
Research Article: New Research | Disorders of the Nervous System

Sex and estrous cycle stage shape left-right asymmetry in chronic hippocampal seizures in mice

<https://doi.org/10.1523/ENEURO.0041-23.2023>

Cite as: eNeuro 2023; 10.1523/ENEURO.0041-23.2023

Received: 1 February 2023

Revised: 13 April 2023

Accepted: 1 May 2023

This Early Release article has been peer-reviewed and accepted, but has not been through the composition and copyediting processes. The final version may differ slightly in style or formatting and will contain links to any extended data.

Alerts: Sign up at www.eneuro.org/alerts to receive customized email alerts when the fully formatted version of this article is published.

Copyright © 2023 Cutia et al.

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license, which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

Sex and estrous cycle stage shape left-right asymmetry in chronic hippocampal seizures in mice

Abbreviated title: Left-right asymmetry in chronic seizures in mice

Author names: Cathryn A. Cutia¹, Leanna K. Leverton², and Catherine A. Christian-Hinman^{1,2,3*}

Author affiliations: ¹Neuroscience Program; ²Department of Molecular and Integrative Physiology; ³Beckman Institute for Advanced Science and Technology, University of Illinois Urbana-Champaign, Urbana, IL, 61801 USA

Cathryn A. Cutia ORCID ID: 0001-8738-9255
Leanna K. Leverton ORCID ID: 0003-2590-2599
Catherine A. Christian-Hinman ORCID ID: 0003-3475-2166

Author contributions: C.A.C.-H. designed research; C.A.C. and L.K.L. performed research; C.A.C. analyzed data; C.A.C. and C.A.C.-H. wrote the paper.

Corresponding author:
Catherine A. Christian-Hinman, PhD
407 S. Goodwin Ave.
Urbana, IL 61801 USA
Phone: (217) 244-8230
Email: cathchri@illinois.edu

[Number of figures: 7](#)
[Number of tables: 1](#)
[Number of multimedia: 0](#)
[Number of words for Abstract: 241](#)
[Number of words for significance statement: 120](#)
[Number of words for Introduction: 632](#)
[Number of words for Discussion: 1573](#)

Acknowledgements: We thank Jiang Li for assistance with pilot studies and Victoria Daniels for help with estrous cycle monitoring.

Conflict of Interest: The authors declare no competing financial interests.

Funding Sources: This work was supported by the National Institutes of Health (NIH)/National Institute of Neurological Disorders and Stroke and the NIH Office of Research on Women's Health through grants R03 NS103029 and R01 NS105825 (C.A.C.-H.) and by a CURE Epilepsy Research Continuity Fund Grant (C.A.C.-H.).

43 **Abstract**

44 Lateralization of hippocampal function is indicated by varied outcomes of patients with
45 neurological disorders that selectively affect one hemisphere of this structure, such as temporal
46 lobe epilepsy (TLE). The intrahippocampal kainic acid (IHKA) injection model of TLE allows for
47 targeted damage to the left or right hippocampus, enabling systematic comparison of effects of
48 left-right asymmetry on seizure and non-seizure outcomes. Although varying non-seizure
49 phenotypic outcomes based on injection side in dorsal hippocampus were recently evaluated in
50 this model, differences in chronic seizure patterns in left- (IHKA-L) vs. right-injected (IHKA-R)
51 IHKA animals have yet to be evaluated. Here, we assessed hippocampal seizure incidence in
52 male and female IHKA-L and IHKA-R mice. Females displayed increased electrographic seizure
53 activity compared to males at both 2 and 4 months post-injection. In addition, IHKA-L females
54 showed higher seizure frequency than IHKA-R on diestrus and estrus at 2 months post-
55 injection, but seizure duration and percent time in seizures were only higher in IHKA-L females
56 on diestrus. These cycle stage-associated changes, however, did not persist to 4 months post-
57 injection. Furthermore, this lateralized difference in seizure burden was not observed in males.
58 These results indicate for the first time that the side of IHKA injection can shape chronic
59 electrographic seizure burden. Overall, these results demonstrate a female-specific left-right
60 asymmetry in hippocampal function can interact with estrous cycle stage to shape chronic
61 seizures in mice with epilepsy, with implications for neural activity and behavior in both normal
62 and disease states.

63

64

65 **Significance Statement**

66 Seizures in temporal lobe epilepsy often originate in the hippocampus, and patient outcomes
67 can depend on whether the seizures initiate in the left or right hippocampus. Although rodent
68 brain function appears less lateralized than in humans, emerging evidence indicates stronger
69 lateralization of hippocampal function in mice than previously thought. Here, we systematically
70 compared chronic epilepsy profiles in mice based on whether left or right hippocampus is the
71 main site of seizure generation. Males did not show a left-right asymmetry in epilepsy severity,
72 but females showed effects of seizure initiation side that varied with estrous cycle stage. These
73 results thus suggest a female-specific lateralization of hippocampal function can interact with the
74 estrous cycle to shape chronic seizures in mice with epilepsy.

75

76

77 **Introduction**

78 The human brain is structurally and functionally lateralized (Gazzaniga, 1995). Although
79 the hippocampus shows structural symmetry, neuroimaging studies suggest distinct functional
80 roles of the two human hippocampi (Howard et al., 2011; Maguire and Frith, 2003). Importantly,
81 several neurological diseases affect the hippocampus. The damage inflicted from neurological
82 disorders such as stroke and epilepsy can be unilaterally localized. Furthermore, patient
83 outcomes can vary based on whether this damage is present in the left or right hippocampus.
84 For example, patients with ischemic stroke injury in the left hippocampus have more apparent
85 memory dysfunction than those with damage in the right (Schaapsmeeders et al., 2015).
86 Additionally, people with epilepsy whose seizures arise from the left hippocampus show a
87 higher degree of cognitive impairment than with right hippocampal seizure foci (Addis et al.,
88 2007; Alessio et al., 2006; Phuong et al., 2021). This variation in outcomes based on the
89 hemisphere containing the unilateral seizure focus may result from underlying functional
90 lateralization in the hippocampus.

91 Temporal lobe epilepsy (TLE) is a common form of focal epilepsy in which seizures arise
92 from a specific subregion and hemisphere of the temporal lobe, particularly the hippocampus
93 (Engel, 2001). Interestingly, clinical observations in women with TLE suggest that the
94 lateralization of a patient's seizure focus can impact seizure patterning in relationship to the
95 menstrual cycle. For instance, clinical evidence suggests left-sided seizure foci are associated
96 with higher incidence of seizures that cluster in the few days prior to and during menstruation,
97 known as perimenstrual catamenial seizures, whereas right-sided seizure foci are associated
98 with non-catamenial patterning of seizures spread across the menstrual cycle (Herzog, 2008;
99 Quigg et al., 2009). Women with catamenial epilepsy are at higher risk for resistance to
100 antiseizure medications (Choi et al., 2020), underscoring the importance of identifying the

101 underlying mechanisms. Furthermore, recent imaging studies have suggested that shifting
102 levels of ovarian hormones across the menstrual cycle can have structural and functional
103 ramifications on the human hippocampus (Gloe et al., 2021; Taylor et al., 2020), although it
104 remains unclear how these effects may interact with seizure focus lateralization to shape
105 seizure patterning in relation to the ovarian cycle.

106 Recent studies demonstrating lateralization in the rodent hippocampus (Jordan, 2020)
107 support the use of rodent models in investigating the mechanisms that underlie these
108 differences. The intrahippocampal kainic acid (IHKA) mouse model of TLE, which allows for
109 epileptogenic insults to be selectively targeted to the left or right hemisphere, shows
110 neuropathological changes similar to human TLE (Bouilleret et al., 1999; Gröticke et al., 2008;
111 Riban et al., 2002). This model also displays epileptiform discharges and spontaneous recurrent
112 seizures (Bouilleret et al., 1999) that are hallmarks of human TLE (French et al., 1993; Mathern
113 et al., 1995; Rusina et al., 2021). To date, researchers using this model have arbitrarily targeted
114 the left or right hippocampus for KA injection, in the absence of systematic examination of
115 differential outcomes of left and right IHKA injections. In a recent study, however, non-seizure
116 phenotypic outcomes in C57BL/6J females injected with KA in the left or the right dorsal
117 hippocampus were compared (Cutia et al., 2022). It was determined that dentate gyrus granule
118 cell dispersion was altered in an injection site-specific manner (Cutia et al., 2022), indicating
119 lateralized phenotypes at the level of the hippocampus. However, recent work has suggested
120 that granule cell dispersion does not correlate to chronic seizure severity in left-injected IHKA
121 mice (Lisgaras and Scharfman, 2022). Therefore, whether the lateralization effect in granule cell
122 dispersion contributes to functional differences in hippocampal seizure incidence remains
123 unclear. Furthermore, it is unknown whether potential lateralized differences in seizure
124 occurrence are shaped by animal sex and, in females, estrous cycle stage. Here, the hypothesis

125 that the laterality of IHKA injection leads to differential patterning of subsequent spontaneous
126 recurrent seizures, both based on sex and estrous cycle stage, was tested.

127

128 **Methods**

129 *Animals and estrous cycle monitoring*

130 Animal procedures used in this study complied with the ARRIVE guidelines and were
131 approved by the Institutional Animal Care and Use Committee of the University of Illinois
132 Urbana-Champaign. Female and male C57BL/6J mice (#000664, Jackson Laboratories) were
133 purchased for delivery at 6 weeks of age. Mice were then housed in a 14:10 h light:dark cycle
134 (lights off at 1900 h) and given food and water *ad libitum*. Animals were group-housed (2-5 mice
135 per cage) until the time of electrode implantation, after which all mice were housed singly.

136 Beginning one week after arrival, estrous cycle monitoring in females was performed
137 between 0900 and 1100 h using a vaginal cytology protocol previously described (Pantier et al.,
138 2019). Female mice were assessed for at least 14 days to verify regular cycles prior to entering
139 the study and resumed estrous cycle monitoring for the duration of the local field potential (LFP)
140 recording periods. Cycle lengths for each mouse at each recording period were calculated as
141 the average time to progress from one stage of estrus through all other stages to another phase
142 of estrus for all captured cycles (Li et al., 2018, 2017). To evaluate the development of
143 differences in seizures over time, mice were recorded and underwent estrous cycle monitoring
144 at both 2 and 4 months post-injection (mpi).

