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BRIEF REPORT



Greater female than male resilience to mortality and morbidity in the Scn8a mouse model of pediatric epilepsy

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ABSTRACT

Aims: Females and males of all ages are affected by epilepsy; however, unlike many clinical studies, most preclinical research has focused on males. Genetic variants in the voltage-gated sodium channel gene, SCN8A, are associated with a broad spectrum of neurological and epileptic syndromes. Here we investigate sex differences in the natural history of the Scn8a-N1768D knockin mouse model of pediatric epilepsy.

Methods: We utilize 24/7 video to monitor juveniles and adults of both sexes to investigate variability in seizure activity (e.g. onset and frequency), mortality and morbidity, response to cannabinoids, and mode of death. We also monitor sleep architecture using a noninvasive piezoelectric method in order to identify factors that influence seizure severity and outcome.

Results: Both sexes had nearly 100% penetrance in seizure onset and early mortality. However, adult heterozygous (D/+) females were more resilient as exhibited by the ability to tolerate more seizures over a longer lifespan. Homozygous (D/D) juveniles did not exhibit a sex difference in overall survival. Female estrus cycle was disrupted before seizure onset, while sleep was disrupted in both sexes in association with seizure onset. Females typically died while in convulsive status epilepticus; however, a high proportion of males died while not experiencing behavioral seizures. Only juvenile and adult males benefited from cannabinoid administration.

Conclusions: These results support the hypothesis that factors associated with sexual differentiation play a role in the neurobiology of epilepsy and point to the importance of including both sexes in the design of studies to identify new epilepsy therapies.

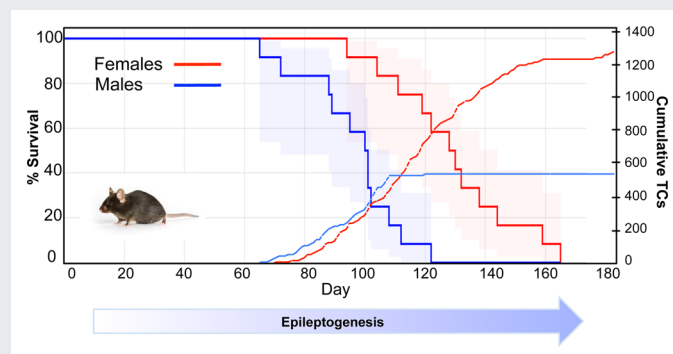
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GRAPHICAL ABSTRACT




Introduction

The epilepsies are common neurologic disorders characterized by spontaneous recurrent seizures. Females

and males of all ages are affected by epilepsy and associated comorbidities. Epidemiological studies suggest that gender may affect susceptibility to epilepsy and its prognosis [1] with a growing appreciation that

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sex differences in underlying brain function are important in the design and development of new epilepsy therapies [2,3]. Unlike clinical studies that include both sexes, most preclinical animal studies have primarily focused on males [3–6]. For those studies that have included both sexes, the results differ depending on the method used to anesthetize and/or induce seizures, or collect seizure measurements (e.g. latency to onset, severity, duration). Studies also differ depending on species, strain, age, and time of the female estrous cycle [1]. Therefore, a consensus has been difficult to obtain on the manner and mechanisms by which sex differences contribute to seizure susceptibility, as well as to mortality and morbidity in epilepsy.

The increasing number of transgenic mouse models established over the last three decades offers the opportunity for careful and controlled investigation of sex differences in the epilepsies. Unfortunately, there have not been many systematic studies of sex differences with knock-in alleles associated with pediatric epilepsy. In the case of the Dravet Syndrome model (e.g. *Scn1a*^{+/-} mice) there have been reports of females showing greater mortality than males [7], and others that found no sex differences [8]. More comprehensive screening for sex differences across genetic models is clearly needed.

De novo mutations of the voltage-gated sodium channel gene *SCN8A* have recently been associated with a wide spectrum of pediatric syndromes ranging from benign familial seizures to severe developmental and epileptic encephalopathy (DEE) [9,10]. Seizures typically develop in the first year of life with ensuing development delay, movement disorders, intellectual disability, and increased risk of sudden unexpected death in epilepsy (SUDEP) in patients [11,12]. A preclinical model was constructed in which the variant of the index patient (p.N1768D) was introduced into the mouse genome by TALEN targeting [13]. In characterizing this model, Wagnon et al. [14] reported that heterozygous (D/+) mice developed tonic-clonic seizures (TCs) between 3 to 4 months of age, exhibited ictal discharges coinciding with convulsive seizures, and suffered high rates of SUDEP [14]. However, they did not investigate sex differences in these features [14]. Here we investigate seizure characteristics (penetrance, frequency, lifetime number, and age at onset), mortality and morbidity, mode of death, over the entire lifespan of juvenile (D/D) and adult (D/+) mice of both sexes. We also examine associations between seizures and estrus cycle, sleep architecture and sex differences in responses to treatment with cannabinoids.

Materials and methods

Female and male mice on the C57BL/6J (B6) background were housed in sex-specific groups of 3–4 per cage in a pathogen-free mouse facility with a 14h light/10h dark cycle (lights turned on at 5am). Transparent polycarbonate cages were provided with bedding and a small amount of enrichment material to allow mice to be observable by camera mounted over the enclosure. All efforts were made to minimize animal stress and suffering and to reduce the number of mice used. Experiments received formal approval from the University of Arizona Institutional Animal Care and Use Committee Program (IACUC #16–160). Genotyping at the *Scn8a*-N1768D site to distinguish wildtype (+/+), heterozygous mutant (D/+), or homozygous mutant (D/D) mice was carried out as previously described [14]. A 24/7 video monitoring system was utilized to collect seizure data, with infrared illumination to monitor behavior during the dark period. Seizures were counted as individual tonic-clonic events (TCs), whether they occurred as single isolated events, as clusters (2 or more TCs per event), or as a series of TCs in a status event. The relationship between the estrus cycle and the seizure profile of female mice was examined by visual observation and on the basis of published criteria [15,16] (Figure S1). Ovariectomy (OVX) and orchietomy (ORX) surgery was performed at 4 weeks of age according to standard procedures.

Hemp oil administration

Dosing was initiated either in the neonatal period or after seizure onset when D/+ mice were adults. Oral dosing was performed by thoroughly mixing hemp-derived CBD oil (distilled CO₂ extracted Plus CBD Oil, 26:1 CBD:THC, CV Sciences, San Diego CA) with pure peanut butter in a petri dish. Mice were allowed to eat the peanut butter pellet with or without the hemp oil for a period of 24h before being given the next dose. The amount of pellet fed to mice (~1g per day per mouse) was adjusted to permit the mice to receive 100mg/kg body weight/day. A subset of mice were given 100mg/kg/day 98% pure CBD intraperitoneally (I.P.) (CV Sciences, San Diego CA). Supported by the finding that CBD is efficiently transmitted from the dam's blood to the pups through her milk [17,18], and given that untreated D/D mice have a life expectancy below 30 days, we began treatment by dosing pregnant (T0) and nursing dams (T1). This protocol allowed us to produce early treated D/+ and D/D mice and to continue dosing both D/D and D/+

mice after they were weaned. A total of 11 D/+ females and 15 D/+ males were administered cannabinoids beginning treatment at postnatal day (P) 3 (chronically dosed group). Another seven males were part of an earlier dosed group in which the dam was treated at embryonic day (E) 15–17. Juvenile D/D mice dosed at P3 included 25 female and 19 male mice. A female late-dosed group also included 24 dams that were administered hemp oil shortly after seizure onset (T2). The male late-dosed group included 9 mice, 4 of which were injected intraperitoneally with pure CBD after seizure onset (T2). No difference in survival was observed between oral and I.P. treated mice (mean survival = 112.3 ± 8.2 days (95% CI: 99.3, 125.4) versus 113.8 ± 8.6 days (95% CI: 103.1, 124.5), respectively; *t*-test *p* value = 0.396); so data from these two groups were combined for further analyses.

