Life-span characterization of epilepsy and comorbidities in Dravet syndrome mice carrying a targeted deletion of exon 1 of the Scn1a gene

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ABSTRACT

Objective: Dravet Syndrome (DS) is a catastrophic form of paediatric epilepsy associated with multiple comorbidities mainly caused by mutations in the SCN1A gene. DS progresses in three different phases termed febrile, worsening and stabilization stage. Mice that are haploinsufficient for Scn1a faithfully model each stage of DS, although various aspects have not been fully described, including the temporal appearance and sex differences of the epilepsy and comorbidities. The aim of the present study was to investigate the epilepsy landscape according to the progression of DS and the long-term co-morbidities in the Scn1a+/− tm1kea DS mouse line that are not fully understood yet.

Methods: Male and female F1.Scn1a(+/+) and F1.Scn1a+/− tm1kea mice were assessed in the hyperthermia model or monitored by video electroencephalogram (vEEG) and wireless video-EEG according to the respective stage of DS. Long-term comorbidities were investigated through a battery of behaviour assessments in ~6 month-old mice.

Results: At P18, F1.Scn1a(+/+)tm1kea mice showed the expected sensitivity to hyperthermia-induced seizures. Between P21 and P28, EEG recordings in F1.Scn1a(+/−)tm1kea mice combined with video monitoring revealed a high frequency of SRS and SUDEP (sudden unexpected death in epilepsy). Power spectral analyses of background EEG activity also revealed that low EFG power in multiple frequency bands was associated with SUDEP risk in F1.Scn1a(+/−)tm1kea mice during the worsening stage of DS. Later, SRS and SUDEP rates stabilized and then declined in F1.Scn1a(+/−)tm1kea mice. Incidence of SRS ending with death in F1.Scn1a(+/−)tm1kea mice displayed variations with the time of day and sex, with female mice displaying higher numbers of severe seizures resulting in greater SUDEP risk. F1.Scn1a(+/−)tm1kea mice ~6 month-old displayed fewer behavioural impairments than expected including hyperactivity, impaired exploratory behaviour and poor nest building performance.

Significance: These results reveal new features of this model that will optimize use and selection of phenotype assays for future studies on the mechanisms, diagnosis, and treatment of DS.

1. Introduction

Dravet Syndrome (DS) is a rare, intractable and catastrophic form of childhood epilepsy with an estimated incidence of 1 in 15,700–40,000 births (Brunklaus et al., 2012; Wu et al., 2015). Nearly 90% of DS patients carry de novo homozygous mutations in the SCN1A gene leading to haploinsufficiency of the type 1 voltage-gated sodium channel α subunit (Nav1.1) (Bender et al., 2012). Loss of Nav1.1 protein mainly affects parvalbumin-expressing interneurons, causing disruption of excitation and inhibition balance in several neuronal circuits (Yu et al., 2006). Recent studies show this impairment is transient, however, and the mechanisms by which SCN1A deficiency contributes to cognitive
and other phenotypes remains incompletely understood (Favero et al., 2018; Almog et al., 2021).

DS is primarily characterized by hyperthermia sensitivity, spontaneous recurrent seizures (SRS) and premature death. Apart from epilepsy, a global development delay, hyperactivity, intellectual disability, and autistic-like behaviour may be also present (Anwar et al., 2019). The behavioural impairments and epilepsy phenotype emerge in an age-dependent manner according to three different stages of DS termed ‘febrile’, ‘worsening’ and ‘stabilisation’ stage (Gataullina and Dulac, 2017). Thus, a comprehensive understanding of DS features in each disease stage is crucial for successful preclinical development and evaluation of new targeted therapies or biomarker discovery.

Several genetic mouse lines have been developed to model DS (Yu et al., 2006; Ogiwara et al., 2007; Ricobaraza et al., 2019). This includes the Scn1a(+/-);tm1Kea mouse line harbouring a targeted deletion of exon 1 of Scn1a (Miller et al., 2014). This model has demonstrated translational value in identifying known and novel anti-seizure molecules and has helped define the molecular landscape upon Scn1a deletion (Hawkins et al., 2019). While the broad features of the model are resolved, various phenotypes remain incompletely defined. This includes epilepsy-related features of Scn1a(+/-);tm1Kea mice at the different stages of DS progression and the influence, if any, of sex. Furthermore, to the best of our knowledge, long-term assessment of neuropsychiatric comorbidities of this Dravet mouse line have not been evaluated.