145

146 *Stereotaxic IHKA and LFP electrode implantation surgeries*

147 All stereotaxic surgeries were carried out under isoflurane anesthesia (2-3%, vaporized
148 in 100% oxygen) with carprofen (0.5 mg/ml) for analgesia. Female mice underwent stereotaxic
149 unilateral injection of KA (Tocris Bioscience; 50 nl of 20 mM prepared in 0.9% sterile saline) on
150 the first day of diestrus following the first estrous cycle monitoring period. Age-matched males
151 (> postnatal day 60) were injected in the same manner. Injections were randomly targeted to the
152 left or right dorsal hippocampal region (relative to Bregma: 2.0 mm posterior, 1.5 mm lateral, 1.4
153 mm ventral to cortical surface) as previously described (Li et al., 2017). Age-matched controls
154 were injected with the same volume at the same location with saline. Saline-injected animals
155 (female: left n = 5, right n = 5; males: left n = 3, right n = 4) showed no seizures and thus were
156 not included in analyses.

157 All animals were allowed two weeks to recover from the injection before undergoing a
158 second surgery for LFP electrode implantation. Two twisted bipolar electrodes (P1
159 Technologies) were implanted into the ipsilateral hippocampus just dorsal and lateral to the
160 injection site (relative to Bregma: 2.0 mm posterior, 1.75 mm lateral, 1.25 mm ventral)
161 (Armstrong et al., 2013; Cutia et al., 2022; Li et al., 2020; Li and Christian-Hinman, 2022).
162 Anchor micro-screws (J.I. Morris Co.) were placed into the skull and stabilized with dental
163 cement (Teets “Cold Cure” Dental Cement). Mice were singly housed after electrode
164 implantation and for the duration of the remaining experimental period.

165

166 *LFP recording and analysis*

167 One week after electrode implantation, the mice were tethered to an electrical
168 commutator (P1 Technologies) connected to a Brownlee 440 amplifier (NeuroPhase) with gain

169 set at 1 K. LFP signals were recorded as the local field potential differential between the two
170 electrodes (Armstrong et al., 2013), sampled at 2 KHz and digitized to recorder software written
171 in MATLAB (Armstrong et al., 2013). Mice were recorded at 1, 2, and 4 mpi. The 2 and the 4
172 mpi recording periods were evaluated in the current study; the LFP data collected at 1 mpi from
173 the female animals in the present study were included in another study (Cutia et al., 2022), and
174 are therefore not shown here. In addition, some female mice (IHKA-R = 2, IHKA-L = 5) included
175 in this study were included in a previous publication (Li et al., 2020), but the analysis of the data
176 from these animals carried out in the previous work is distinct from that reported here.

177 All recordings were analyzed with an automated electrographic seizure analyzer (Zeidler
178 et al., 2018) with the minimal seizure duration set at five seconds and the interictal interval at six
179 seconds. It should be noted that most seizures detected in the IHKA mouse model are
180 electrographic and do not generalize to behavioral convulsive seizures (Bouilleret et al., 1999;
181 Gröticke et al., 2008; Klein et al., 2015).

182

183 *Hippocampal granule cell dispersion visualization and quantification*

184 Brains from a randomly selected subset of females and males (8-10 mice per group)
185 were sectioned into 40 μm -thick coronal sections using a freezing microtome (Leica SM 2010R).
186 Every third section from the dorsal hippocampal region was used for histology, with a total of
187 four to six sections evaluated for each mouse. Sections were stained with cresyl violet (Sigma-
188 Aldrich C5042) for 12 minutes at room temperature, dehydrated using graded ethanol solutions
189 (70-100%), before being cleaned with Xylene and coverslipped with Permount. Images were
190 collected using an Olympus BX43 brightfield microscope with an Infinity 3-6UR Teledyne
191 Lumenera Camera and Infinity Capture software (Lumenera). Dentate gyrus granule cell

192 dispersion ipsilateral to the KA injection was quantified as previously described (Cutia et al.,
193 2022; Lisgaras and Scharfman, 2022).

194

195 *Ovarian hormone assays*

196 After completion of LFP recordings at 4 mpi, trunk blood was collected from the female
197 mice at the time of euthanasia. All female mice were euthanized by decapitation on diestrus
198 between 1200 and 1600 h. Trunk blood was stored at 4°C for 24 h, and centrifuged for 15
199 minutes at 4°C. Serum was then isolated and stored at -80°C until the time of assay. Enzyme-
200 linked immunosorbent assays (ELISAs) for progesterone (IBL America, IB79105) and estradiol
201 (American Laboratory Products Co., 11-ESTHU-E01) were performed on serum samples using
202 commercial kits according to the manufacturers' instructions and evaluated using a Bio-Tek
203 800TS Microplate Absorbance Reader. One serum sample from the IHKA-L and one from the
204 IHKA-R group were excluded from the study based on low quality. One IHKA-R sample was
205 tested for progesterone but not for estradiol due to limited sample volume. Additionally, given
206 limited sample volume, all samples were run in singlet to accommodate multiple hormone
207 assays. Intra-assay coefficient of variation values calculated from controls run in duplicate were
208 5.1 for estradiol and 6.8 for progesterone.

209

210 *Statistical analysis*

211 All statistical comparisons were performed using R software. Comparisons of seizure
212 parameters between male and female IHKA-L and -R animals were made using two-way
213 ANOVA and Tukey's *post hoc* tests. Comparisons of seizure parameters between IHKA-L and -

214 R females within individual estrous cycle stages were made using two-sample t-tests, or
215 Wilcoxon rank sum tests depending on the normality of the data. Comparisons of progesterone,
216 estradiol, and the progesterone-to-estradiol ($P_4:E_2$) ratio between IHKA-L and IHKA-R groups
217 were made using Wilcoxon rank sum tests. Normality was evaluated using Shapiro-Wilks tests,
218 and homogeneity of variance was evaluated using Levene's tests. Correlations between percent
219 of time in seizures and granule cell dispersion, estrous cycle length, and hormone levels were
220 made using Pearson's correlations. Information on the estimation statistics used to generate
221 statistically significant results in the study is provided in **Table 1**.

222

223 **Results**

224 *Seizure burden is increased in IHKA females compared to males at 2 months after injection*

225 Both male (IHKA-L = 9, IHKA-R = 8) and female (IHKA-L = 17, IHKA-R = 20) mice were
226 evaluated to characterize sex differences in general seizure patterning and in response to the
227 side of targeted injection (**Figure 1A**). Recordings collected across a 7-day period at 2 mpi were
228 evaluated for each mouse and averaged for group comparisons. Seizure frequency (seizures
229 per hour) differed between groups based on both injection site ($F(1,51) = 4.12$, $p = 0.047$) and
230 sex ($F(1,51) = 12.40$, $p = 0.0009$, **Figure 1B**). Specifically, IHKA-L females displayed elevated
231 seizure frequency compared to both IHKA-L ($p = 0.005$) and IHKA-R males ($p = 0.002$, **Figure**
232 **1B**). IHKA-R females also showed higher seizure frequency than IHKA-R males ($p = 0.005$).
233 Seizure duration also displayed a sex difference ($F(1,51) = 9.79$, $p = 0.002$, **Figure 1C**), with
234 longer seizure duration in IHKA-L females compared to both IHKA-L ($p = 0.02$) and IHKA-R
235 males ($p = 0.03$, **Figure 1C**). IHKA-R females also showed higher duration compared to IHKA-R
236 males ($p = 0.02$, **Figure 1C**). The mean percent time in seizures also differed by injection site

237 (F(1,51) = 4.45, p = 0.04) and sex (F(1,51) = 17.14, p = 0.0001, **Figure 1D**), as IHKA-L females
238 spent more time in seizures than IHKA-L (p = 0.0007) and IHKA-R males (p = 0.0004), and
239 IHKA-R females spent more time in seizures than IHKA-R males (p = 0.0007, **Figure 1D**).

240

241 *Sex differences in seizure duration and percent time in seizures persist to 4 months after*
242 *injection*

243 To characterize the progression of seizure activity over time in these animals, the same
244 animals evaluated at 2 mpi were also evaluated at 4 mpi, with the exception of two IHKA-R
245 females that died prior to the 4-month time point. By 4 mpi, there was no effect of injection site
246 (F(1,49) = 0.0001, p = 0.99) or sex (F(1,48) = 2.17, p = 0.15, **Figure 2A**) on seizure frequency.
247 Seizure duration, however, was still affected by sex and higher in females compared to males
248 (F(1,49) = 11.84, p = 0.001, **Figure 2B**). Percent time in seizures was also influenced by sex
249 (F(1,49) = 8.95, p = 0.004, **Figure 2B**), as IHKA females displayed higher time in seizures than
250 male counterparts (IHKA-L: p = 0.02, IHKA-R: p = 0.02, **Figure 2B**). Together, these results
251 indicate that IHKA females maintain higher seizure duration and percent time in seizures than
252 males when recorded at 4 mpi.

253

254 *Left-right asymmetry in seizure burden in IHKA females is most pronounced on diestrus at 2*
255 *months after injection*

256 To investigate the relationship of estrous cycle phase to seizure patterns, female
257 animals were evaluated across diestrus, estrus, and proestrus. Recordings from three days of
258 each estrous cycle stage from at least three different cycles per mouse were averaged to

259 quantify seizure parameters present on each estrous cycle stage. At 2 mpi, IHKA-L females
260 displayed higher seizure frequency than IHKA-R during diestrus and estrus (diestrus: $t = 2.05$,
261 p -value = 0.05; estrus $t = 2.41$, p -value = 0.02, **Figure 3A**), with a trend towards elevated
262 seizure frequency in IHKA-L females during proestrus ($t = 1.97$, $p = 0.06$, **Figure 3A**). However,
263 seizure duration and percent time in seizures were higher in IHKA-L compared to IHKA-R
264 females only during diestrus (seizure duration: $W = 261$, p -value = 0.04, **Figure 3B**; time in
265 seizure: $W = 286$, p -value = 0.004, **Figure 3C**). These results suggest that there are distinct
266 elevations in seizure parameters in IHKA-L compared to IHKA-R females at 2 mpi, and that
267 these differences are more pronounced during diestrus.

268

269 *Left-right asymmetries and effects of estrous cycle stage on seizure burden in females are*
270 *dampened by 4 months after injection*

271 In contrast to the pattern of asymmetric seizure burden at 2 mpi, there were no
272 appreciable differences between IHKA-L and IHKA-R females in any seizure parameters on
273 diestrus or estrus at 4 mpi (**Figure 4**). During proestrus, however, IHKA-R animals showed
274 trends for elevated seizure frequency ($t = -2.00$, $df = 32$, $p = 0.05$, **Figure 4A**) and percent time
275 in seizures ($t = -1.99$, $df = 32$, $p = 0.06$, **Figure 4C**). These results suggest that the influences of
276 injection side on seizure parameters in females are less distinct at 4 mpi.