Sleep analysis

Sleep analysis was carried out using the method of Yaghoubi et al. [19]. The PiezoSleep Mouse Behavioral Tracking System with integrated sensor, hardware and software tracking (Signal Solutions, Lexington KY) provided real-time scoring of sleep and wake. Pre-seizure and post-seizure mice were examined individually in cages for a minimum of 24 h. SleepStats Data Explorer (Signal Solutions, Lexington KY) was used to visualize the collected data and create reports.

Data analysis

Results of statistical summaries were generally expressed as mean \pm SD. Kaplan-Meier survival curves were used to test for differences in survival. One-way analyses of variance (ANOVA) were used to analyze the lifespans of females in different stages of estrus and CBD-dosed males. In cases where groups did not have the same variance, we performed two-sample *t*-tests. Chi-square tests were applied to test for sex differences in modes of deaths. Finally, for sleep data, unpaired *t*-tests were exploited to compare different sleep fragments between males and females as well as pre-epilepsy and post-epilepsy.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Brains of mice were dissected to yield tissue samples from the neocortex (NCX) and hippocampus (HIP). Total RNA was isolated from post-seizure D/+ females and males—each of which had between 15

and 20 tonic-clonic seizures—and from age-matched wildtype (+/+) controls. RNA extraction, quantification, and cDNA synthesis procedures were followed as described by Sprissler et al. [20]. Taqman probes were obtained from Life Technologies (Waltham, MA) for the *Scn8a* gene, and all samples were run with the endogenously expressed control gene, *Actb* [21].

Results

Seizures and survival

Table 1 displays survival and seizure statistics for 12 adult heterozygous (D/+) virgin females and males (*n* = 24) monitored 24/7 by video beginning at P30 and continuing for the remainder of their entire life span. All mice perished prematurely (mean = 115.5 ± 24.7 days, max = 165 days; 95% CI: 105.07, 125.93) after experiencing many tonic-clonic seizures (TCs) (mean = 75.0 ± 49.5 ; 95% CI: 54.1, 95.9). TCs were experienced either as a singleton (an isolated TC), a cluster (two or more TCs within 2–3 min), or a series of 3–20 TCs over a period of an hour or more (convulsive status epilepticus or CSE). Virgin females and males (Table 1) experienced a similar proportion of singletons, clusters and CSE events (females percentages: 67.4, 28.6, and 0.04, respectively; male percentages: 68.0, 30.1, and 0.019, respectively) (χ^2 statistic = 3.165, *df* = 2, *p* value = 0.206).

We note several key features that distinguish adult virgin females and males. Although age at seizure onset was slightly lower in males (75.8 ± 9.1 ; 95% CI: 70.0, 81.6) than females (82.8 ± 14.9 ; 95% CI: 73.3, 92.3) (*t*-test *p* value = 0.092), we found a statistically significant longer lifespan for females (mean 128.8 ± 21.0 days; 95% CI: 115.5, 142.1) than for males (96.3 ± 16.0 days; 95% CI: 86.1, 106.5) (*t*-test *p* value < 0.001). We also found that females had longer post-TC survival (47.5 ± 20.5 days; 95% CI: 34.5, 60.5) than males (20.4 ± 16.0 days; 95% CI: 10.2, 30.6) (*t*-test *p* value < 0.001). Kaplan-Meier survival curves are shown for these virgin mice in Figure 1A, along with the cumulative number of TCs these 24 mice had over their lifespans. Unequal survival was strongly supported in a goodness of fit test using the χ^2 distribution (right-tailed) (*p* value < 0.001), which also indicated a large observed standard effect size of 0.69 (Figure 1A). In their lifetime, the total number of TCs experienced by females (105.5 ± 31.9 ; 95% CI: 85.2, 125.8) was statistically significantly higher than the total number experienced by males (44.8 ± 45.9 ; 95% CI: 15.6, 73.9) (*t*-test *p* value < 0.001), although post-onset seizure

Table 1. Virgin female and male mice followed by video recording for entire lifespan.

	#	Age at Death	#TCs ¹	#TCs/day (Life)	#TCs/day (PostTC)	Age at Onset	#Days survived postTC	#gaps	mean gap length	MOD ²
Females										
	1	165	140	0.85	2.75	114	51	3	8.3	CSE
	2	94	59	0.63	1.90	63	31	3	5.3	CSE
	3	144	157	1.09	2.91	90	54	6	5.5	CSE
	4	111	95	0.86	3.06	80	31	2	6.0	CSE
	5	104	57	0.55	1.73	71	33	2	7.0	CSE
	6	132	96	0.73	3.56	105	27	2	6.0	CSE
	7	122	133	1.09	2.89	76	46	2	9.0	CSE
	8	128	83	0.65	1.63	77	51	3	8.0	SUD
	9	119	88	0.74	2.93	89	30	2	5.0	CSE
	10	130	101	0.78	1.66	69	61	5	7.6	CSE
	11	159	135	0.85	1.34	74	101	5	12.4	CSE
	12	138	119	0.86	2.20	85	54	6	5.3	CSE
Males										
	1	101	43	0.43	1.65	75	26	2	8.0	SUD
	2	95	46	0.48	1.77	69	26	2	4.5	DEC
	3	122	139	1.14	2.62	68	53	4	6.0	DEC
	4	72	9	0.13	4.50	70	2	1	0.0	DEC
	5	88	18	0.20	3.00	82	6	1	3.0	DEC
	6	102	28	0.27	1.75	85	16	1	7.0	DEC
	7	112	11	0.10	1.22	85	27	1	4.0	DEC
	8	65	4	0.06	1.33	62	3	0	0.0	DEC
	9	108	103	0.95	3.32	77	31	1	13.0	CSE
	10	101	108	1.07	2.84	64	38	3	5.7	CSE
	11	100	18	0.18	1.20	86	15	1	10.0	SUD
	12	89	11	0.12	5.50	87	2	0	0.0	DEC

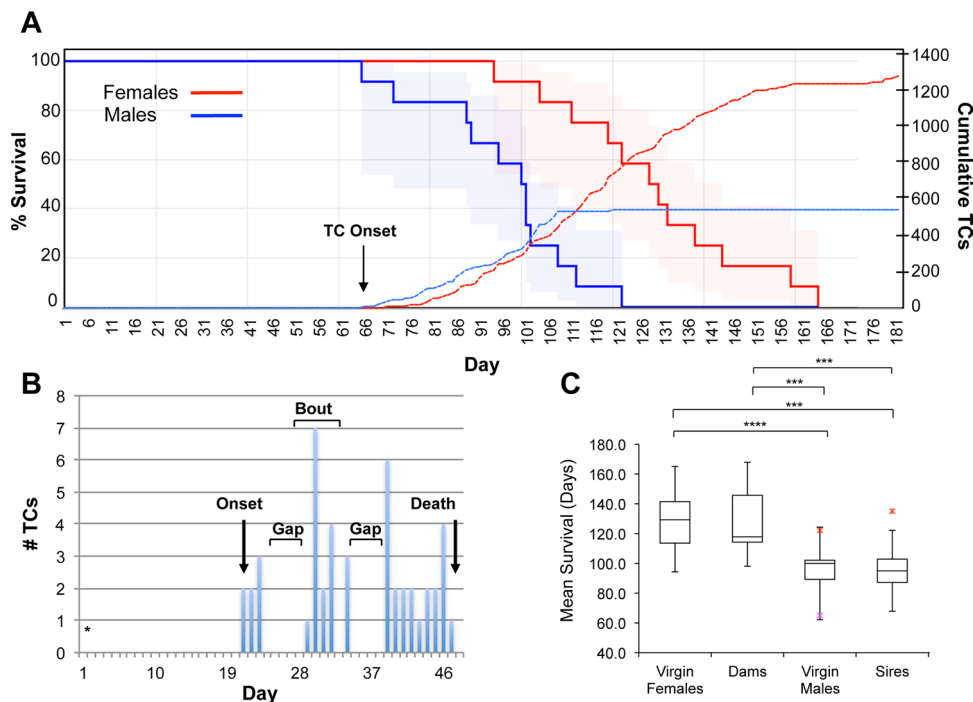
¹TC, tonic-clonic seizure.²MOD, mode of death.

