bicuculline. O. Cumulative probability distribution plots of sIPSC amplitude and P. IEI confirming previous findings. Dots represent individual data points. Graphs represent mean ± SEM. Significant differences are represented by Significant differences are represented by * - $p < 0.05$; ** - $p < 0.01$, *** - $p < 0.001$.

elevated CORT levels on hyperthermia-induced seizure in male and female rat pups during development. The data demonstrated this chronic stress model affect weight gain, basal plasma CORT levels before HS, body temperature threshold of HS, GC latency, recovery time, number, and duration of electrographic seizures in both male and female rat pups. Sex-specific differences were found in basal plasma CORT levels after HS, recovery time, number, and duration of electrographic seizures in CORT animals. Interestingly, sex-differences were also observed in baseline conditions in GC latency and duration of electrographic seizures. Overall, while also affected by CORT, female rat pups were more resistant than males to this chronic stress model and appeared significantly less likely to develop FS that could possibly lead to mTLE. Consequently, effects of CORT were observed in evoked potential responses, as well as spontaneous inhibitory currents in the more susceptible male juvenile rats.

4.1. CORT administration as a chronic model of stress

Glucocorticoids (CORT in rodents and cortisol in humans) are the primary end product of the HPA axis, and chronic stress states are associated with a sustained elevation of this hormone into the bloodstream (Munck et al., 1984). In the present study, daily postnatal injections of CORT in rat pups mimic a chronically stressed state during development. The use of CORT administration was chosen because it reduces inter-subject variability as physical, environmental or emotional stress paradigms can all cause individual differences in the HPA axis regulation (Kott et al., 2016; van Campen et al., 2018), as well as mimic normal temporal variation in hormone levels (Claflin et al., 2017). Although the current approach is not a natural physiological stressor, it standardizes the effects of constant or elevated exposure to glucocorticoids in vivo. Moreover, the current injection regimen maintained a CORT increase pattern that was comparable to those found following psychosocial or maternal deprivation stress; these models represent better models of early-life stress in rodents, where a hormone increase between two to four folds of basal physiological level values is expected (Brummelte and Galea, 2010; Desgent et al., 2012; Kumar et al., 2007; Morales-Medina et al., 2009).

4.2. Stress hyporesponsive period (SHRP)

The secretion of glucocorticoids in rats begins during the fetal period and before birth; the basal levels of CORT are similar to those found during adulthood (Condon et al., 1998). However, they markedly decrease after the first two days of life to remain at very low levels until the end of the second postnatal week, a phase called stress SHRP (Dent et al., 2000; Levine, 1994; Schoenfeld et al., 1980). This suggests that the HPA axis of the neonate is not only less responsive to the stimulatory effects of stressors, but also to the inhibitory mechanisms that regulate the neuroendocrine response to stress for their life span. It has been suggested that the SHRP constitutes a protective mechanism as it ensures low and stable levels of glucocorticoids during the early postnatal brain development (Sapolsky and Meaney, 1986; Walker and Scribner, 1991), given that different lines of research evidence demonstrate that exposure to high levels of CORT during the neonatal period leads to irreversible changes that persist in adulthood (Shors, 2006).

To date, very few preclinical and clinicals studies have reported sex differences in HPA axis functionality during the SHRP. Our results indicate that males have higher levels of CORT whereas females undergoing similar brain insults do not have such changes in plasma CORT. These results corroborate previous studies demonstrating that perinatal cortical malformations, such as a freeze lesion combined with HS at P10, led to a rise in their plasma CORT levels and to the development of mTLE in male pups and testosterone-treated females. Despite undergoing similar brain insults, untreated females were not affected (Desgent et al., 2012). Furthermore, in a clinical study looking at stress reactivity in healthy term neonates, higher cortisol response was found in male neonates compared to the female ones, suggesting neonatal sex differences in physiological stress reactivity prior to socialization (Davis and Emory, 1995). However, different types of stressors can affect females more than males. Evidence comes from models of stress due to an immune challenge at P3 in which results showed that HPA axis activity was greater in intact females relative to male rats, whereas this sex difference was reversed with gonadectomy on the day of birth (Shanks et al., 1994). Similarly, female rat pups at P8 exhibited an enhanced adrenocorticotropic hormone (ACTH) response to inhalation of ether when compared to males and when given a testosterone injection at birth, yielding comparable ACTH responsivity to males (Hary et al., 1986). Therefore, it is inappropriate to assume that circuits and regulatory processes are common to males and females. Our data suggest that sex differences during SHRP clearly indicate biological differences between males and females during the organizational period possibly because of prenatal gonadal hormones (androgens or estrogens) that exert a permanent, organizing effect on brain tissue.