Here we sought to characterize the epilepsy-related features according to the progression of DS in F1 Scn1a(+/-);tm1Kea mice. Moreover, we investigated the long-term behaviour impairments to characterize the comorbidities of the Scn1a(+/-);tm1Kea DS mouse line.

Our findings uncover previously unknown features. We identify two seizure types in F1 Scn1a(+/-);tm1Kea mice subject to hyperthermia at P18. Sex differences and the time of the day may influence the occurrence of SRS and SUDEP in F1 Scn1a(+/-);tm1Kea mice. Finally, long-term comorbidities in F1 Scn1a(+/-);tm1Kea mice are characterized by hyperactivity, impaired exploratory behaviour, poor nest building ability but not changes in memory performance, sociability or anxiety levels.

2. Materials and methods

2.1. Mice and ethics statement

All animal experiments were performed in accordance with the European Communities Council Directive (2010/63/EU) and approved by the Research Ethics Committee of the Royal College of Surgeons in Ireland (REC 13022bhb) under license from the Department of Health (AE19127/P064), Dublin Ireland. Animals were maintained in a light 20:00/dark cycle (20:00–08:00) with food and water ad libitum. F1 Scn1a(+/-);tm1Kea mice used in the experiments were generated by crossing Scn1a(+/-);tm1Kea male on the 129S6/SvEvTac background (Jackson Laboratory, USA) with inbred female mice C57BL/6JOlalHsd (Envigo, UK) (Miller et al., 2014).

2.2. Experimental design

All animals were genotyped before P7 and then assigned to four cohorts to investigate the three different stages of DS: Stage 1 (Febrile): At P18, the first cohort of mice were subjected to a hyperthermia-induced seizure threshold assay to determine their sensitivity to febrile seizures. Stage 2 (Worsening): At P21, a second cohort of mice were implanted with cortical EEG electrodes to be recorded by vEEG followed by video monitoring until P28 to investigate SRS and SUDEP (Note: due to the high mortality rate, an expanded cohort of mice in this experiment was not undertaken). Stage 3 (Stabilization): At P36, another group of mice were implanted with a wireless telemetry device for continuous vEEG recording to detect SRS and SUDEP for 2 weeks until P49. Finally, another cohort of naïve mice were monitored from P0 to 6 months of age for the occurrence of SUDEP. Animals that survived and reached 5–6 months old were subject to a battery of behavioural assessments to investigate the long-term comorbidities in DS (Fig. 1).

2.3. Hyperthermia-induced seizures

Hyperthermia-induced seizure threshold assay was performed at P18 as previously described (Hawkins et al., 2017). First, the mouse was gently hand-restrained in a supine position with tail lifted. Then, a temperature probe (RET-4, physiostim, Clifton, New Jersey) positioned above and the rectal probe attached to a TCAT-2DF thermoc oupler (physiostim, Clifton, New Jersey). Mice were held at 37.5°C for 5 min to become accustomed to the chamber. Then core body temperature was gradually elevated by 0.5°C every 2 min until a seizure occurred or until reaching 42.5°C. If reaching that temperature, animals were held for 3 min before turning off the heat lamp. After that, mice remained 5 min in the chamber for observation of any late occurring seizures before they were removed, cooled down and considered seizure free. If the mouse had a seizure during the hyperthermia challenge, the heating process was stopped immediately to cool down the mouse to 37°C on a cold metal surface. Seizure severity was classified according to the Racine scale scoring system with few modifications (Racine, 1972; Van Erum et al., 2019).

2.4. Seizure semiology

All seizures detected in each stage of DS were scored according to the Racine scale with a few modifications, as reported (Racine, 1972; Van Erum et al., 2019). No behaviour changes (0), mouth and facial movements (1), head nodding (2), unilateral forelimb clonus (3), bilateral forelimb clonus with rearing (4), rearing and falling (loss of posture) (5), wild running or jumping (6) and Tonic hindlimb extension possibly leading to death (7).

2.5. Behaviour phenotype

Naïve 5–6-month-old mice were submitted to a battery of behavioural assessments. Spatial reference, working and recognition memory was assessed with the Y maze and novel object recognition test, respectively (Wolf et al., 2016; Lueptow, 2017). Spontaneous locomotion, stereotyped behaviour, and anxiety-like behaviour was investigated in the open field. Further investigation related to anxiety-like behaviour was performed in the elevated plus maze and light dark box test as previously described (Almeida Silva et al., 2020). Autism-like behaviour and motor coordination were assessed in the three chamber and rotarod test, respectively (Mandillo et al., 2008; Yang et al., 2011). Finally, innate exploratory behaviour and welfare parameters were assessed through marble burying and the nest building task (Deacon, 2006; Neely et al., 2019). Behavioural tests were performed with multiple rest day intervals and according to the increasing order of interventional complexity.