Figure 1. Survival, lifetime number of TCs and seizure frequency patterns. A. Kaplan-Meier survival curves (solid lines) for females and males listed in Table 1. Log rank test p value <0.0001 . Cumulative number of TCs is shown in dotted lines. B. Pattern of TC frequency by day for a representative individual (male #2 in Table 1) illustrating seizure onset, bouts, gaps and time of death. C. Mean survival times for virgin females and males, as well as for dams and sires shown in Table 2.

frequencies did not differ significantly for females (2.4 ± 0.8 seizures/day; 95% CI: 1.9, 2.9) and males (2.6 ± 1.4 seizures/day; 95% CI: 1.7, 3.5) (t -test p value = 0.346).

Figure 1B shows the seizure pattern (onset, seizures per day) in the life of a single individual (male #2 in Table 1). We define a seizure *bout* as a cluster of seizures on consecutive days, and a *gap* as a seizure-free

Table 2. Mean survival of untreated and treated virgins, dams and sires.

Category	N	Mean (days)	STDEV
A. Untreated D/+			
Female virgin	12	128.8	21.0
Male virgin	13	96.3	16.0
Dams	15	127.3	23.4
Sires	18	99.2	19.8
All M	41	94.0	17.2
B. CBD Treated D/+			
Female all	35	115.6	14.5
Female early (T1)	11	108.4	12.9
Female late (T2)	24	118.9	14.3
Male preg (T0)	7	98.3	15.9
Male nursing (T1)	15	105.9	14.0
Male late (T2)	9	113.1	7.9
C. Juveniles D/D			
Female Untreated	20	24.1	2.9
Female CBD	25	23.0	3.3
Male untreated	17	24.6	2.6
Male CBD	19	27.1	4.6
D. Gonadectomy D/+			
Ovariectomy	4	164.3	16.1
Orchiectomy	5	92.6	48.7
	2	141.0	15.6

period of 3 days or more. Adult females have more than a two-fold greater number of gaps (3.4 ± 1.6 ; 95% CI: 2.3, 4.4) than males (1.4 ± 1.2 ; 95% CI: 0.6, 2.2) (*t*-test *p* value= 0.001), and spend more days in gaps (24.7 ± 15.0 days; 95% CI: 15.2, 34.2) than males (8.6 ± 7.8 days; 95% CI: 3.6, 13.6) (*t*-test *p* value= 0.002). In addition to monitoring virgin females and males, we also recorded the length of lifespan for 15 dams and 17 sires (Table 2). Figure 1C shows that virgin females and dams (128.8 ± 21.0 ; 95% CI: 115.5, 142.1 and 127.3 ± 23.4 days; 95% CI: 114.3, 140.3, respectively) on the one hand, and virgin males and sires (96.3 ± 16.0 days; 95% CI: 86.1, 106.5, and 99.2 ± 19.8 days; 95% CI: 89.4, 109.1, respectively) on the other, show very similar patterns of survival. Survival times are also given for these and additional mice in Table 2A.

Figure S2 displays the number of TCs occurring in female and male D/+ adults by hour of day during the 10h dark and 14h light cycles. In general, both sexes had more seizures in light *versus* dark cycles. This is mainly due to the fact the >80% of TCs are experienced in sleep (data not shown), and the proportion of time spent in sleep is greater during the light cycle. The mean number of female TCs in light and dark is 73.8 ± 23.2 (95% CI: 58.2, 89.4) and 27.6 ± 10.5 (95% CI: 20.6, 34.7), respectively. This was similarly shown with males experiencing more TCs during light than dark 32.4 ± 34.0 (95% CI: 10.8, 54.0) and 12.1 ± 12.1 (95% CI: 4.4, 19.8), respectively. In terms of the TC light:dark cycle ratio, females and males are nearly identical to each other (2.68 *versus* 2.69, respectively). Finally, females appear to experience peak TCs around the

dark-light boundary (4–6 am), while males appear to experience peak TCs later in the afternoon before the light-dark boundary (4–6pm).

Mode of death

Mode of death was recorded for each of our continuously monitored mice, as well as for additional mice monitored at the time of death. We noted three different modes of death: 1) convulsive status epilepticus (CSE), 2) sudden unexpected death (SUD), and 3) decompensation (DEC). CSE is defined as death after a continuous cluster of seizures with the absence of intervening recovery. Mice will often have a series of 10–30 clusters of TCs repeated over the course of 24–48h before succumbing to a final tonic seizure. We define SUD as death after a single seizure or cluster of 2 TCs that follow a seizure-free period of 24–48h. As in the case of CSE, the final seizure typically lacked the clonic phase. DEC is characterized as a death process that—while following a period of TCs—does not involve observable motor seizures in the last 24–36h of life. Reduced ambulation and a ceasing of drinking and eating occur before death. Figure 2A shows the relative prevalence of these three modes of death. Of the 21 females that were monitored, 19 died by CSE and 2 died by SUD (Table 1). The pattern was quite different for males with only 3 of 17 dying by CSE and SUD, respectively, while the remaining 11 experienced DEC (Freeman-Halton extension of Fisher exact test, *p* value < 0.0001). Figure 2B shows the declining breath rate (measured *via* piezoelectric) of a representative male in the process of decompensating.

Juvenile (D/D) seizure and survival patterns

Female and male (D/D) juvenile mice exhibited very similar seizure and survival patterns. A cohort of 16 juveniles (6 females and 10 males) was monitored over their entire lifespan. All mice were observed to perish by CSE. The age at onset was similar for females and males (23.3 ± 4.6 ; 95% CI: 18.5, 28.1 and 22.8 ± 3.6 days; 95% CI: 20.2, 25.4, respectively) (*t*-test *p* value= 0.383), as was the total number of TCs (86.0 ± 14.5 ; 95% CI: 70.8, 101.2) *versus* 76.0 ± 29.1 ; 95% CI: 55.2, 96.8) (*t*-test *p* value= 0.209). A larger cohort of juveniles (20 females and 17 males) that were monitored for survival resulted in similar survival times for females (24.1 ± 2.9 days; 95% CI: 22.7, 25.5) and males (24.6 ± 2.6 days; 95% CI: 23.3, 25.9) (*t*-test *p* value= 0.256). Kaplan-Meier survival curves for juveniles are shown in Figure 3A.