277

278 *Degree of granule cell layer dispersion is not correlated to seizure burden severity*

279 Once LFP recordings were completed, brains from a subset of IHKA-L (male $n = 9$;
280 female $n = 10$) and IHKA-R (male $n = 8$; female $n = 10$) mice were collected and sectioned for
281 analysis of granule cell layer dispersion through visualization with Nissl stain (**Figure 5A**).
282 Granule cell dispersion was calculated as the ratio between the value for the hippocampus

283 ipsilateral to the site of injection and the equivalent portion of hippocampus contralateral to the
284 side of injection (I:C ratio) (19). There were no differences between IHKA groups in proportions
285 of mice with and without granule cell dispersion (**Figure 5B**). As all brain samples were
286 collected after completion of recordings at the 4 mpi timepoint, these ratios were compared to
287 the average percent of time that each animal spent in seizures at the 4 mpi recording period.
288 There were no correlations between percent time spent in seizures and I:C ratio in IHKA-L
289 (males: $r^2 = 0.60$, $p = 0.08$; females $r^2 = 0.1$, $p = 0.77$) nor IHKA-R mice (males: $r^2 = -0.34$, $p =$
290 0.38 ; females: $r^2 = -0.28$, $p = 0.5$) (**Figure 5C**). These results indicate that the degree of granule
291 cell dispersion and seizure severity are not correlated when assessed at 4 mpi in the IHKA
292 model.

293

294 *Estrous cycle prolongation is not correlated to seizure burden*

295 Estrous cycle disruption is common in a majority of female IHKA mice (Cutia et al., 2022;
296 Li et al., 2017). To determine if the degree of estrous cycle disruption is correlated to the
297 severity of seizure burden, and if this relationship changes over time, estrous cycle lengths of
298 each mouse were quantified from the cycle monitoring data collected during LFP recordings and
299 correlated to the percentage of time in seizures of each mouse over a 7-day period
300 encompassing all cycle stages. There were no correlations found between estrous cycle length
301 and percent of time in seizures for IHKA-R mice at either 2 ($r^2 = 0.3$, $p = 0.20$) or 4 mpi ($r^2 =$
302 0.08 , $p = 0.75$) (**Figure 6**). IHKA-L females showed a weak correlation at 2 mpi ($r^2 = 0.5$, $p =$
303 0.04) but showed no correlation at 4 mpi ($r^2 = 0.15$, $p = 0.56$). These data align with previous
304 work that reported no significant correlation between cycle length and seizure burden in IHKA-R
305 mice at 2 mpi (Li et al., 2020).

306

307 *Circulating estradiol and progesterone levels are not correlated with seizure burden at 4 mpi*

308 Circulating ovarian hormones fluctuate in rodents across the estrous cycle, and seizure
309 activity can also change with estrous cycle stage (Christian et al., 2020; Nilsson et al., 2015). To
310 evaluate whether the chronic seizure burden correlated with circulating ovarian hormone levels
311 in the present cohort, we measured estradiol and progesterone in serum collected on diestrus
312 from the IHKA female animals following completion of LFP recordings at 4 mpi. There were no
313 differences in estradiol (IHKA-L 13.23 ± 1.79 pg/ml, $n = 16$; IHKA-R 10.00 ± 1.27 pg/ml, $n = 17$,
314 $p = 0.2$) or progesterone (IHKA-L 3.36 ± 0.44 ng/ml, $n = 16$; IHKA-R 4.04 ± 0.84 ng/ml, $n = 18$, p
315 $= 0.86$) levels based on the side of IHKA injection. Additionally, there was no difference in the
316 $P_4:E_2$ ratio between IHKA-L (315.20 ± 52.04 pg/ml) and IHKA-R groups (455.58 ± 106.45 pg/ml,
317 $p = 0.56$), consistent with previous findings (Cutia et al., 2022). Furthermore, there was no
318 correlation between circulating progesterone levels and the percent time in seizure in the 7 days
319 prior to euthanasia for IHKA-L ($r^2 = -0.14$, $p = 0.60$) or IHKA-R females ($r^2 = 2.4 \times 10^{-5}$, $p = 0.99$,
320 **Figure 7A**). There were also no correlations between estradiol (IHKA-L: $r^2 = -0.27$, $p = 0.32$;
321 IHKA-R: $r^2 = 0.31$, $p = 0.23$, **Figure 7B**) or the $P_4:E_2$ ratio (IHKA-L: $r^2 = 0.09$, $p = 0.74$; IHKA-R:
322 $r^2 = -0.54$, $p = 0.17$, **Figure 7C**) and the percent time in seizure.

323

324 **Discussion**

325 Clinical evidence indicates that the hemisphere of a temporal lobe seizure focus may
326 influence seizure cluster patterning in women with epilepsy (Herzog, 2008; Quigg et al., 2009).
327 Preclinical evidence suggests that the IHKA model of TLE holds validity for investigating
328 mechanisms of the aforementioned clinical findings, as differences in hippocampal granule cell
329 dispersion and pituitary gene expression are altered based on site of IHKA injection (Cutia et al.,
330 2022). Despite this evidence, the relationship between seizure patterns in IHKA animals and the
331 site of injection has yet to be documented. In the present study, we evaluated the development

332 and presentation of seizures recorded with LFP at 2 and 4 mpi in male and female IHKA-L and -
333 R mice. The results indicate a distinct sex difference in seizure parameters between male and
334 female IHKA mice, with higher seizure burden in females. In addition, IHKA-L females display
335 higher overall seizure burden compared to IHKA-R females on diestrus at 2 mpi. On proestrus
336 and estrus, however, this effect is only present for seizure frequency. These results suggest that
337 the phase of the estrous cycle influences left-right asymmetry in seizure burden in IHKA
338 females.

339 Sex differences have been observed across multiple animal models of TLE and seizure
340 induction (Christian et al., 2020). For example, female rats typically have longer latency to acute
341 seizures and lower mortality rates after systemic pilocarpine injection (Mejías-Aponte et al.,
342 2002). In another study, male rats treated systemically with pilocarpine developed longer lasting
343 seizures over a period of several months, whereas female rats tended to have higher frequency
344 of seizures (Matovu and Cavalheiro, 2022). In a systemic kainic acid injection seizure induction
345 model, male rats had greater susceptibility to seizures than their female counterparts (Mejías-
346 Aponte et al., 2002). In mice, however, the results regarding systemic KA injection have been
347 mixed, with one study reporting higher mortality rates, more severe seizures, and increased
348 neurodegeneration in females (Zhang et al., 2008), but another study describing higher
349 mortality, seizure severity, cognitive impairment, hippocampal neuron loss, and reactive gliosis
350 in males (Li and Liu, 2019). With respect to chronic epilepsy in the IHKA model, one report
351 indicated that female mice do not show the same latent period duration following IHKA injection
352 as males, and that hippocampal paroxysmal discharges were rare in females when examined 4
353 weeks after injection (Twele et al., 2016). By contrast, another study conducted using the IHKA
354 model did not indicate an effect of sex on seizures, cognitive impairment, or histopathology
355 (Zeidler et al., 2018), although this latter study was not powered to evaluate sex differences.
356 Here, males showed reduced overall seizure burden compared to females, in contrast to other

357 IHKA studies in mice. However, the effect of sex in animal models of epilepsy can vary greatly,
358 influenced by factors such as methodology of seizure induction, measurement criteria for
359 seizures, the species and/or strain of animal, and the age at which seizures are induced
360 (Christian et al., 2020; Scharfman and MacLusky, 2014).

361 Once brain tissue was harvested at 4 mpi, the level of granule cell dispersion was
362 quantified and correlated to the percentage of time spent in seizures for each animal. The lack
363 of correlations observed between granule cell dispersion and seizure burden is in agreement
364 with other recent studies (Lisgaras and Scharfman, 2022). Although pyramidal cell layer length
365 has been correlated with seizure severity in IHKA mice, this relationship was shown in a cohort
366 in which seizure activity was monitored at the cortical surface using subdural screw electrodes
367 (Lisgaras and Scharfman, 2022). A similar analysis could not be done using the present dataset
368 due to the location of the LFP electrode in the hippocampus. Another limitation is that the tissue
369 could only be collected after all LFP recordings were complete at 4 mpi. In this regard,
370 lateralized differences in granule cell dispersion at 2 mpi were previously shown in a cohort of
371 animals that were not implanted with LFP electrodes (Cutia et al., 2022). As we observed a
372 decrease in lateralized effects in seizure parameters at 4 mpi, it remains possible that granule
373 cell dispersion (and/or other signs of hippocampal sclerosis) and seizure burden are correlated
374 at 2 mpi.

375 The differences in seizure patterns of IHKA-L and -R mice on certain estrous cycle
376 stages may arise due to interactions between fluctuating ovarian hormones and basal
377 differences in the left and right hippocampi. For example, the left and right murine hippocampi
378 have distinct populations of synapses (El-Gaby et al., 2015; Shinohara et al., 2008), and there is
379 evidence for left-right asymmetry in hippocampal function in mice (Shipton et al., 2014). There is
380 also evidence that gonadal hormones can impact hippocampal lateralization, with androgen
381 receptor signaling suggested to underlie larger granule cell layer volume in the right

382 hippocampus of both male and female C57BL/6J mice (Tabibnia et al., 1999). Therefore, it is
383 possible that the fluctuations in ovarian hormones across the estrous cycle, and lateralized
384 interactions between ovarian hormones and the hippocampus, produce left-right asymmetry in
385 emergent hippocampal phenotypes. In mice, the $P_4:E_2$ ratio is higher on diestrus than on
386 proestrus and estrus (Christian et al., 2020; Walmer et al., 1992), and lower $P_4:E_2$ may promote
387 seizure activity in humans (Bonuccellia et al., 1989; Herzog, 2015; Herzog et al., 1997). A
388 limitation of the lack of correlation between chronic seizure burden and progesterone/estradiol
389 levels in the present study is that the hormones were measured in blood samples collected at 4
390 mpi, whereas the left-right asymmetry in seizure burden was most prominent at 2 mpi. However,
391 previous work indicated a lack of difference in serum progesterone and estradiol, and $P_4:E_2$
392 ratio, between IHKA-L and IHKA-R diestrous females at 2 mpi (Cutia et al., 2022), suggesting
393 that a relationship between circulating levels of these hormones and left-right asymmetry
394 seizure burden at 2 mpi is unlikely. However, a differential response of left vs. right
395 hippocampus to estradiol and/or progesterone actions cannot be ruled out, nor can differences
396 in local hippocampal synthesis of these hormones or their metabolites (Brann et al., 2022; Sato
397 and Woolley, 2016; Tokuda et al., 2011).