2.6. Statistical analyses

The normality of the data was analysed using D’Agostino and Pearson’s omnibus normality test. Data were analysed using unpaired two-tailed Student t-test, Mann-Whitney U test, Kaplan-Meier method, Spearman’s rank-order correlation, Two-way repeated measures ANOVA, one and two-way analysis of variance (ANOVA), Fisher’s exact test, Wilcoxon signed-rank test, Friedman test or Permutation test followed by Tukey’s post hoc test, as appropriate. Number of SRS by sex was analysed using Permutation test, since the data is based on the frequency of SRS with small and equal sample size and unequal standard
deviation, conditions where the Permutation test provides a greater power than the Mann-Whitney U test.

Genotype differences were investigated using a mix of male and female animals in each group F1. Scn1a(+/+)+/−)tm1kea mice. Further analyses on sex differences related to the epilepsy phenotype were focused on male vs female F1. Scn1a(+/+)+/−)tm1kea mice. Sex influence on behaviour phenotype was investigated performing a two-way analysis of variance (ANOVA) considering sex a factor with the respective groups F1. Scn1a(+/+)+/−)tm1kea mice. Finally, sub-group analyses comparing male vs female F1. Scn1a(+/+)+/−)tm1kea mice were performed to completely rule out sex influence on behaviour phenotype. The specific statistical test used for each experiment are indicated in the figure legends. Data are expressed as mean ± SEM or median with interquartile range (IQR), as appropriate. Differences between groups were considered statistically significant when p < 0.05.

Experiments and data were analysed blind to genotype.

3. Results

3.1. F1. Scn1a(+/+)/−)tm1kea P18 mice display sensitivity to hyperthermia-induced seizures during the febrile stage of DS

Febrile seizures as a result of sensitivity to hyperthermia are a hallmark of DS onset. Thus, we first investigated whether F1. Scn1a(+/+)+/−)tm1kea mice display sensitivity to hyperthermia-induced seizures at an early age (P18). Fig. 2 shows the susceptibility of F1. Scn1a(+/+)+/−)tm1kea mice to hyperthermia-induced seizures.

As body temperature was elevated, all F1. Scn1a(+/+)+/−)tm1kea animals developed tonic-clonic seizures, with an onset averaging ~40 °C whereas no F1. Scn1a(+/+)+/−)tm1kea mice developed seizures up to the cut-off temperature of 42.5 °C. Median duration of seizures was ~17 s (p < 0.0001, Fig. 2B). Interestingly, we noted seizures fell into two distributions based on duration in F1. Scn1a(+/+)+/−)tm1kea mice. Half (50%) of F1. Scn1a(+/+)+/−)tm1kea exhibited seizures...
lasting 10–14 s whereas the remaining 50% displayed longer seizures around 20 s ($p = 0.007$, Fig. 2C) (Movies S1,S2). The seizure duration sub-phenotypes did not correlate with the threshold temperature for seizures during the hyperthermia challenge (Fig. S1). Most seizures in F1.Scn1a(+/−)tm1kea mice reached severity 5 without progressing to wild jumping/running, hindlimb extension ($p = 0.002$, Fig. 2D) or sudden death (Fig. 2E). Analysis of male and female F1.Scn1a (+/−)tm1kea mice revealed there were no significant sex differences in any of these parameters.

3.2. F1.Scn1a(+/−)tm1kea mice develop frequent and severe SRS during the worsening stage of DS

Within weeks of febrile seizure symptoms, Dravet patients experience SRS with increasing severity and frequency, reflecting the worsening stage (stage 2) of DS. Fig. 3 shows the frequency, duration and severity of SRS observed during vEEG recordings and video-only monitoring from P21 to P28.