4.3. Sex differences in HS: males are more affected than females

Sex differences have been observed in several animal models when combining diverse stress paradigms with experimental induction of seizures. Despite this, most animal models used in epilepsy have used only males, do not specify sex, or pool sexes. However, there are new National Institutes of Health (NIH) and Canadian Institutes of Health Research (CIHR) regulations in place to change this for pre-clinical and clinical studies(Health Canada, 2009; National Institutes of Health, 2015).

Alterations in hippocampal structure and function of CORT might be one of the results underlying hyperthermia-induced seizures in stressed rats. There is increasing evidence showing that chronic stressors that increase glucocorticoid levels lower the threshold for seizure induction, accelerate ictogenesis and promote epileptiform discharges in several animal models of epilepsy (Joëls et al., 2007a, 2007b, Joëls, 2009). For example, CORT in the WAG/Rij rat model of childhood absence epilepsy was associated with rapid increases in spike-wave-discharges (Schridde

and Van Luijtelaar, 2004). Similarly, in the Kainic Acid (KA) model of TLE, pre-exposure to CORT led to increased seizure susceptibility and frequency in male mice and rat species (Roberts and Keith, 1994). Furthermore, in offspring of unspecified sex from pregnant Sprague Dawley dams, results showed prolonged or more severe seizure response in the lipopolysaccharide (LPS) + KA-induced rat model of epileptogenesis in P14 rats that were exposed to prenatal stress in comparison to controls (Qulu et al., 2012). The amplitude of population spikes in the CA1 area was also increased in hippocampal kindled male Wistar rats after exposure to high CORT levels (Karst et al., 1999). Further evidence supporting the role of chronic stress in epileptic activity has been shown in experiments using postnatal stressors comparable to those used in the current experiment. For example, neonatal isolation for one hour per day between P2 and P9 enhanced plasma CORT and exacerbated the neurological consequences of status epilepticus induced by lithium-pilocarpine at P10 (Lai et al., 2006). However, this was done in Sprague Dawley rats of unspecified sex.

Recently, using the amygdala kindled model, O'Brien and colleagues highlighted a potential influence of sex and stress hormones in seizure susceptibility, which has been reported in several prior studies as well (Jones et al., 2013; Jones and O'Brien, 2013). For example, a study using hippocampal kindling in P14 Wistar rats found that mid- or late-gestational stress *via* prenatal restraint of the mother increased the rate of kindled seizure development in rat pups and later adult males, but not in female littermates (Edwards et al., 2002b; Edwards et al., 2002a). Stress also increased the rate and duration of tonic-clonic pilocarpine-induced seizures on P18–19 more severely in males, which showed more elevated CORT levels and epileptic behaviours than female rats (Ahmadzadeh et al., 2011; Sadaghiani and Saboory, 2010). Similarly, increased kindling rates were also observed in adult male rats pre-exposed to chronic postnatal cross-fostering stress during a similar period (*i.e.*, P1-P23) (Gilby et al., 2009). Furthermore, in adult female rats, adrenalectomy compared to CORT replacement or sham-operated controls can delay the kindling process, while testosterone in males can enhance it (Edwards et al., 2001; Edwards et al., 1999). However, it should also be noted that certain circumstances do lead to increased seizure susceptibility in females more than males. For example, daily maternal separation (chronic stress) compared to handling (acute stress), between P2–14, was shown to lower seizure threshold and increase amygdala kindling rates in adult Wistar rats of both sexes, but with more potent effects seen in females (Jones et al., 2009; Kumar et al., 2011; Salzberg et al., 2007).

Hence, our model corroborates with previous studies in which a resistance to HS may be due to the animal body size. More specifically, Barrett et al., 2016 found a positively correlation between body weight and seizure latency in a heat-sensitive transient receptor potential vanilloid-1 (TRPV1) KO mice (Barrett et al., 2016). Accordingly, a slower weight gain in our model of CORT increased seizure susceptibility when compared to VEH-treated animals. Furthermore, our results define a sex-dependent activity of the HPA axis in the SHRP and the male susceptibility for seizures may be influenced by different factors. Our results show that CORT-treated animals have a decreased latency to reach seizure threshold although no sex differences were found between groups. This suggests that CORT affects temperature regulation. Recent literature on FS and breathing reposes to HS shows that vagal TRPV1-driven thermal hyperpnea, a breathing pattern during HS characterized by an increase in tidal volume and breathing frequency, possibly rises susceptibility to HS in pups (Barrett et al., 2018). Therefore, CORT may produce sex-specific effects on breathing responses to HS leading to males being more affected than females, which corroborates with sex-based differences in the consequences of neonatal stress on cardio-respiratory system (Baldy et al., 2018; Tenorio-Lopes and Kinkead, 2021).