398 Previous work established that the estrous cycle can have impacts on interictal spike
399 presentation in systemic kainic acid and pilocarpine models of TLE in rats (D'Amour et al.,
400 2015), and IHKA mice can exhibit elevated seizure duration and time spent in seizures on
401 proestrus and estrus combined compared with diestrus (Li et al., 2020). Importantly, most IHKA
402 female mice exhibit elongated estrous cycles (Cutia et al., 2022; Ingram et al., 2022; Li et al.,
403 2020, 2018, 2017; Li and Christian-Hinman, 2022). Here, estrous cycle length was not
404 significantly correlated to seizure burden in either IHKA-L or -R mice, consistent with previous
405 reports (Li et al., 2020). Therefore, it does not appear that higher epilepsy severity drives
406 greater estrous cycle disruption, nor is there a reciprocal relationship of cycle disruption

407 promoting increased seizure burden in the IHKA model. In previous work examining mice
408 without LFP recordings, estrous cycle disruption persisted to 4 mpi in IHKA-R but not IHKA-L
409 females (Cutia et al., 2022). The dissipation in phenotypes in IHKA-L females suggests that
410 asymmetries may be influenced by the progression of the disease, the increased age of the
411 animals, or both. The progressive decline of certain physiological functions as mice age could
412 shape the varied patterns seen at different timepoints. It is also possible that the observed
413 differences at 2 mpi are indicative of differential rates of disease progression that eventually
414 normalize, such that seizure severity lacks overt sex- and hemisphere-based differences by 4
415 mpi. In total, these findings suggest that injection site and time of recording after injection in
416 IHKA female mice are factors to be considered in experimental design that can shape overall
417 seizure burden outcomes.

418 The female-specific presence of seizure burden asymmetries aligns with clinical findings
419 of reproductive endocrine disorder development among TLE patients. Male and female patients
420 with TLE often develop reproductive endocrine dysfunction (Herzog et al., 1986a, 1986b), but
421 females more consistently develop higher rates of dysfunction than the general population
422 (Herzog et al., 1986b; Herzog and Schachter, 2001). Moreover, the propensities for developing
423 polycystic ovary syndrome or hypothalamic amenorrhea are differentially based on the
424 hemisphere of the seizure focus in females (Herzog, 1993; Kalinin and Zheleznova, 2007).
425 These findings indicate that there may be differential mechanisms in the left and right
426 hemispheres that contribute to the ability of seizures to promote reproductive endocrine
427 dysfunction. Furthermore, the different patterns of seizure clustering in association with
428 menstrual cycle phase in females with left- or right-sided TLE (Herzog, 2008; Quigg et al., 2009)
429 suggest that left-right asymmetry can shape the presentation of both epilepsy and associated
430 comorbidities. The present findings of female-specific asymmetry in seizure presentation are
431 thus consistent with clinical reports, and support the translational validity of the IHKA mouse

432 model in recapitulating at least some mechanistic differences in structural or functional changes
433 due to seizures in the left and right hippocampus, as well as sex differences in this lateralization.

434 The IHKA mouse model displays many characteristics recapitulating human epilepsy,
435 underscoring the validity of its use in investigating neural mechanisms of TLE. The present work
436 indicates that seizure burden outcomes within this preclinical model are influenced by sex and,
437 in females, laterality of epileptogenic insult. Female mice also show estrous cycle-associated
438 changes in seizure activity that vary depending on the hemisphere of targeted hippocampal
439 damage. This work thus reveals female-specific left-right asymmetry in functional properties of
440 the murine hippocampus, with implications for both normal and disease states.

441

442 **References**

- 443 Addis DR, Moscovitch M, McAndrews MP (2007) Consequences of hippocampal damage
444 across the autobiographical memory network in left temporal lobe epilepsy. *Brain*
445 130:2327–2342.
- 446 Alessio A, Bonilha L, Rorden C, Kobayashi E, Min LL, Damasceno BP, Cendes F (2006)
447 Memory and language impairments and their relationships to hippocampal and perirhinal
448 cortex damage in patients with medial temporal lobe epilepsy. *Epilepsy & Behavior*
449 8:593–600.
- 450 Armstrong C, Krook-Magnuson E, Oijala M, Soltesz I (2013) Closed-loop optogenetic
451 intervention in mice. *Nat Protoc* 8:1475–1493.
- 452 Bonuccella U, Melis GB, Paoletti AM, Fioretti P, Murri L, Muratorio A (1989) Unbalanced
453 progesterone and estradiol secretion in catamenial epilepsy. *Epilepsy Research* 3:100–
454 106.
- 455 Bouillere V, Ridoux V, Depaulis A, Marescaux C, Nehlig A, Le Gal La Salle G (1999) Recurrent
456 seizures and hippocampal sclerosis following intrahippocampal kainate injection in adult
457 mice: electroencephalography, histopathology and synaptic reorganization similar to
458 mesial temporal lobe epilepsy. *Neuroscience* 89:717–729.
- 459 Brann DW, Lu Y, Wang J, Zhang Q, Thakkar R, Sareddy GR, Pratap UP, Tekmal RR,
460 Vadlamudi RK (2022) Brain-derived estrogen and neural function. *Neuroscience &*
461 *Biobehavioral Reviews* 132:793–817.
- 462 Choi H, Detyniecki K, Bazil C, Thornton S, Crosta P, Tolba H, Muneeb M, Hirsch LJ, Heinzen
463 EL, Sen A, Depondt C, Perucca P, Heiman GA, EPIGEN Consortium (2020)
464 Development and validation of a predictive model of drug-resistant genetic generalized
465 epilepsy. *Neurology* 95:e2150–e2160.
- 466 Christian CA, Reddy DS, Maguire J, Forcelli PA (2020) Sex Differences in the Epilepsies and
467 Associated Comorbidities: Implications for Use and Development of Pharmacotherapies.
468 *Pharmacol Rev* 72:767–800.
- 469 Cutia CA, Leverton LK, Ge X, Youssef R, Raetzman LT, Christian-Hinman CA (2022)
470 Phenotypic differences based on lateralization of intrahippocampal kainic acid injection
471 in female mice. *Experimental Neurology* 355:114118.
- 472 D'Amour J, Magagna-Poveda A, Moretto J, Friedman D, LaFrancois JJ, Pearce P, Fenton AA,
473 MacLusky NJ, Scharfman HE (2015) Interictal spike frequency varies with ovarian cycle
474 stage in a rat model of epilepsy. *Exp Neurol* 269:102–119.
- 475 El-Gaby M, Shipton OA, Paulsen O (2015) Synaptic Plasticity and Memory: New Insights from
476 Hippocampal Left–Right Asymmetries. *Neuroscientist* 21:490–502.
- 477 Engel J (2001) Mesial temporal lobe epilepsy: what have we learned? *Neuroscientist* 7:340–
478 352.
- 479 French JA, Williamson PD, Thadani VM, Darcey TM, Mattson RH, Spencer SS, Spencer DD
480 (1993) Characteristics of medial temporal lobe epilepsy: I. Results of history and
481 physical examination. *Annals of Neurology* 34:774–780.
- 482 Gazzaniga MS (1995) Principles of human brain organization derived from split-brain studies.
483 *Neuron* 14:217–228.
- 484 Gloe LM, Kashy DA, Jacobs EG, Klump KL, Moser JS (2021) Examining the role of ovarian
485 hormones in the association between worry and working memory across the menstrual
486 cycle. *Psychoneuroendocrinology* 131:105285.
- 487 Gröticke I, Hoffmann K, Löscher W (2008) Behavioral alterations in a mouse model of temporal
488 lobe epilepsy induced by intrahippocampal injection of kainate. *Exp Neurol* 213:71–83.

- 489 Herzog AG (2015) Catamenial epilepsy: Update on prevalence, pathophysiology and treatment
490 from the findings of the NIH Progesterone Treatment Trial. *Seizure, Gender Issues in*
491 *Epilepsy* 28:18–25.
- 492 Herzog AG (2008) Catamenial epilepsy: definition, prevalence pathophysiology and treatment.
493 *Seizure* 17:151–159.
- 494 Herzog AG (1993) A relationship between particular reproductive endocrine disorders and the
495 laterality of epileptiform discharges in women with epilepsy. *Neurology* 43:1907–1910.
- 496 Herzog AG, Klein P, Ransil BJ (1997) Three patterns of catamenial epilepsy. *Epilepsia*
497 38:1082–1088.
- 498 Herzog AG, Schachter SC (2001) Valproate and the polycystic ovarian syndrome: final
499 thoughts. *Epilepsia* 42:311–315.
- 500 Herzog AG, Seibel MM, Schomer DL, Vaitukaitis JL, Geschwind N (1986a) Reproductive
501 endocrine disorders in men with partial seizures of temporal lobe origin. *Arch Neurol*
502 43:347–350.
- 503 Herzog AG, Seibel MM, Schomer DL, Vaitukaitis JL, Geschwind N (1986b) Reproductive
504 endocrine disorders in women with partial seizures of temporal lobe origin. *Arch Neurol*
505 43:341–346.
- 506 Howard LR, Kumaran D, Ólafsdóttir HF, Spiers HJ (2011) Double Dissociation between
507 Hippocampal and Parahippocampal Responses to Object–Background Context and
508 Scene Novelty. *J Neurosci* 31:5253–5261.
- 509 Ingram RJ, Leverton LK, Daniels VC, Li J, Christian-Hinman CA (2022) Increased GABA
510 transmission to GnRH neurons after intrahippocampal kainic acid injection in mice is
511 sex-specific and associated with estrous cycle disruption. *Neurobiol Dis* 172:105822.
- 512 Jordan JT (2020) The rodent hippocampus as a bilateral structure: A review of hemispheric
513 lateralization. *Hippocampus* 30:278–292.
- 514 Kalinin VV, Zheleznova EV (2007) Chronology and evolution of temporal lobe epilepsy and
515 endocrine reproductive dysfunction in women: Relationships to side of focus and
516 catameniality. *Epilepsy Behav* 11:185–191.
- 517 Klein S, Bankstahl M, Löscher W (2015) Inter-individual variation in the effect of antiepileptic
518 drugs in the intrahippocampal kainate model of mesial temporal lobe epilepsy in mice.
519 *Neuropharmacology* 90:53–62.
- 520 Li F, Liu L (2019) Comparison of kainate-induced seizures, cognitive impairment and
521 hippocampal damage in male and female mice. *Life Sciences* 232:116621.
- 522 Li J, Christian-Hinman CA (2022) Epilepsy-associated increase in gonadotropin-releasing
523 hormone neuron firing in diestrous female mice is independent of chronic seizure burden
524 severity. *Epilepsy Research* 184:106948.
- 525 Li J, Kim JS, Abejuela VA, Lamano JB, Klein NJ, Christian CA (2017) Disrupted female estrous
526 cyclicity in the intrahippocampal kainic acid mouse model of temporal lobe epilepsy.
527 *Epilepsia Open* 2:39–47.
- 528 Li J, Leverton LK, Naganathanahalli LM, Christian-Hinman CA (2020) Seizure burden fluctuates
529 with the female reproductive cycle in a mouse model of chronic temporal lobe epilepsy.
530 *Exp Neurol* 334:113492.
- 531 Li J, Robare JA, Gao L, Ghane MA, Flaws JA, Nelson ME, Christian CA (2018) Dynamic and
532 Sex-Specific Changes in Gonadotropin-Releasing Hormone Neuron Activity and
533 Excitability in a Mouse Model of Temporal Lobe Epilepsy. *eNeuro* 5:ENEURO.0273-
534 18.2018.
- 535 Lisgaras CP, Scharfman HE (2022) Robust chronic convulsive seizures, high frequency
536 oscillations, and human seizure onset patterns in an intrahippocampal kainic acid model
537 in mice. *Neurobiology of Disease* 166:105637.