F1.Scn1a(+/−)tm1kea mice showed an increasing SRS frequency ranging from 4 to 20 seizures, often accompanied by death (SUDEP), whereas no seizures were observed in F1.Scn1a(+/+) control mice ($p < 0.0001$). F1.Scn1a(+/−)tm1kea mice showed an increasing SRS frequency ranging from 4 to 20 seizures, often accompanied by death (SUDEP), whereas no seizures were observed in F1.Scn1a(+/+) control mice ($p < 0.0001$).
0.0001, Fig. 3A,B). F1.Scn1a(+/-)tm1kea mice displayed SRS with a median duration of 32 s (p < 0.0001, Fig. 3C) without a statistical difference between sexes. However, analysis of the number of SRS in F1. Scn1a(+/-)tm1kea mice revealed a significant sex difference, with more seizures in females (p = 0.043, Fig. 3D). F1.Scn1a(+/-)tm1kea mice showed seizures with severity scoring an average of 5.81 on the adapted Racine Scale (p = 0.002, Fig. 3E). Spontaneous seizures were least severe at P21. From P22 to P25 F1.Scn1a(+/-)tm1kea mice experienced the most severe SRS (Fig. 3F). The total number of SRS with severity 6 and 7 observed in females F1.Scn1a(+/-)tm1kea mice was twice that in males (p = 0.015 and p = 0.041 respectively, Fig. 3G). We also observed that SRS with severity 6 and 7 were of a longer duration than SRS with severity 5 (p = 0.016; p = 0.0008, Fig. 3H), and a strong positive correlation was found between SRS duration and seizure severity (r = 0.7019, p < 0.0001, Fig. 3I). Next, we investigated the time of day during which SRS were most likely to occur. Fig. 3J shows a plot of 107 SRS recorded in F1.Scn1a(+/-)tm1kea mice during monitoring between P21 and P28 according to the time of day. Interestingly, the number of SRS peaked just before the light-dark cycle lights went off (20:00 h). However, no difference was found on the number of SRS when dividing the day into 8 segments (Fig. 3K) and the average number of SRS over the course of a full day did not differ between light and dark phases (Fig. 3L).

3.3. F1.Scn1a(+/-)tm1kea mice display higher incidence of SUDEP and reduction in background EEG power during the worsening stage of DS

SUDEP is a prominent feature of DS especially during the worsening stage of the disease (stage 2). A Kaplan-Meier plot of deaths revealed that F1.Scn1a (+/-)tm1kea mice display a critical period of SUDEP risk from P22 to P25, with a survival rate of 20% at P28 (p = 0.0003, Fig. 4A).

There was no sex difference in the occurrence of SUDEP in F1.Scn1a (+/-)tm1kea EEG-implanted mice between P21 to P28 (Fig. 4B). All sudden deaths experienced by F1.Scn1a(+/-)tm1kea mice were preceded by a severe SRS following a stereotypical progression. All pre-SUDEP SRS began with forelimb clonus, rearing and loss of balance/posture (GTCS-Racine scale 5) followed by wild running or jumping (Racine scale 6) ending with tonic hindlimb extension and possibly death (Racine scale 7) (Movie S3). No difference in the number of hindlimb extension seizures was found in any period of the day (Fig. 4C). However, SUDEP incidence was higher in the last period of the day (16:00-00:00) (p = 0.003, Fig. 4D). EEG power spectral analyses in the interictal period revealed low EEG power in multiple frequency bands in the period of high SUDEP incidence from P22 to P24/P25 in F1.Scn1a (+/-)tm1kea mice (Fig. 4E,F,G). Consistently, no SUDEP or reduction in EEG background power was observed at P21 in F1.Scn1a(+/-)tm1kea mice. Fig. 4H,I,J shows representative EEG traces of F1.Scn1a(+/-) and F1.Scn1a(+/-)tm1kea mice respectively. As can be observed, SUDEP in F1.Scn1a(+/-)tm1kea mice was preceded by a severe SRS ending with hindlimb extension in both sexes.

3.4. Reduced SRS and SUDEP incidence during the stabilization stage in F1.Scn1a(+/-)tm1kea mice

SRS and SUDEP tend to reduce across adulthood during the stabilization stage of DS (Kalume et al., 2013; Shimuedy et al., 2016; Gataullina and Dulac, 2017). Thus, we next equipped F1.Scn1a mice with implantable EEG telemetry units to track the occurrence of SRS and SUDEP in young adult mice (P36-P49). Monitoring during this period detected SRS in only 1 out of 5 F1.Scn1a(+/-)tm1kea mice (Fig. 5A).

As expected, no seizures were observed in F1.Scn1a(+/-) control mice. Furthermore, all SRS observed over this period were less severe and did not reach the maximum score on the Racine scale (Fig. 5B) and no deaths were recorded (Fig. 5C). Fig. 5D-F show representative EEG traces of one F1.Scn1a(+/-), one F1.Scn1a(+/-)tm1kea mice (without SRS) and one F1.Scn1a(+/-)tm1kea mouse experiencing a SRS respectively. Later, using another batch of animals, we investigated the long-term survival in naïve mice. A Kaplan Meier plot revealed that the premature death commences at P20 culminating in overall mortality of ~40% at the end of 6 months (p = 0.002, Fig. 5G). More than half of the deaths (56.25%) occurred within a short interval between P20 to P32 (worsening stage). Thereafter, the occurrence dropped, tending to stabilize over the period until 6 months (Fig. 5G). Interestingly, a sub-group analysis of SUDEPs found a sex difference with higher mortality rates in female F1.Scn1a(+/-)tm1kea mice (p = 0.035, Fig. 5H).