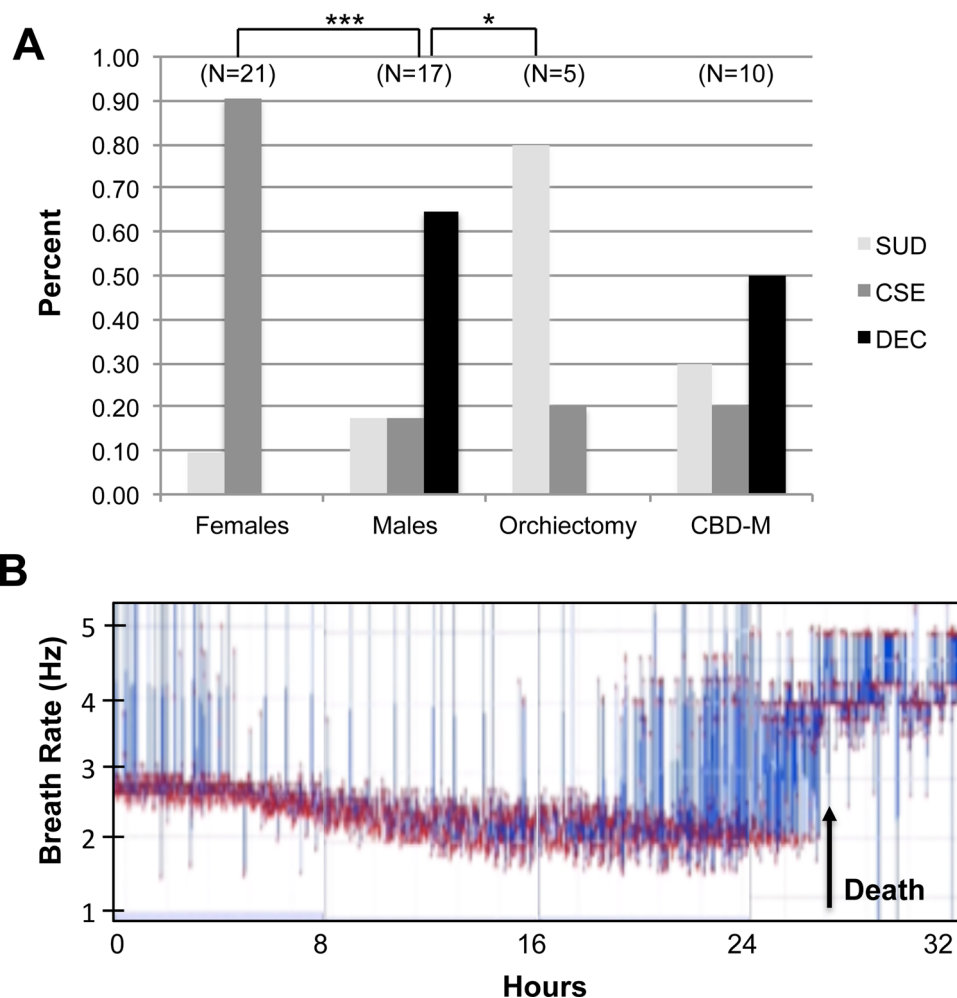


Figure 2. Mode of death. A. Number of mice that died from convulsive status epilepticus (CSE), sudden death (SUD), and decompensation (DEC) in intact and females and males, as well as males that were orchietomized or treated with cannabinoids (CBD-M). B. Breath rate in male undergoing decompensation. The gradual slowing in breath rate is highlighted over several hours before death.

Estrus cycle and seizures

Figure S3 shows the average number of seizures per each phase of the estrus cycle along with the inferred levels of progesterone [22]. The highest frequency of TCs per day is observed in proestrus with 6.4 ± 2.9 (95% CI: 3.9, 8.8) and the lowest frequency is observed during metestrus (3.6 ± 2.6 ; 95% CI: 1.5, 5.8) and estrus (4.0 ± 2.0 (95% CI: 2.5, 5.5)). One-way ANOVA testing assuming equal variance for all four stages does not indicate statistically significant differences in rates of TCs (f-ratio value= 2.91, p value= 0.079). However, the number of TCs/day was statistically significantly higher in pairwise t-tests for proestrus compared with metestrus (p value= 0.034) and estrus (p value= 0.034).

Figure S4 shows the number of TCs by day along with the phase of the estrus cycle for two

representative virgin females (#892 and #924 in Table 1) over the course of their lifetimes. For #892, we note that the regularity of the estrus cycle was altered just before seizure onset. Specifically, the regular cycle of proestrus, estrus, metestrus, and two days of diestrus (PEMDD) was interrupted about 5 days before seizure onset. In the case of #892, the cycle shifted from one day of proestrus and metestrus, followed by continuous diestrus for most of the remaining days before CSE. The beginning of the repetition of diestrus corresponded with TC onset. Similar disruptions in the estrus cycle were noted for several virgin females. For virgin female #924, the regularity in estrus cycling was also interrupted just before seizure onset; however, in this case the PEMDD pattern reappeared in the midst of several days of repeated diestrus, possibly as a result of partial recovery between seizure bouts.

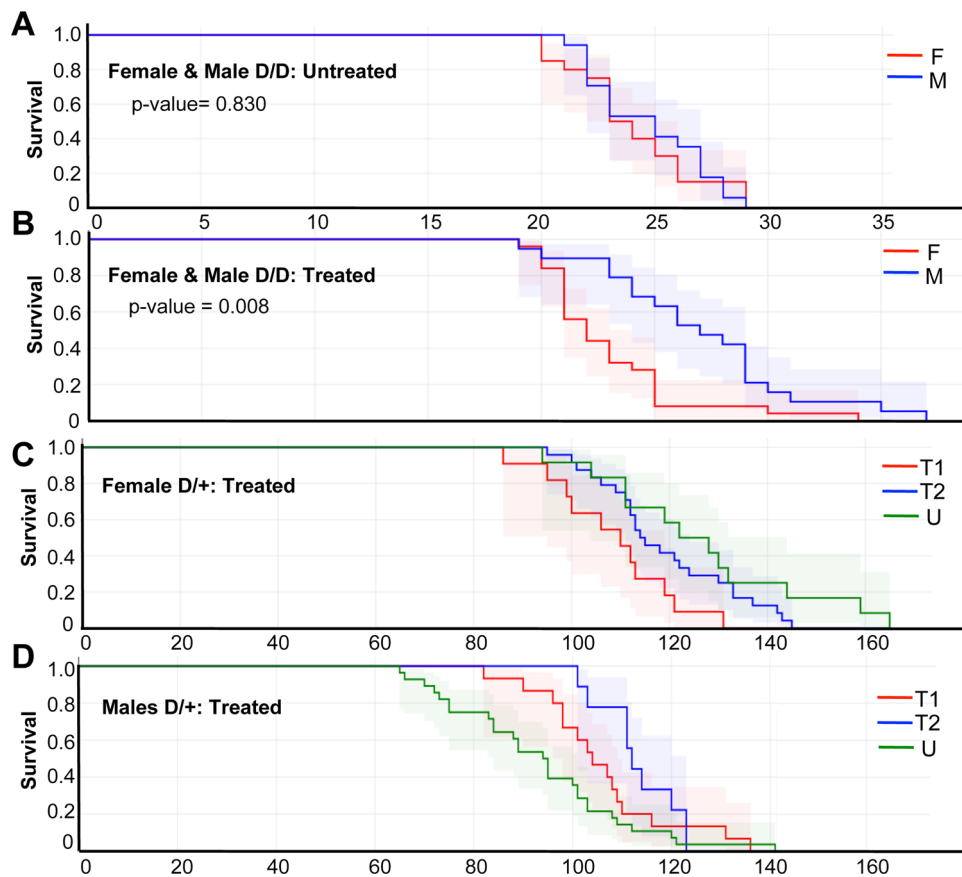


Figure 3. Survival curves for untreated and CBD-treated mice. A. Untreated D/D females and males. B. Treated D/D females and males. D/+ adult females (C) and males (D): untreated (U), treated during nursing (T1) and in adulthood (T2).

Seizures and sleep

Figure 4A shows the average percentage of time spent in light and dark cycle sleep for two females before and after seizure onset, as well as the percent of time spent in sleep bouts at increments of 1, 2, 4, 8, 32, and 64 min. A similar plot for the time spent in sleep bouts averaged for two males before and after seizure onset is shown in Figure 4B. In considering 9 females ($n=23$ pre-seizure and 26 post-seizure sessions), and 7 males ($n=26$ pre-seizure and 21 post-seizure sessions), we found that both sexes showed a large reduction in percent of time spent in longer sleep bouts after seizure onset in both light (t -test p value <0.001 and 0.005 , respectively) and dark cycles (t -test p value = 0.003 and 0.027 , respectively). After seizure onset, the time spent in ≥ 32 min light and dark cycle sleep bouts declined by 50.0% and 48.0%, respectively, for females, and by 5.5% and 50%, respectively, for males. When examined in association with seizures, it is apparent that long sleep bouts decline during seizure bouts and recover partially in longer seizure-free gaps (e.g. Figure S4). In general,

both sleep deprivation and increased numbers of TCs are ongoing at the time of death for cases of CSE and SUD.