- 538 Maguire EA, Frith CD (2003) Lateral Asymmetry in the Hippocampal Response to the
539 Remoteness of Autobiographical Memories. *J Neurosci* 23:5302–5307.
- 540 Mathern GW, Babb TL, Vickrey BG, Melendez M, Pretorius JK (1995) The clinical-pathogenic
541 mechanisms of hippocampal neuron loss and surgical outcomes in temporal lobe
542 epilepsy. *Brain* 118:105–118.
- 543 Matovu D, Cavalheiro EA (2022) Differences in Evolution of Epileptic Seizures and
544 Topographical Distribution of Tissue Damage in Selected Limbic Structures Between
545 Male and Female Rats Submitted to the Pilocarpine Model. *Front Neurol* 13:802587.
- 546 Mejías-Aponte CA, Jiménez-Rivera CA, Segarra AC (2002) Sex differences in models of
547 temporal lobe epilepsy: role of testosterone. *Brain Research* 944:210–218.
- 548 Nilsson ME, Vandenput L, Tivesten Å, Norlén A-K, Lagerquist MK, Windahl SH, Börjesson AE,
549 Farman HH, Poutanen M, Benrick A, Maliqueo M, Stener-Victorin E, Ryberg H, Ohlsson
550 C (2015) Measurement of a Comprehensive Sex Steroid Profile in Rodent Serum by
551 High-Sensitive Gas Chromatography-Tandem Mass Spectrometry. *Endocrinology*
552 156:2492–2502.
- 553 Pantier LK, Li J, Christian CA (2019) Estrous Cycle Monitoring in Mice with Rapid Data
554 Visualization and Analysis. *Bio Protoc* 9:e3354.
- 555 Phuong TH, Houot M, Méré M, Denos M, Samson S, Dupont S (2021) Cognitive impairment in
556 temporal lobe epilepsy: contributions of lesion, localization and lateralization. *J Neurol*
557 268:1443–1452.
- 558 Quigg M, Smithson SD, Fowler KM, Sursal T, Herzog AG, NIH Progesterone Trial Study Group
559 (2009) Laterality and location influence catamenial seizure expression in women with
560 partial epilepsy. *Neurology* 73:223–227.
- 561 Riban V, Bouillieret V, Pham-Lê BT, Fritschy J-M, Marescaux C, Depaulis A (2002) Evolution of
562 hippocampal epileptic activity during the development of hippocampal sclerosis in a
563 mouse model of temporal lobe epilepsy. *Neuroscience* 112:101–111.
- 564 Rusina E, Bernard C, Williamson A (2021) The Kainic Acid Models of Temporal Lobe Epilepsy.
565 *eNeuro* 8:ENEURO.0337-20.2021.
- 566 Sato SM, Woolley CS (2016) Acute inhibition of neurosteroid estrogen synthesis suppresses
567 status epilepticus in an animal model. *eLife* 5:e12917.
- 568 Schaapsmeeders P, van Uden IWM, Tuladhar AM, Maaijwee NAM, van Dijk EJ, Rutten-Jacobs
569 LCA, Arntz RM, Schoonderwaldt HC, Dorresteyn LDA, de Leeuw F-E, Kessels RPC
570 (2015) Ipsilateral hippocampal atrophy is associated with long-term memory dysfunction
571 after ischemic stroke in young adults. *Human Brain Mapping* 36:2432–2442.
- 572 Scharfman HE, MacLusky NJ (2014) Sex differences in the neurobiology of epilepsy: a
573 preclinical perspective. *Neurobiol Dis* 72 Pt B:180–192.
- 574 Shinohara Y, Hirase H, Watanabe M, Itakura M, Takahashi M, Shigemoto R (2008) Left-right
575 asymmetry of the hippocampal synapses with differential subunit allocation of glutamate
576 receptors. *Proc Natl Acad Sci U S A* 105:19498–19503.
- 577 Shipton OA, El-Gaby M, Apergis-Schoute J, Deisseroth K, Bannerman DM, Paulsen O, Kohl
578 MM (2014) Left–right dissociation of hippocampal memory processes in mice. *PNAS*
579 111:15238–15243.
- 580 Tabibnia G, Cooke BM, Breedlove SM (1999) Sex difference and laterality in the volume of
581 mouse dentate gyrus granule cell layer. *Brain Res* 827:41–45.
- 582 Taylor CM, Pritschet L, Olsen RK, Layher E, Santander T, Grafton ST, Jacobs EG (2020)
583 Progesterone shapes medial temporal lobe volume across the human menstrual cycle.
584 *Neuroimage* 220:117125.

- 585 Tokuda K, Izumi Y, Zorumski CF (2011) Ethanol Enhances Neurosteroidogenesis in
586 Hippocampal Pyramidal Neurons by Paradoxical NMDA Receptor Activation. *J Neurosci*
587 31:9905–9909.
- 588 Twele F, Töllner K, Brandt C, Löscher W (2016) Significant effects of sex, strain, and anesthesia
589 in the intrahippocampal kainate mouse model of mesial temporal lobe epilepsy. *Epilepsy*
590 & Behavior 55:47–56.
- 591 Walmer DK, Wrona MA, Hughes CL, Nelson KG (1992) Lactoferrin expression in the mouse
592 reproductive tract during the natural estrous cycle: correlation with circulating estradiol
593 and progesterone. *Endocrinology* 131:1458–1466.
- 594 Zeidler Z, Brandt-Fontaine M, Leintz C, Krook-Magnuson C, Netoff T, Krook-Magnuson E
595 (2018) Targeting the Mouse Ventral Hippocampus in the Intrahippocampal Kainic Acid
596 Model of Temporal Lobe Epilepsy. *eNeuro* 5.
- 597 Zhang X-M, Zhu S-W, Duan R-S, Mohammed AH, Winblad B, Zhu J (2008) Gender differences
598 in susceptibility to kainic acid-induced neurodegeneration in aged C57BL/6 mice.
599 *NeuroToxicology* 29:406–412.

600

601

602

603 **Figure Legends**

604 **Figure 1. Sex differences in seizure parameters at 2 months after IHKA injection.** A) Example seizure traces. B-D) Circles represent individual values of the average number of seizures per hour (B), average seizure duration (C), and average percent time in seizure (D) for each mouse plotted with mean \pm SEM in black lines. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ two-way ANOVA and Tukey's *post hoc* tests.

609

610 **Figure 2. Higher seizure duration in females at 4 months after IHKA injection.** A-C) Circles represent individual values of the average number of seizures per hour (A), average seizure duration (B), and average percent time in seizure (C) for each mouse plotted with mean \pm SEM in black lines. * $p < 0.05$, ** $p < 0.01$ two-way ANOVA and Tukey's *post hoc* tests.

614

615 **Figure 3. Seizure burden is elevated in IHKA-L females on diestrus 2 months after IHKA injection.** A-C) Circles represent individual values of the average number of seizures per hour (A), average seizure duration (B), and average percent time in seizure (C) for each IHKA female, plotted with mean \pm SEM in black lines. * $p < 0.05$, ** $p < 0.01$ two-sample t-test or Wilcoxon ranked sum test based on data normality.

620

621 **Figure 4. Lateralization and cycle stage effects on seizure burden in females are dampened at 4 months after IHKA injection.** A-C) Circles represent individual values of the average number of seizures per hour (A), average seizure duration (B), and average percent time in seizure (C) for each IHKA female, plotted with mean \pm SEM in black lines.

625 **Figure 5. Seizure burden severity is not correlated to the degree of granule cell**
626 **dispersion at 4 mpi.** A) Representative images of Nissl staining, illustrating granule cell
627 dispersion, CA1 cell death, and damage from LFP electrode implantation. Scale bar = 1 mm. B)
628 Proportions of mice in each group that did (colored bars) or did not (black bars) display granule
629 cell dispersion by visual inspection. C) Relationship of the percent time in seizure as a function
630 of the ipsilateral-to-contralateral (I:C) granule cell area ratio. Circles indicate individual mice.
631 Solid lines indicate the linear regression of best fit.