4.4. Possible compensatory alteration in the CORT juvenile male rats

While CORT increased synaptic efficacy as measured by eEPSP, the CA1 pyramidal cells into the epileptic environment received augmented inhibitory input with an increase of the amplitude of sIPSCs. Changes in $GABA_A$ receptor subunit composition, as well as enhanced sensitivity or activation of postsynaptic $GABA_A$ receptors, might explain the elevated inhibitory drive of CORT juvenile male rats. This is supported by studies demonstrating that $GABA_A$ receptors exhibit enhanced efficacy after SE (Brooks-Kayal et al., 1998; Cohen et al., 2003; Gibbs et al., 1997). We speculate that changes in the amplitude of inhibitory currents may maintain homeostatic balance in the face of changing E/I balance and characterize a compensatory alteration in the CORT treated animals.

Two main limitations are noted in this study. First, the electrophysiology experiments performed at P18-P22, included only males. Even if previous studies showed a preferential KCC2 upregulation in a model of freeze lesion combined with HS probably due to sustained neural activity, which in turn may increase the risk for mTLE in juvenile male rats (Awad et al., 2016; Fiumelli et al., 2005; Fiumelli and Woodin, 2007), future studies in females will be needed to determine the implications of sex-specific susceptibility to the emergence of mTLE following FS. Second, this series of experiments are exploratory and suggest that males are more affected in the short term but long-term vEEG recordings are needed to confirm the sex differences in the two-hit model of mTLE, as described in previous studies (Desgent et al., 2012).

4.5. Crosstalk between HPA and hypothalamic-pituitary-gonadal (HPG)

There exists a reciprocal relationship between the HPA and the HPG axes wherein the activation of one affects the function of the other and *vice versa* (Oyola and Handa, 2017). By using CORT administration as a chronic model of stress, our results suggest that this resistance could be due to a lower susceptibility of the female HPA axis to excessive CORT exposure during this specific postnatal window, supporting our hypothesis that sex hormones influence the HPA activity during development and seizure severity and outcome. Androgens generally exert proconvulsant effects that can underlie sex differences in the expression of seizures, and these are mediated in part by their actions on the hippocampus, where HS originate (Frye, 2008; Goel and Bale, 2009; Hamed, 2008). Thus, this study suggests that the surge of sexual hormones (testosterone and its estrogenic metabolites) during the end of the embryonic period and until birth in male Sprague Dawley rat pups is involved in their more severe phenotype during HS and that this effect is potentiated by the presence of excessive plasma CORT levels in male rat pups.

5. Conclusion

These results suggest that repeated exposure to elevated CORT levels exacerbates HS and that males are more affected than females. This provides additional evidence that an exposure to early life stress, by increasing glucocorticoid levels early in life, may act as a first hit to predispose to complex febrile seizures and possibly ensuing epileptogenesis. The results of this experiment are consistent with the two-hit hypothesis of epileptogenesis and suggest early-life stress is a significant first hit due to an increased hippocampal vulnerability to febrile seizures. However, further work is required to investigate the long-term outcome (*e.g.*, at P90) using the current model. Furthermore, the results encourage continued studies on sex-specific biological effects on the development of epilepsy in animal models, especially as they pertain to the developing brain. Converging data indicate that elevated expression of glucocorticoids constitutes an important mechanism, through HPA axis modifications, for generating developmentally regulated alterations that could increase excitability in the hippocampus that in turn would trigger and worsen seizure responses early in life. Our data adds additional insights to the important relationship between sex, early-life stress

and epileptogenesis, and may lead to refinement of our clinical surveillance and therapeutic strategies in the neonatal period.

Author's contributions

DCW, SD, NTS, designed the study and wrote the manuscript. DCW, SD, NTS, CMB, PA, AS, SD, MTS, MS, GAB performed experiments. DCW, SD, JSC analyzed data. DCW, JSC, LME, ECL, AGW reviewed manuscript.

Declaration of Competing Interest

None.

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