Cannabinoid treatment

Table 2 Lists the mice tested with cannabinoids. A total of 35 adult females were dosed orally with 26:1 hemp oil, either starting during nursing (T1, $n=11$) or as adults shortly after seizure onset (T2, $n=24$). a total of 31 adult males were dosed with cannabinoids. Of these 31 males, 7 started treatment in utero by orally dosing the pregnant dam, 15 were orally dosed at birth (P1–P3) by initially dosing the dam and making a pellet available to pups as they matured, and 9 were dosed as adults. Four of the latter were given injections of pure CBD I.P. Overall, CBD-treated females (115.6 ± 14.5 days; 95% CI: 110.6, 120.6) had shorter survived times compared with untreated females (128.8 ± 21.0 days; 95% CI: 115.5, 142.1) (t -test p value = 0.010). early and chronically treatment females had an even shorter average survival time (108.4 ± 12.9 days; 95% CI: 99.7, 117.1) (t -test, p value = 0.006). survival curves shown in Figure 3C are concordant with mean

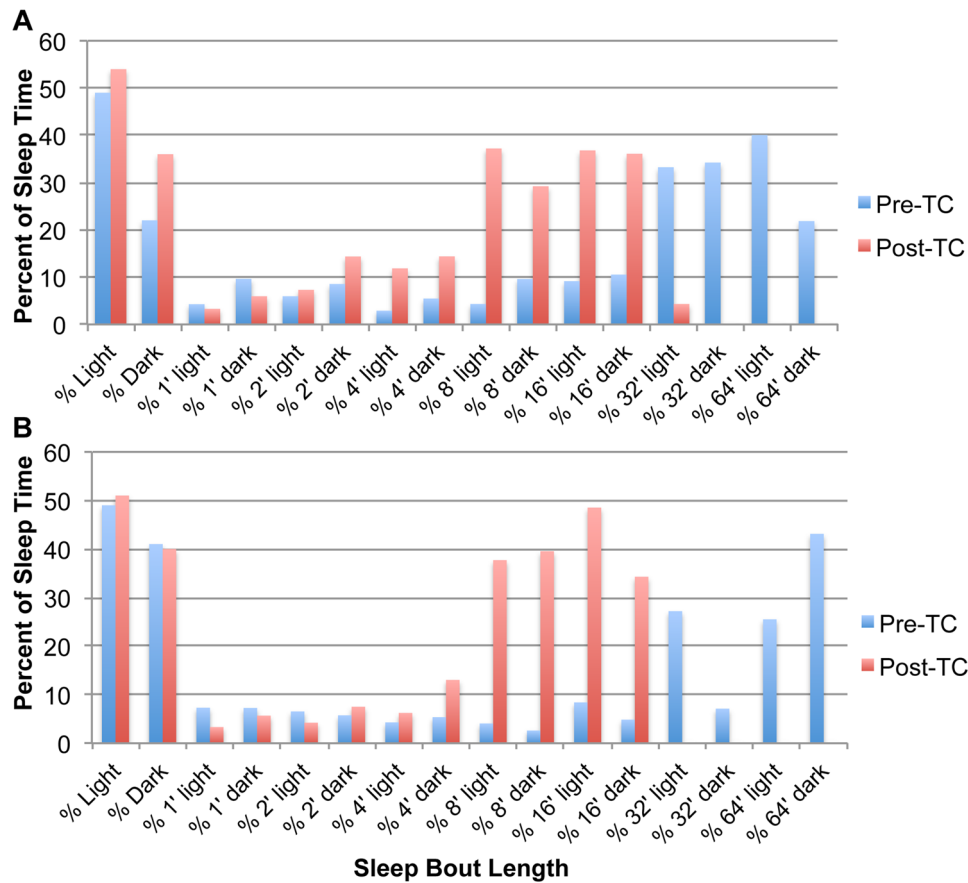


Figure 4. Percent of time spent in sleep during light and dark cycles, and by amount of time in sleep bouts of varying length. Average time spent in sleep bouts for (A) two females and (B) two males before and after seizure onset.

survival, showing a trend toward a ranking in survival rates such that untreated > T2 > T1.

The same pattern did not hold for CBD-treated males (Figure 3D). Compared with untreated males (U, 96.3 ± 16.0 ; 95% CI: 86.1, 106.5), survival times for chronically treated males (T2, 113.1 ± 7.9 ; 95% CI: 107.0, 119.2) had marginally and statistically significantly longer survival times (*t*-test, *p* value = 0.003). However, survival times for early treated (T1, 105.9 ± 14.0 days; 95% CI: 98.2, 113.7) were only slightly longer than those for untreated males (*t*-test, *p* value = 0.053); however, D/+ males treated while the dam was pregnant (T0, 98.3 ± 15.9 ; 95% CI: 83.6, 113.0) fared no better than untreated males (*t*-test *p* value = 0.792). Survival curves in Figure 3D indicate decreasing rates of survival such that T2 > T1 > U.

Unlike untreated juveniles, CBD-treated juveniles showed a sex difference in survival. CBD had little effect in extending lifespan for treated females (23.0 ± 3.3 days; 95% CI: 21.6, 24.4) (*t*-test, *p* value = 0.126) (Table 2C). On the other hand, CBD-treated D/D males lived longer than untreated males (27.1 ± 4.6 days; 95% CI: 24.9, 29.3) (*t*-test *p* value = 0.031). Kaplan-Meier survival curves yielded similar results (Figure 3A, B).

Gonadectomy

Mean survival times for mice submitted to ovariectomy ($n=4$) and orchietomy ($n=5$) at P28 are shown in Table 2D. One of the OVX females never experienced a seizure and lived for the full period of study (180 days). The mean survival for the remaining OVX females was 164.3 ± 16.1 days (95% CI: 138.7, 189.0), a statistically significant increase in survival compared with intact virgin females (128.8 ± 21.0 days; 95% CI: 115.5, 142.1) (*t*-test *p* value = 0.004). Three of the five ORX males experienced their first TC within 1 week after surgery (i.e. at P29, P33 and P35), while the other two had typical onset ages (i.e. P72 and P80). The three early-onset ORX males experienced a similar number of TCs (66.7 ± 19.8 ; 95% CI: 17.5, 115.9) as intact males, yet they lived many fewer days than intact males (60.3 ± 12.7 days; 95% CI: 28.8, 91.9) (*t*-test *p* value = 0.001). On the other hand, the two ORX males with typical onset ages lived longer than intact males (141.0 ± 36.8 days; *t*-test *p* value = 0.003), and experienced an increased number of TCs (136.0 ± 31.1) relative to intact males (*t*-test *p* value = 0.008). Indeed, survival times and TC numbers for the two ORX males were not distinguishable from those of intact virgin females (*t*-test *p* value = 0.214 and 0.118,

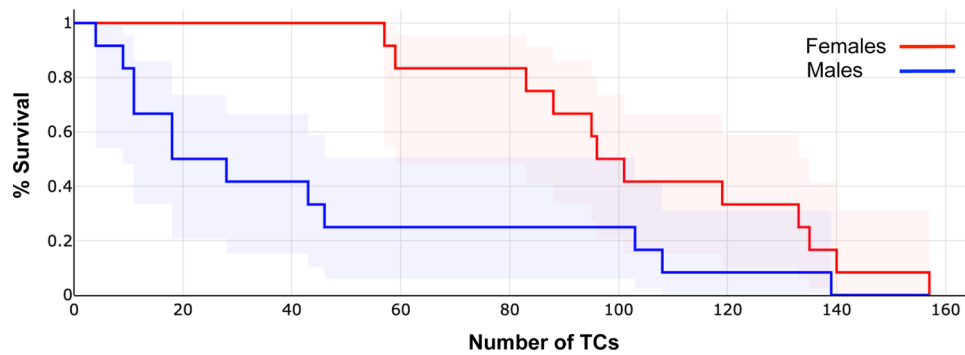


Figure 5. Concept of resilience. Survival curves based on the number of TCs experienced in the lifespan of virgin D/+ females and males listed in Table 1 (goodness of fit test using the χ^2 distribution, p value= 0.024 and a medium standard effect size of 0.46). after 42 TCs only 25% of males remained alive compared with 100% of females.

respectively). Gap numbers and lengths were also similar to those of intact females.

Scn8a gene expression

Its possible that Scn8a expression differences between females and males could account for observed sex differences in seizure patterns and survival, etc. To determine this, we isolated RNA from the neocortex and hippocampus of three D/+ females and males, each of which had experienced between 15 and 20 TCs. We compared levels of mRNA abundance in the post-seizure mice with age-matched wildtype (+/+) controls. Post-seizure heterozygotes exhibited a slight increase in expression levels compared with age-matched controls for female and male hippocampus and neocortex tissues (Table 3). The only statistically significant increase in post-seizure Scn8a expression was found for the female hippocampus (1.23-fold, t -test p value = 0.037). The male to female ratio of expression was 0.83 for the hippocampus and 1.12 for neocortex.