632

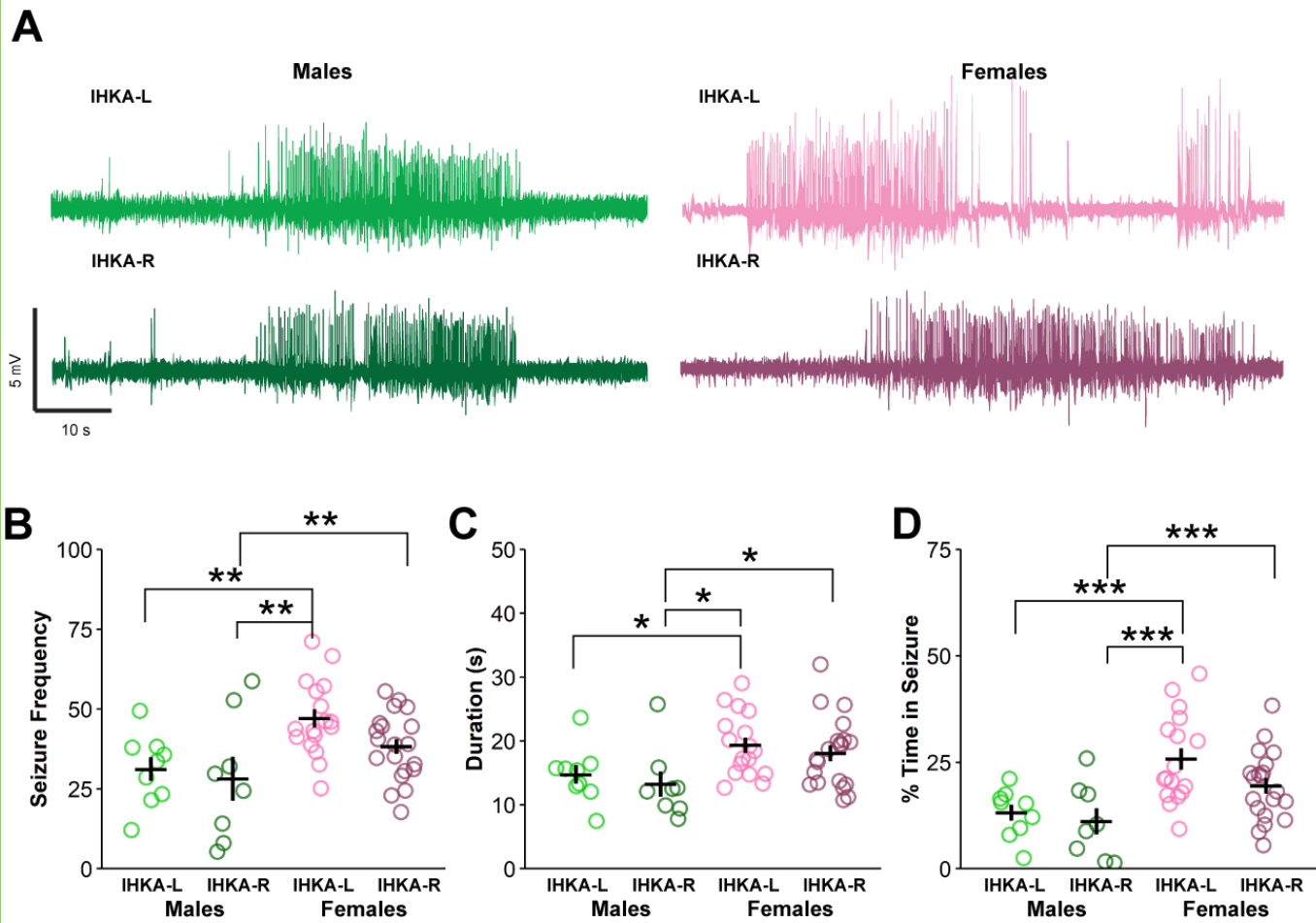
633 **Figure 6. Seizure burden severity is not correlated to the degree of estrous cycle**
634 **disruption.** Percent of time in seizure as a function of estrous cycle length for each mouse
635 evaluated at 2- (left) and 4-months post injection (right) in IHKA-L (top) and IHKA-R (bottom)
636 groups. Circles indicate individual mice. Solid lines indicate the linear regression of best fit.

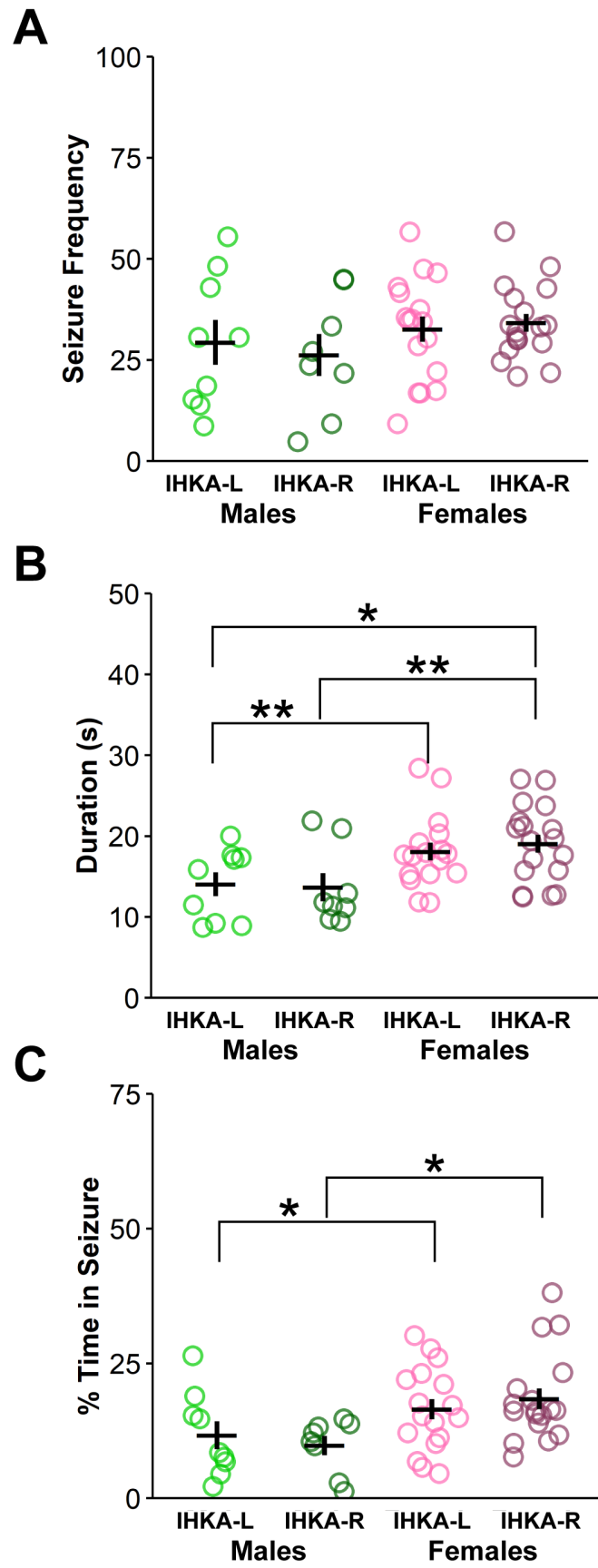
637

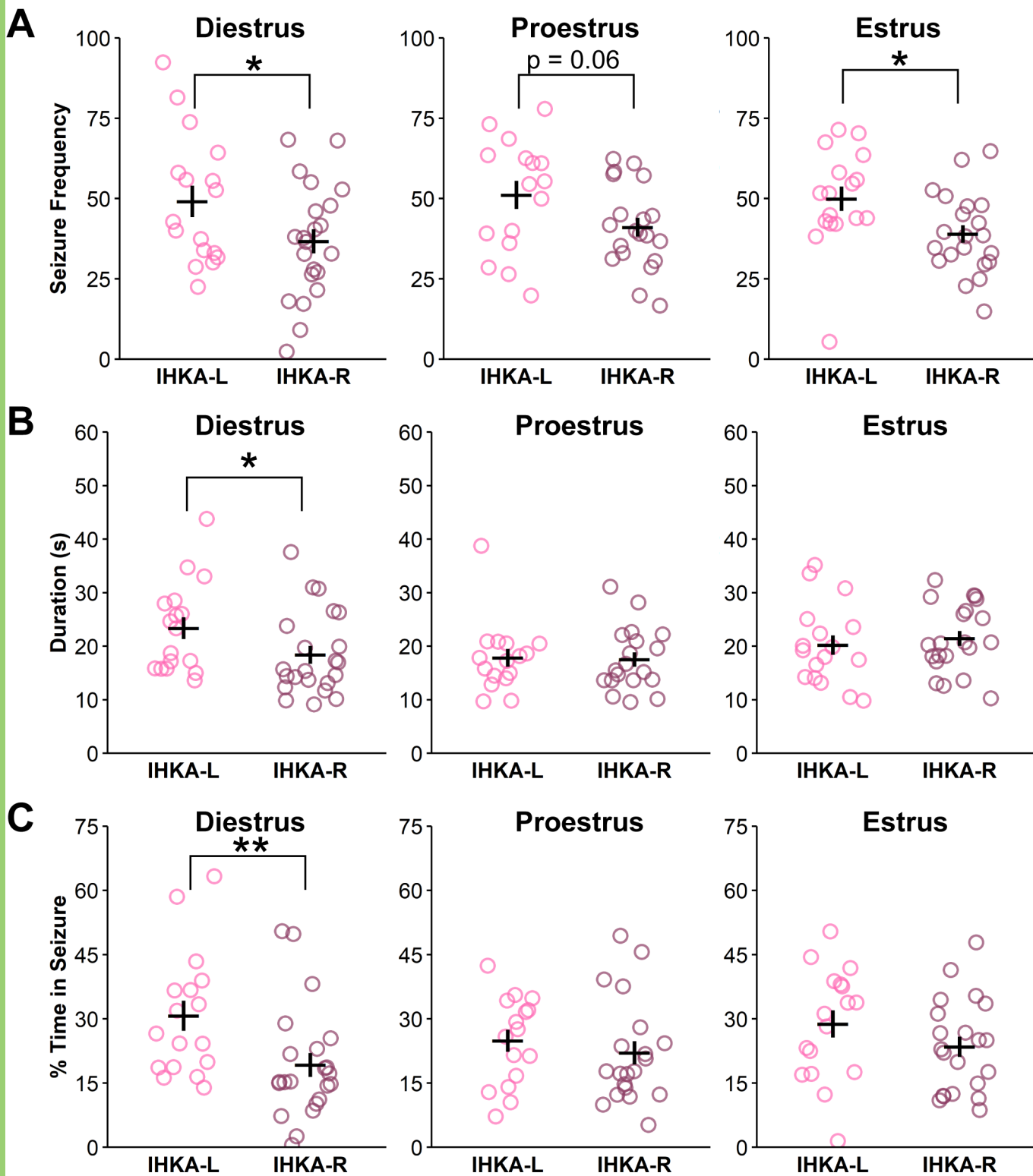
638 **Figure 7. Circulating levels of ovarian hormones on diestrus are not correlated with**
639 **seizure burden severity at 4 mpi.** A-C) Percentage of time spent in seizures as a function of
640 serum progesterone (A), estradiol (B), and $P_4:E_2$ ratio (C) levels of hormones in circulation for
641 each mouse in IHKA-L (left) and IHKA-R (right) groups. Circles indicate individual mice. Solid
642 lines indicate the linear regression of best fit.