Table 3. qRT-PCR results.

	Het vs WT	p -value
Female HIP	1.23	0.037
Male HIP	1.02	0.435
Female NCX	1.16	0.299
Male NCX	1.30	0.088

Discussion

Sex differences have been observed in many human studies with accumulating evidence that men exhibit greater overall susceptibility to seizures while women exhibit greater seizure threshold fluctuations related to menstrual cycle alterations in steroid hormones [3,5,23,24]. Several contributing factors that have been discussed in the literature include differences in steroid hormones or neurosteroid levels in females *versus* male brains, differences in brain development and

neurogenesis, and sexual dimorphism in specific receptors [3,5,4,25]. Moreover, it remains to be understood how genetic differences (i.e. at the levels of the sex chromosomes and at individual genes across the genome that exhibit sex differences in gene expression) contribute to differential vulnerability to seizures and epileptogenic cascades [26].

Attempts to understand sex differences in rodent models have been confounded by the methodology to induce seizures, which involve different chemoconvulsants or electrical stimulation. Here we study a mouse model with a 'natural' onset of seizures utilizing non-invasive methods to observe seizures and measure associated sleep parameters (*via* video and piezoelectric monitoring) [19]. We documented over 3,780 TCs occurring over the lifespans of 25 adult (D/+) virgin females and males, as well as in cohorts of homozygous (D/D) juvenile mice, gonadectomized and cannabidiol-treated animals. The results indicate several key differences between females and males, as well as some important similarities. A major finding is that adult females live more than 30% longer than adult males. Age at seizure onset was similar for adults of both sexes (~80 days), yet female lifespan post-onset was more than 2.4-fold longer than that of males (Table 1). Females also experienced ~2.3-fold more lifetime TCs than males. We refer to the observed increase in the ability to tolerate TCs and live longer as increased "resilience". Figure 5 illustrates increased female resilience in a survival curve where the x-axis is cumulative number of lifetime TCs. For example, at the ~40TC point only 25% of males remained alive compared with 95% of females. Interestingly, there is no such increase in female resilience in juvenile (D/D) mice, with both sexes experiencing similar age at onset and length of lifespan. In the following sections we explore possible explanations for increased adult female resilience.

Role of hormones

We found that disruptions in the estrous cycle began just prior to seizure onset and that females spent more time in diestrus during periods of spontaneous recurrent seizures (e.g. Figure S4). Female rodents tested in various models of epilepsy have shown a high propensity for developing disrupted estrous cycles and other indicators of reproductive endocrine dysfunction [3]. Our results are consistent with recent studies examining female mice in the intrahippocampal kainic acid (KA) model in which prolongation of estrous cycle period was characterized by more time spent in diestrus [27]. These differences did not appear for at least 6 weeks after the KA injection, indicating that it was the chronic epileptic condition, and not the acute effects of KA excitotoxicity that drove the cycle disruption. Our results suggest that hormonal fluctuation is a factor in increasing excitation in the brain prior to the onset of seizures in adult females.

Unlike adults, immature female and male mice were equally resilient in terms of survival. This finding supports the hypothesis that circulating levels of steroid hormones such as estradiol, progesterone, and testosterone play a role in the observed differences between adult females and males. An alternate, non-mutually exclusive, explanation is that juvenile D/D mice are more severely neurologically impaired such that sex differences that exist at the juvenile stage (e.g. differences in brain development and neurogenesis, genetic factors, etc.) do not overcome the pathophysiology directly related to *Scn8a*. Our results are consistent with those of Akman et al. [28] who observed little to no evidence for sex differences in immature animals with respect to induction, seizure threshold, or mortality in a variety of chemoconvulsant models.

Despite small sample sizes of gonadectomized mice, we obtained a statistically significant increase in survival of OVX females compared with intact virgin females. The simplest explanation is that fluctuations in gonadal hormones (or their metabolites) have an overall negative impact on lifespan in mice experiencing spontaneous recurrent seizures in our model. In the case of catamenial epilepsy, the occurrence of increased seizures during particular phases of the menstrual cycle is attributed to cyclic fluctuations of hormones, and as a result, corresponding neurosteroid levels. Along these lines, we found a trend toward increased seizure frequency in proestrus females, although the increase did not reach statistical significance. Reddy et al. [24] developed rodent models of catamenial epilepsy that mimicked the surge and precipitous drop of progesterone and corresponding neurosteroids during the perimenstrual period. These studies

showed an inverse relationship between seizure susceptibility and neurosteroid levels, which was attributed to the antiepileptogenic effects of neurosteroids (i.e. after conversion from progesterone) that bind to GABA_A receptors and enhance phasic and tonic inhibition in the brain [29]. Interestingly, inhibitory somatostatin-positive interneurons were recently shown to contribute to seizures in a similar *Scn8a* mouse model [30].

Unfortunately, three of the males submitted to orchiectomy experienced seizure onset within 1 week of surgery, making it difficult to infer whether the premature onset was due to a hormonal change or to the direct effects of the surgery. It is interesting to note that the two remaining ORX males had a statistically significant increase in survival, living as long as intact virgin females and experiencing a similar number of seizures and gaps between seizure bouts. None of the ORX males perished by decompensation in stark contrast to intact males (Freeman-Halton extension of Fisher exact test, *p* value= 0.010). This suggests that decompensation may be related to the presence of testosterone or one of its metabolites and raises the possibility that the overall effect of testosterone is negative with respect to seizure resilience. However, we are cautious in making such an inference because of the small sample size, along with the fact that testosterone is synthesized by both the testes and the ovaries (and to a much lesser degree, the adrenal gland), and it can also be synthesized *de novo* in the brain from cholesterol.

Sex difference in response to cannabinoids

Males (both juvenile and adult), but not females, benefited from treatment with cannabinoids as evidenced by statistically significantly longer survival times compared with untreated age-matched controls (see Table 2, Figure 3). The exception to this pattern was the cohort of males treated earlier *in utero* (i.e. *via* dosing the pregnant dam), which showed no survival advantage (Table 2). Treated females had shorter lifespans than untreated females, a finding that was exacerbated with earlier administration of cannabinoids. On average, cannabinoid-treated *versus* untreated males trended toward longer post-TC survival (27.3 ± 17.0 ; 95% CI: 17.0, 37.6 *versus* 20.4 ± 16.0 days; 95% CI: 10.2, 30.6, respectively; *t*-test *p*value= 0.118). Still, cannabinoid-treated males did not live as long as untreated females, seizure frequency was not reduced relative to untreated males, and treated males perished in a manner that was similar to untreated males (i.e. the ratio of deaths by SUD, CSE and DEC were nearly identical) (Table 4).

Table 4. Number of individuals experiencing three modes of death.

	Females	Males	Ovariectomy	Orchiectomy	CBD-F	CBD-M
SUD	2	3	0	4	3	3
CSE	19	3	3	1	5	2
DEC	0	11	0	0	0	5
	21	17	3	5	8	10

CBD: cannabinoid, SUD, SUDEP; CSE: convulsive status epilepticus, DEC: decompensation. Freeman-Halton extension of Fisher exact test, p value <0.0001.

Although we could not find published studies examining sex differences in response to cannabinoid treatment for epilepsy, there are precedents in the literature for sex differences in the effects of cannabis. For example, in rodents, females are more sensitive than males to the effects of cannabinoids [31–33], with exposure during adolescence suggesting that female adolescents are more likely than male adolescents to be deleteriously affected [34]. Part of the explanation for sex differences in response to exogenous cannabidiol observed here may be due to gonadal hormones, which are known to contribute to the sexual dimorphism of cannabinoid sensitivity [32]. Estradiol has been identified as the hormone that contributes most to the sexually dimorphic effects of cannabinoids in adults, and the estrous cycle phase has been reported to significantly influence sex differences for cannabinoid effects [34]. Sexual dimorphism in the endocannabinoid system (ECS), which develops early in life, is also likely to influence the response to exogenous cannabinoids [32]. Compared with females, male rats have a higher density of CB1 receptor (CB1R), which is the predominant cannabinoid receptor within the central nervous system. However, a higher G-protein activation after CB1R stimulation is observed in adolescent females in several brain areas [31]. Additional molecular mechanisms may help explain the observed sex differences. Dimorphism in cannabinoid metabolism may explain the higher sensitivity of females to the deleterious influence of exogenous cannabinoids, and potential age-specific differences in the pharmacokinetics of cannabinoids might explain different responses of juveniles and adults to exogenous cannabinoids [31]. Recent experiments have also shown that CBD-exposed females present a greater vulnerability to gestational CBD than males [35]. Ultimately, cannabinoid receptor activation modulates the activity of most neurotransmitter systems, including GABA, glutamate, dopamine, and serotonin, which could play important roles in resilience to seizures in our mouse model [36].