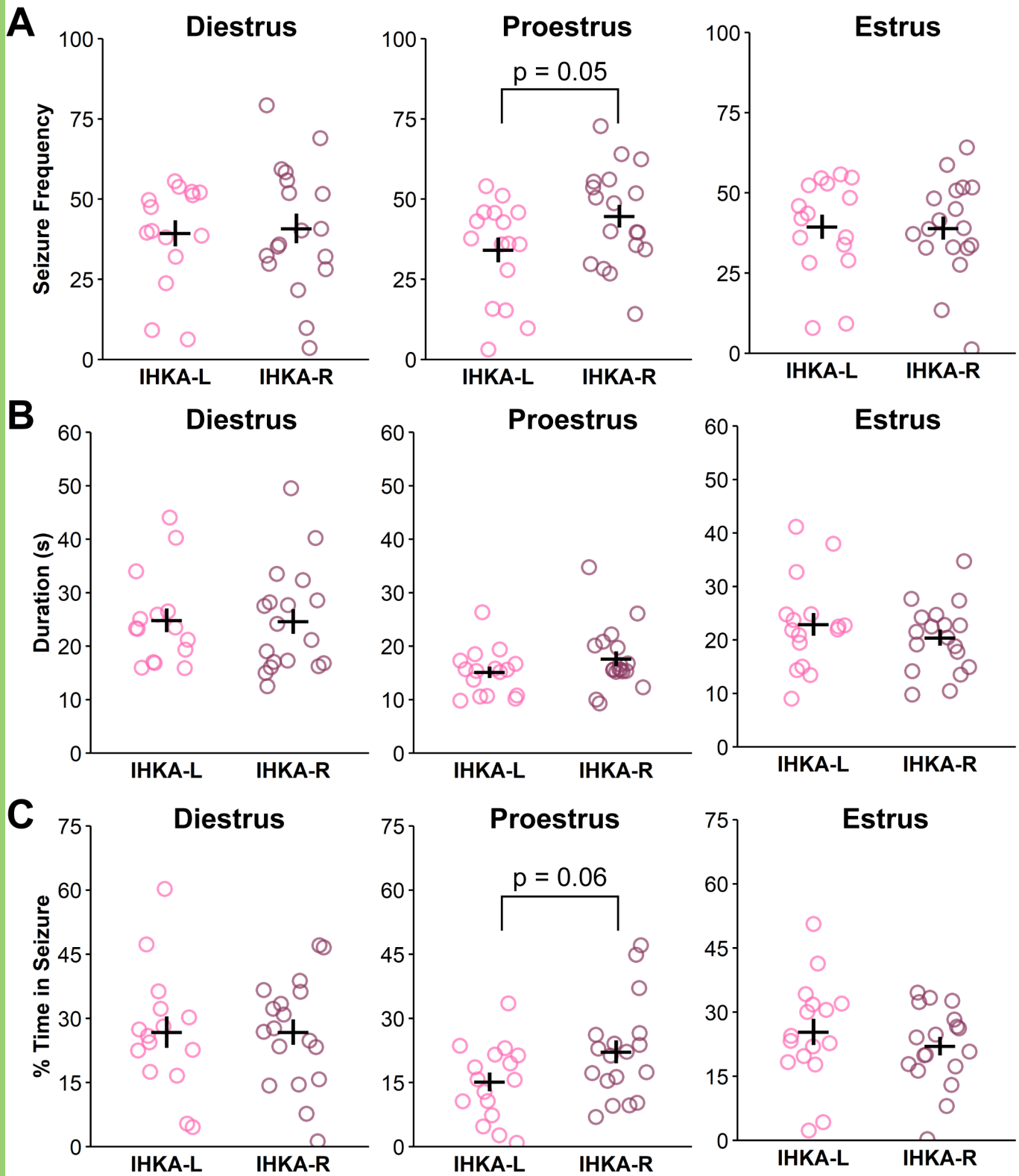
643

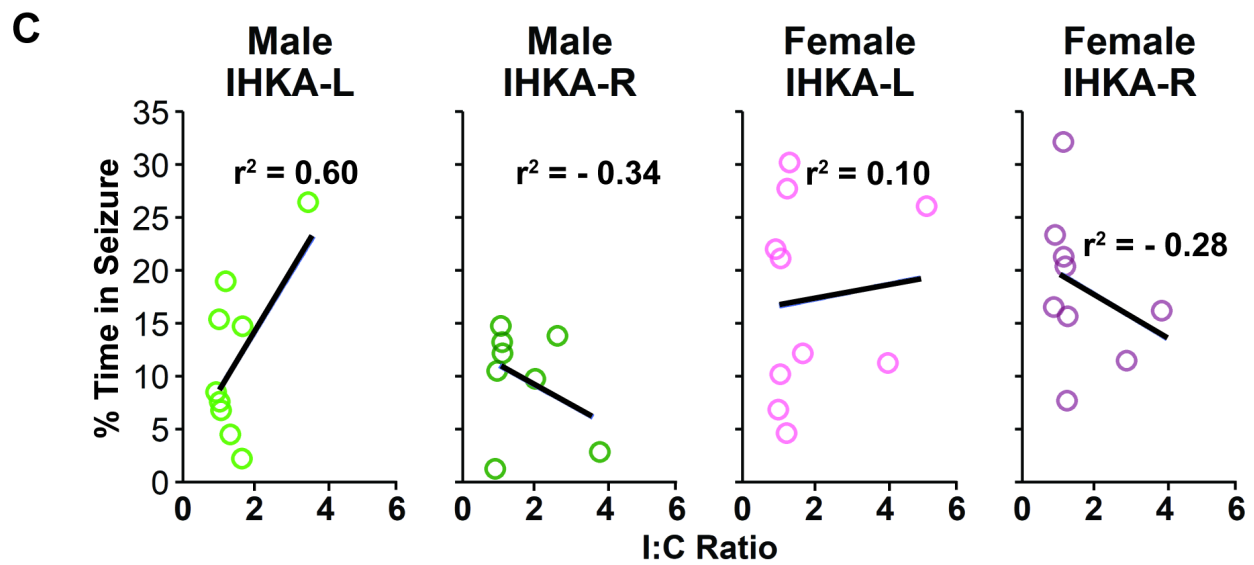
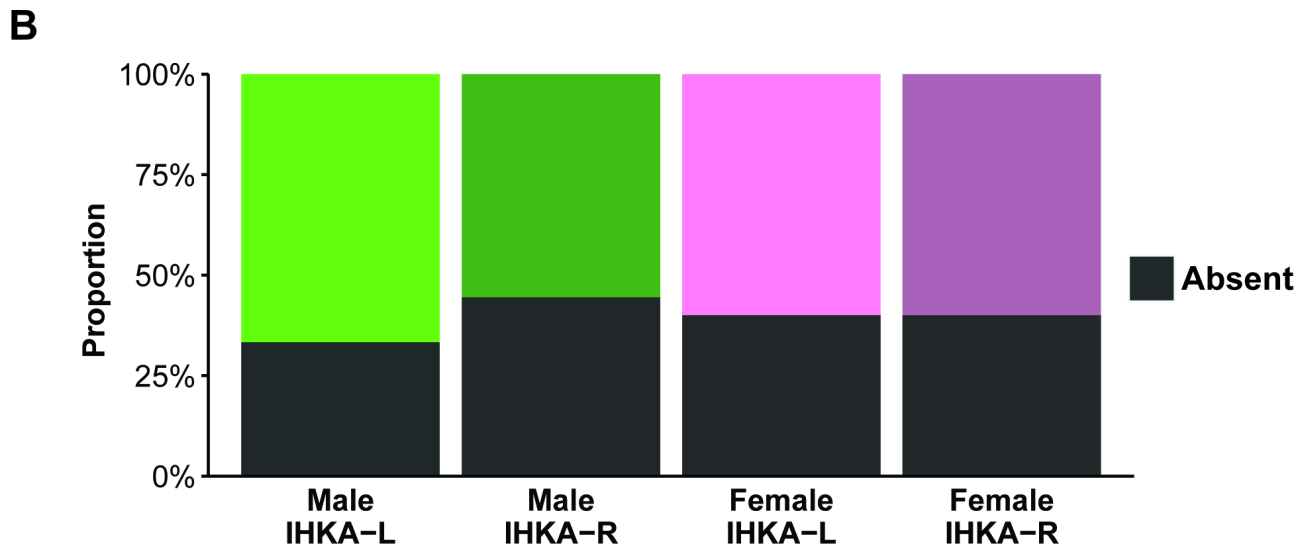
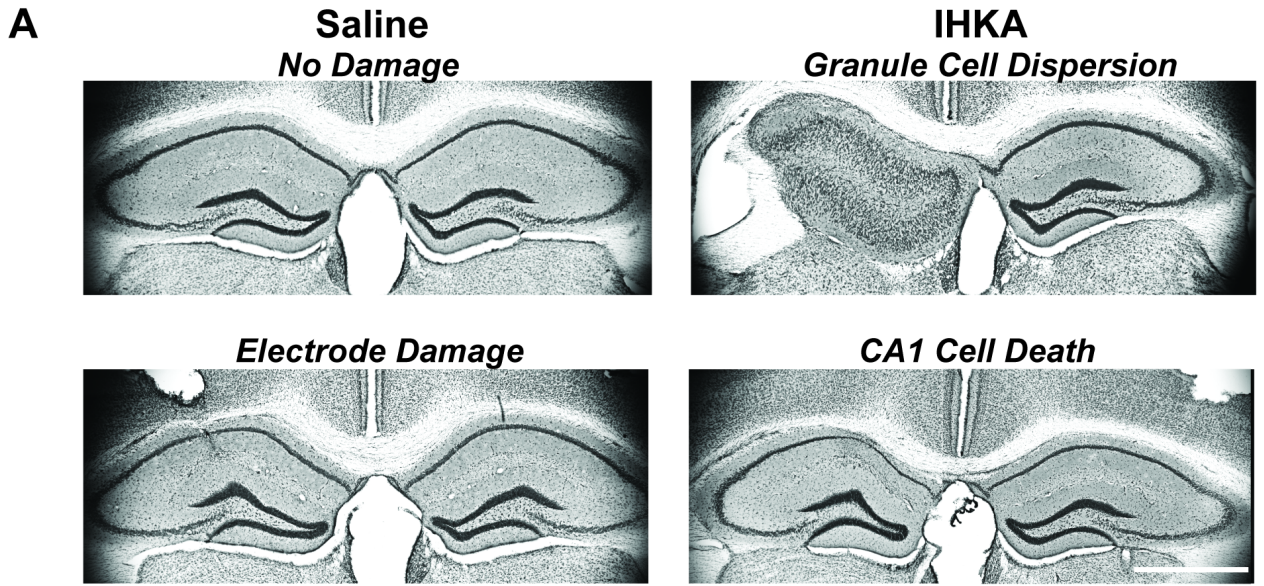
644