Sex-related differences in gene expression may also explain differences in seizure resilience [26]. We can exclude sex-biased expression of *Scn8a* mRNA as the cause of differences in resilience between the sexes

given that our qRT-PCR results showed similar expression levels in both the hippocampus and neocortex of post-seizure females and males (Table 3). Further studies of whole genome transcription in females and males at different stages of epileptogenesis may shed light on sex difference in resilience. We also highlight the many similarities between the sexes in this model, including the role of sleep and its disruption as seizures develop. Seizures and sleep have a bidirectional relationship, with poorer sleep triggering seizures and worse seizure control causing sleep loss [37].

Limitations of study

While this study revealed several sex-related differences in the natural history of epilepsy in the *Scn8a*-N1768D mouse model, we acknowledge several limitations including small samples sizes of gonadectomized mice, levels of cannabinoids received by each individual were not quantified, nor were endogenous levels of hormones. We also acknowledge the multitude of factors that affect seizure severity, and the complex manner in which they may interact. Future studies exploring the hormonal, genomic, epigenetic and non-genomic factors that influence sex differences in the natural history of epilepsy—especially those that lead to increased female resilience—are highly warranted and likely will lead to a better understanding of the pathophysiology of this and other forms of epilepsy.

Disclosure statement

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References

- [1] Twele F, Tollner K, Brandt C, et al. Significant effects of sex, strain, and anesthesia in the intrahippocampal kainate mouse model of mesial temporal lobe epilepsy.

- Epilepsy Behav. 2016;55:47–56. doi: [10.1016/j.yebeh.2015.11.027](https://doi.org/10.1016/j.yebeh.2015.11.027).
- [2] Christensen J, Kjeldsen MJ, Andersen H, et al. Gender differences in epilepsy. *Epilepsia*. 2005;46(6):956–960 doi: [10.1111/j.1528-1167.2005.51204.x](https://doi.org/10.1111/j.1528-1167.2005.51204.x).
 - [3] Christian CA, Reddy DS, Maguire J, et al. Sex differences in the epilepsies and associated comorbidities: implications for use and development of pharmacotherapies. *Pharmacol Rev*. 2020;72(4):767–800. PubMed PMID: 32817274; PubMed Central PMCID: PMCPCMC7495340. doi: [10.1124/pr.119.017392](https://doi.org/10.1124/pr.119.017392).
 - [4] Prendergast BJ, Onishi KG, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. *Neurosci Biobehav Rev*. 2014;40:1–5. Epub 2014/01/25. doi: [10.1016/j.neubiorev.2014.01.001](https://doi.org/10.1016/j.neubiorev.2014.01.001).
 - [5] Scharfman HE, MacLusky NJ. Sex differences in the neurobiology of epilepsy: a preclinical perspective. *Neurobiol Dis*. 2014;72 Pt B:180–192. Epub 2014/07/25. PubMed PMID: 25058745; PubMed Central PMCID: PMCPCMC4252793. doi: [10.1016/j.nbd.2014.07.004](https://doi.org/10.1016/j.nbd.2014.07.004).
 - [6] Will TR, Proano SB, Thomas AM, et al. Problems and progress regarding sex bias and omission in neuroscience research. *eNeuro*. 2017;4(6):ENEURO.0278-17.2017 PubMed PMID: 29134192; PubMed Central PMCID: PMCPCMC5677705. doi: [10.1523/ENEURO.0278-17.2017](https://doi.org/10.1523/ENEURO.0278-17.2017).
 - [7] Niihori Y, Lee SJ, Minassian BA, et al. Sexually divergent mortality and partial phenotypic rescue after gene therapy in a mouse model of dravet syndrome. *Hum Gene Ther*. 2020;31(5–6):339–351. Epub 2019/12/14. PubMed PMID: 31830809; PubMed Central PMCID: PMCPCMC7087406. doi: [10.1089/hum.2019.225](https://doi.org/10.1089/hum.2019.225).
 - [8] Kang SK, Hawkins NA, Kearney JA. C57BL/6J and C57BL/6N substrains differentially influence phenotype severity in the Scn1a (+/-) mouse model of Dravet syndrome. *Epilepsia Open*. 2019;4(1):164–169. Epub 2019/03/15. PubMed PMID: 30868126; PubMed Central PMCID: PMCPCMC6398090. doi: [10.1002/epi4.12287](https://doi.org/10.1002/epi4.12287).
 - [9] Johannesen KM, Liu Y, Koko M, et al. Genotype-phenotype correlations in SCN8A-related disorders reveal prognostic and therapeutic implications. *Brain*. 2022;145(9):2991–3009. Epub 2021/08/26. doi: [10.1093/brain/awab321](https://doi.org/10.1093/brain/awab321).
 - [10] Talwar D, Hammer MF. SCN8A epilepsy, developmental encephalopathy, and related disorders. *Pediatr Neurol*. 2021;122:76–83. Epub 2021/08/07. doi: [10.1016/j.pediatrneurol.2021.06.011](https://doi.org/10.1016/j.pediatrneurol.2021.06.011).
 - [11] Gardella E, Marini C, Trivisano M, et al. The phenotype of SCN8A developmental and epileptic encephalopathy. *Neurology*. 2018;91(12):e1112–e24. Epub 2018/09/02. doi: [10.1212/WNL.0000000000006199](https://doi.org/10.1212/WNL.0000000000006199).
 - [12] Hammer MF, Xia M, Schreiber JM, et al. SCN8A-Related epilepsy and/or neurodevelopmental disorders. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW editors. *Seattle (WA):GeneReviews((R))*. 2023.
 - [13] Jones JM, Meisler MH. Modeling human epilepsy by TALEN targeting of mouse sodium channel Scn8a. *Genesis*. 2014;52(2):141–148. Epub 2013/11/30. PubMed PMID: 24288358; PubMed Central PMCID: PMCPCMC3973152. doi: [10.1002/dvg.22731](https://doi.org/10.1002/dvg.22731).
 - [14] Wagnon JL, Korn MJ, Parent R, et al. Convulsive seizures and SUDEP in a mouse model of SCN8A epileptic encephalopathy. *Hum Mol Genet*. 2015;24(2):506–515. doi: [10.1093/hmg/ddu470](https://doi.org/10.1093/hmg/ddu470).
 - [15] Champlin AK, Dorr DL, Gates AH. Determining the stage of the estrous cycle in the mouse by the appearance of the vagina. *Biol Reprod*. 1973;8(4):491–494. doi: [10.1093/biolreprod/8.4.491](https://doi.org/10.1093/biolreprod/8.4.491).
 - [16] Byers SL, Wiles MV, Dunn SL, et al. Mouse estrous cycle identification tool and images. *PLOS One*. 2012;7(4):e35538. Epub 2012/04/20. PubMed PMID: 22514749; PubMed Central PMCID: PMCPCMC3325956. doi: [10.1371/journal.pone.0035538](https://doi.org/10.1371/journal.pone.0035538).
 - [17] Moss MJ, Bushlin I, Kazmierczak S, et al. Cannabis use and measurement of cannabinoids in plasma and breast milk of breastfeeding mothers. *Pediatr Res*. 2021. Epub 2021/09(4):861–868. doi: [10.1038/s41390-020-01332-2](https://doi.org/10.1038/s41390-020-01332-2).
 - [18] Navarrete F, Garcia-Gutierrez MS, Gasparyan A, et al. Cannabis use in pregnant and breastfeeding women: behavioral and neurobiological consequences. *Front Psychiatry*. 2020;11:586447–58Epub 2020/11/27. PubMed PMID: 33240134; PubMed Central PMCID: PMCPCMC7667667. doi: [10.3389/fpsy.2020.586447](https://doi.org/10.3389/fpsy.2020.586447).
 - [19] Yaghouby F, Donohue KD, O'Hara BF, et al. Noninvasive dissection of mouse sleep using a piezoelectric motion sensor. *J Neurosci Methods*. 2016;259:90–100. Epub 2015/11/20. PubMed PMID: 26582569; PubMed Central PMCID: PMCPCMC4715949. doi: [10.1016/j.jneumeth.2015.11.004](https://doi.org/10.1016/j.jneumeth.2015.11.004).
 - [20] Sprissler RS, Wagnon JL, Bunton-Stasyshyn RK, et al. Altered gene expression profile in a mouse model of SCN8A encephalopathy. *Exp Neurol*. 2017;288:134–141. Epub 2016/11/12. PubMed PMID: 27836728; PubMed Central PMCID: PMCPCMC5215827. doi: [10.1016/j.expneurol.2016.11.002](https://doi.org/10.1016/j.expneurol.2016.11.002).
 - [21] Marques TE, de Mendonca LR, Pereira MG, et al. Validation of suitable reference genes for expression studies in different pilocarpine-induced models of mesial temporal lobe epilepsy. *PLOS One*. 2013;8(8):e71892. Epub 2013/09/07. PubMed PMID: 24009668; PubMed Central PMCID: PMCPCMC3751890. doi: [10.1371/journal.pone.0071892](https://doi.org/10.1371/journal.pone.0071892).
 - [22] Scharfman HE, MacLusky NJ. Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS. *Front Neuroendocrinol*. 2006;27(4):415–435. Epub 2006/10/24. PubMed PMID: 17055560; PubMed Central PMCID: PMCPCMC1778460. doi: [10.1016/j.yfrne.2006.09.004](https://doi.org/10.1016/j.yfrne.2006.09.004).
 - [23] Christensen J, Vestergaard M, Pedersen MG, et al. Incidence and prevalence of epilepsy in Denmark. *Epilepsy Res*. 2007;76(1):60–65. Epub 2007/08/10. doi: [10.1016/j.eplepsyres.2007.06.012](https://doi.org/10.1016/j.eplepsyres.2007.06.012).
 - [24] Reddy DS, Thompson W, Calderara G. Molecular mechanisms of sex differences in epilepsy and seizure susceptibility in chemical, genetic and acquired epileptogenesis. *Neurosci Lett*. 2021;750:135753–13. PubMed PMID: 33610673; PubMed Central PMCID: PMCPCMC7994197. doi: [10.1016/j.neulet.2021.135753](https://doi.org/10.1016/j.neulet.2021.135753).
 - [25] Veliskova J, Desantis KA. Sex and hormonal influences on seizures and epilepsy. *Horm Behav*. 2013;63(2):267–277. Epub 2012/04/17. PubMed PMID: 22504305; PubMed Central PMCID: PMCPCMC3424285. doi: [10.1016/j.yhbeh.2012.03.018](https://doi.org/10.1016/j.yhbeh.2012.03.018).
 - [26] Tower J, Pomatto LCD, Davies KJA. Sex differences in the response to oxidative and proteolytic stress. *Redox Biol*. 2020;31:101488–10Epub 2020/03. /24. PubMed