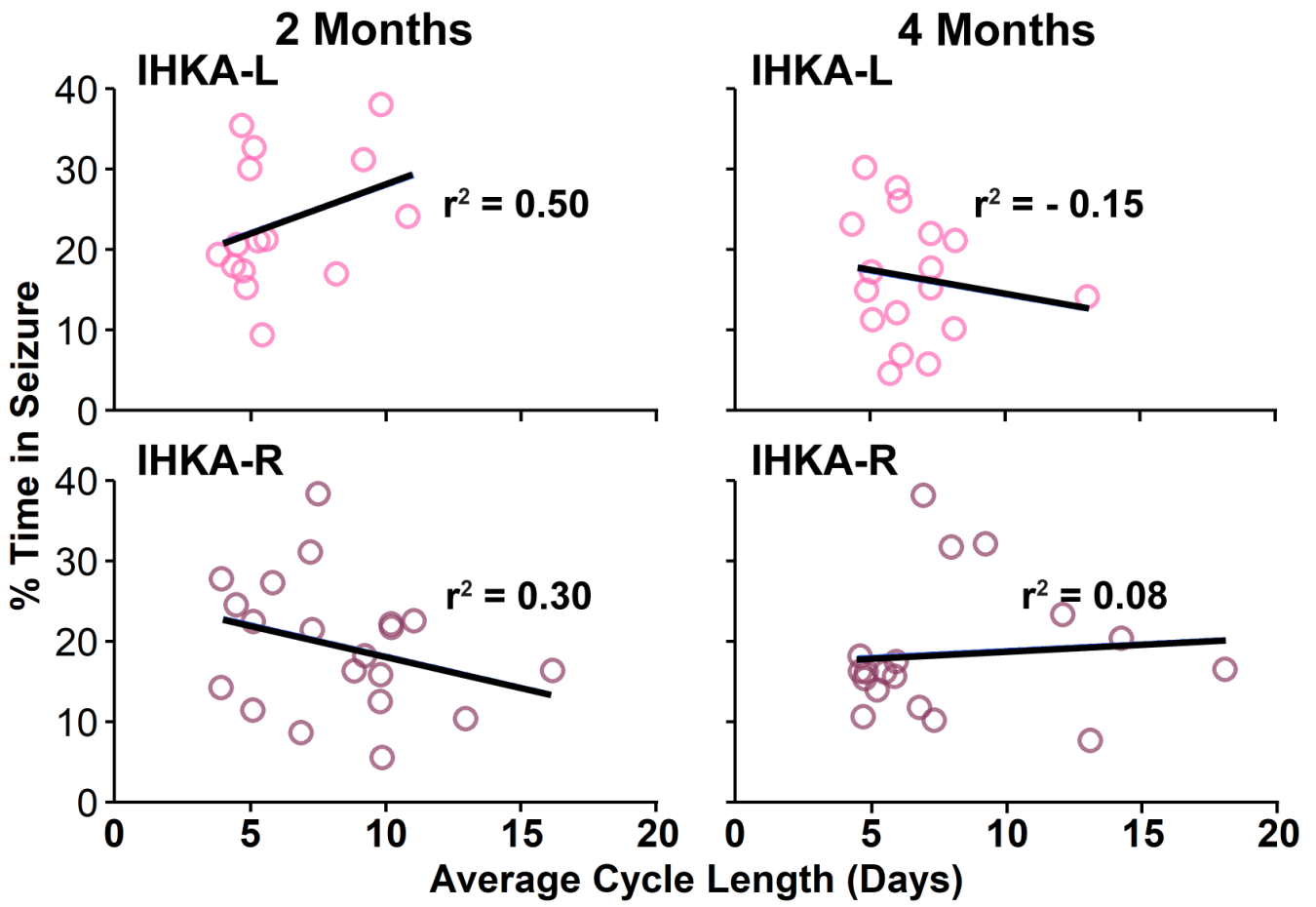












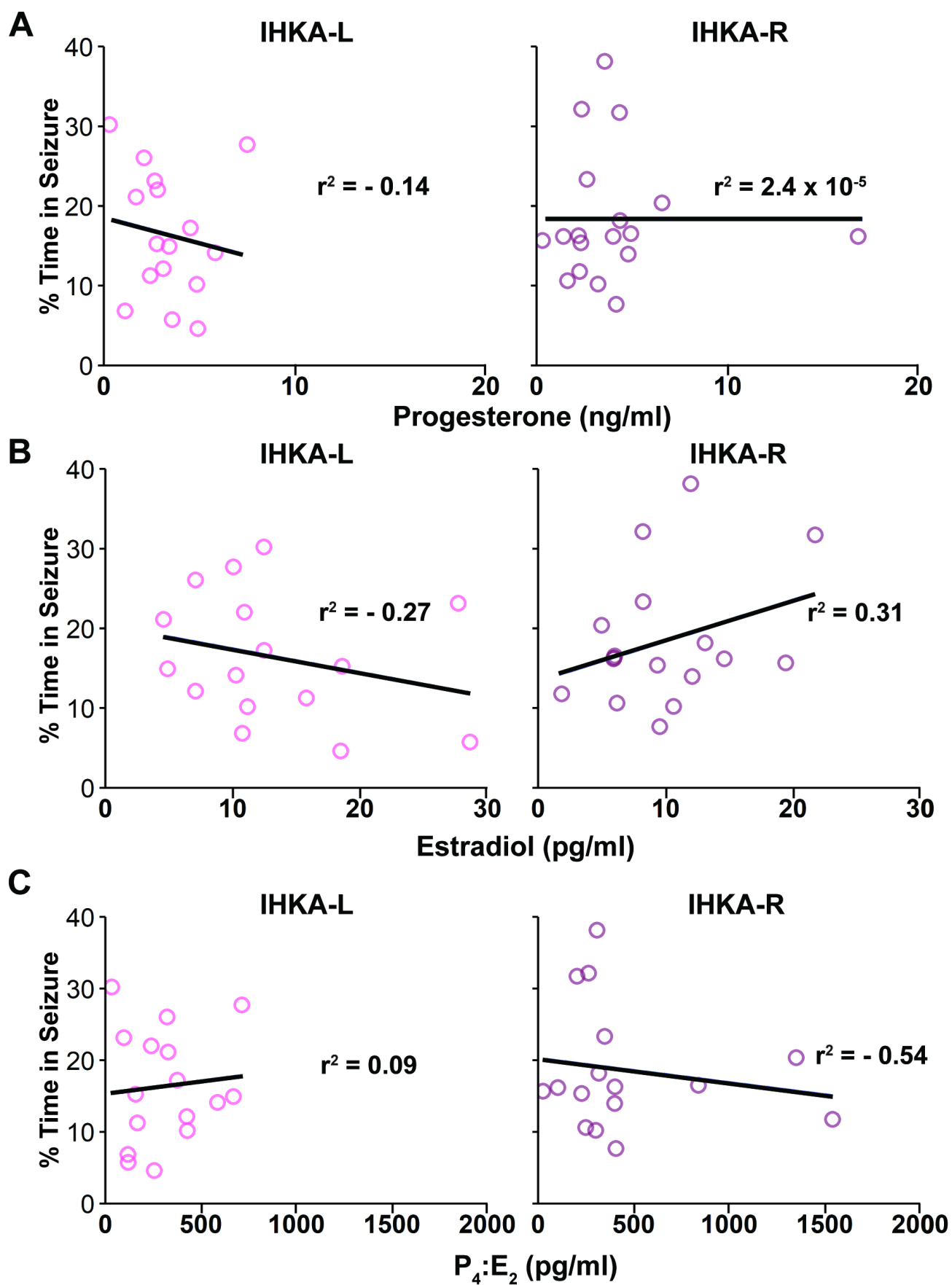


Figure	Graph	Data Structure	Test	p Value	95% Confidence Interval
Figure 1B	IHKA-L males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.005	23.96 to 38.42 and 41.46 to 57.72
Figure 1B	IHKA-R males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.005	14.60 to 41.80 and 33.88 to 42.66
Figure 1B	IHKA-R males vs. IHKA-L females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.002	14.60 to 41.80 and 41.46 to 57.72
Figure 1C	IHKA-L males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.02	11.94 to 17.58 and 17.01 to 21.67
Figure 1C	IHKA-R males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.02	9.36 to 17.16 and 15.65 to 20.51
Figure 1C	IHKA-R males vs. IHKA-L females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.03	9.36 to 17.16 and 17.01 to 21.67
Figure 1D	IHKA-L males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.0007	9.43 to 16.87 and 20.87 to 20.71
Figure 1D	IHKA-R males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.0007	5.03 to 17.23 and 15.90 to 23.00
Figure 1D	IHKA-R males vs. IHKA-L females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.0004	5.03 to 17.23 and 20.87 to 20.71
Figure 2B	IHKA-L males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.006	11.16 to 16.92 and 15.94 to 20.22
Figure 2B	IHKA-R males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.006	10.25 to 17.07 and 16.85 to 21.25
Figure 2B	IHKA-L males vs. IHKA-R females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.03	10.25 to 17.07 and 15.94 to 20.22
Figure 2C	IHKA-L males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.02	6.58 to 16.78 and 12.81 to 20.15
Figure 2C	IHKA-R males vs. females	normally distributed	Two-way ANOVA, <i>Tukey's post hoc test</i>	0.02	6.27 to 13.29 and 14.67 to 22.19
Figure 3A	Diestrus	normally distributed	Two-sample t test	0.05	29.30 to 44.04 and 39.53 to 58.65
Figure 3A	Estrus	normally distributed	Two-sample t test	0.02	33.63 to 44.29 and 42.51 to 57.33
Figure 3B	Diestrus	non-normal distribution	Wilcoxon rank sum test	0.04	15.13 to 21.63 and 19.37 to 27.37
Figure 3C	Diestrus	non-normal distribution	Wilcoxon rank sum test	0.04	13.77 to 24.67 and 23.83 to 37.59

Table 1. Statistical table of significant results.