- PMID: 32201219; PubMed Central PMCID: PMCPMC7212483. doi: [10.1016/j.redox.2020.101488](https://doi.org/10.1016/j.redox.2020.101488).
- [27] Li J, Kim JS, Abejuela VA, et al. Disrupted female estrous cyclicity in the intrahippocampal kainic acid mouse model of temporal lobe epilepsy. *Epilepsia Open*. 2017;2(1):39–47. Epub 2016/12/05. PubMed PMID: 29750212; PubMed Central PMCID: PMCPMC5939433. doi: [10.1002/epi4.12026](https://doi.org/10.1002/epi4.12026).
- [28] Akman O, Moshe SL, Galanopoulou AS. Sex-specific consequences of early life seizures. *Neurobiol Dis*. 2014;72 Pt B(Pt B):153–166. Epub 2014/05/31. PubMed PMID: 24874547; PubMed Central PMCID: PMCPMC6681915. doi: [10.1016/j.nbd.2014.05.021](https://doi.org/10.1016/j.nbd.2014.05.021).
- [29] Reddy DS. Role of hormones and neurosteroids in epileptogenesis. *Front Cell Neurosci*. 2013;7:115–Epub 2013/08./06. PubMed PMID: 23914154; PubMed Central PMCID: PMCPMC3728472. doi: [10.3389/fncel.2013.00115](https://doi.org/10.3389/fncel.2013.00115).
- [30] Wengert ER, Miralles RM, Wedgwood KCA, et al. Somatostatin-positive interneurons contribute to seizures in SCN8A epileptic encephalopathy. *J Neurosci*. 2021;41(44):9257–9273. Epub 2021/09/22. doi: [10.1523/JNEUROSCI.0718-21.2021](https://doi.org/10.1523/JNEUROSCI.0718-21.2021).
- [31] Borsoi M, Manduca A, Bara A, et al. Sex differences in the behavioral and synaptic consequences of a single in vivo exposure to the synthetic cannabimimetic WIN55,212-2 at puberty and adulthood. *Front Behav Neurosci*. 2019;13:23. Epub 2019/03/21. PubMed PMID: 30890922; PubMed Central PMCID: PMCPMC6411818. doi: [10.3389/fnbeh.2019.00023](https://doi.org/10.3389/fnbeh.2019.00023).
- [32] Craft RM, Marusich JA, Wiley JL. Sex differences in cannabinoid pharmacology: a reflection of differences in the endocannabinoid system? *Life Sci*. 2013;92(8–9):476–481. Epub 2012/06/26. PubMed PMID: 22728714; PubMed Central PMCID: PMCPMC3492530. doi: [10.1016/j.lfs.2012.06.009](https://doi.org/10.1016/j.lfs.2012.06.009).
- [33] Farhang B, Diaz S, Tang SL, et al. Sex differences in the cannabinoid regulation of energy homeostasis. *Psychoneuroendocrinology*. 2009;34 Suppl 1(0 1):S237–S46. Epub 2009/05/12. PubMed PMID: 19427130; PubMed Central PMCID: PMCPMC3772082. doi: [10.1016/j.psyneuen.2009.04.007](https://doi.org/10.1016/j.psyneuen.2009.04.007).
- [34] Cooper ZD, Craft RM. Sex-dependent effects of cannabis and cannabinoids: a translational perspective. *Neuropsychopharmacology*. 2018;43(1):34–51. Epub 2017/08/16. PubMed PMID: 28811670; PubMed Central PMCID: PMCPMC5719093. doi: [10.1038/npp.2017.140](https://doi.org/10.1038/npp.2017.140).
- [35] Iezzi D, Caceres-Rodriguez A, Chavis P, et al. In utero exposure to cannabidiol disrupts select early-life behaviors in a sex-specific manner. *Transl Psychiatry*. 2022;12(1):501. Epub 2022/12/05. PubMed PMID: 36470874; PubMed Central PMCID: PMCPMC9722662. doi: [10.1038/s41398-022-02271-8](https://doi.org/10.1038/s41398-022-02271-8).
- [36] Struik D, Sanna F, Fattore L. The modulating role of sex and anabolic-androgenic steroid hormones in cannabinoid sensitivity. *Front Behav Neurosci*. 2018;12:249. Epub 2018/10/26. PubMed PMID: 30416437; PubMed Central PMCID: PMCPMC6212868. doi: [10.3389/fnbeh.2018.00249](https://doi.org/10.3389/fnbeh.2018.00249).
- [37] Gibbon FM, McCormac E, Gringras P. Sleep and epilepsy: unfortunate bedfellows. *Arch Dis Child*. 2019;104(2):189–192. Epub 2018/09/30. PubMed PMID: 30266875; PubMed Central PMCID: PMCPMC6362435. doi: [10.1136/archdischild-2017-313421](https://doi.org/10.1136/archdischild-2017-313421).