3.5. Long-term neuropsychiatric comorbidities in F1.Scn1a(+/-)tm1kea mice

Fig. 6 shows the long-term comorbidities in ~6 month old F1.Scn1a (+/-)tm1kea mice. F1.Scn1a(+/-)tm1kea mice displayed increased spontaneous locomotion activity in the open field test, displaying an increase in total distance travelled (p = 0.021, Fig. 6A), number of crossing (p = 0.015, Fig. 6B), speed (p = 0.025, Fig. 6C) and total time mobile (p = 0.031, Fig. 6D), when compared to F1.Scn1a(+/-) control mice (Fig. 6E). No changes in motor coordination were observed F1.Scn1a(+/-)tm1kea mice during the rotarod test (data not shown). Assays of autism-like behaviour revealed that F1.Scn1a(+/-)tm1kea mice display more stereotyped behaviour (repetitive rotations of animal’s body) in the open field test (p = 0.048, Fig. 6F). F1.Scn1a(+/-)tm1kea mice displayed normal social interaction (p < 0.041, Fig. 6G) and response to social novelty (p = 0.001, Fig. 6H) in the three chamber test. Similarly, F1.Scn1a(+/-)tm1kea mice did not show anxiety-like behaviour in peripheral regions of the open field test (Fig. 52), elevated plus maze (Fig. 6J) or light dark box (Fig. 6K), when compared to F1.Scn1a(+/-) mice. Interestingly, F1.Scn1a(+/-)tm1kea mice also performed normally when compared to F1.Scn1a(+/-) mice in three memory paradigms: Spatial reference memory (y maze forced, Fig. 6L), spatial working memory (y maze spontaneous, Fig. 6M) and recognition memory (novel object recognition, Fig. 6N). Finally, we investigated the exploratory behaviour and nest building activity in the marble burying and nest build task, respectively. F1.Scn1a(+/-)tm1kea mice showed a reduction in the number of marbles buried (p = 0.040, Fig. 6P,R) and displaced (p = 0.030, Fig. 6Q,R), as well as poor nest-building ability at 24 h (p = 0.003, Fig. 6O) and 48 h (p = 0.0003, Fig. 6O) when compared to F1. Scn1a(+/-)mice). Analysis of male and female F1.Scn1a(+/-) and F1. Scn1a(+/-)tm1kea mice revealed no significant sex difference in any behaviour parameters (Fig. S2,S3). Sub group analysis of male and female F1.Scn1a(+/-)tm1kea mice also showed no sex difference in any behaviour assessment performed (Fig. S2,S4).

4. Discussion

In the present study we provide the most comprehensive assessment of the phenotypes over the lifetime in the F1.Scn1a(+/-)tm1kea model of DS and identify a number of features including testing age and sex that may improve the design and execution of therapeutic and biomarker studies using this DS model. DS is commonly classified in three different stages accordingly to the clinical manifestations (Gataullina and Dulac, 2017; Anwar et al., 2019). The ‘febrile stage’ of DS is marked by high incidence of hyperthermia sensitivity often resulting in prolonged seizures (Gataullina and Dulac, 2017; Anwar et al., 2019). Temperature sensitivity is a conserved feature of mouse models of DS and here we confirmed that F1.Scn1a(+/-)tm1kea mice present a reduced threshold to the hyperthermia-induced seizures at early developmental stages (Oakley et al., 2009). Williams et al., 2019 raised the hypothesis that F1. Scn1a(+/-)tm1kea mice have thermally induced seizures only at high temperatures (>42 °C) which is characteristic of heat stroke rather than fever (Williams et al., 2019; Hawkins et al., 2017). Here, most F1.Scn1a (+/-)tm1kea mice displayed seizures at lower temperatures (median

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A

B

C

D

E

F

G

H

I

J

F1.5cn1a (+/+) mouse I - female

EEG representative traces

F1.5cn1a (+/-)tm1Kee mouse I - male

P22

P23

500 µV

30 s

500 µV

30 s

500 µV

30 s

(caption on next page)
40.2 °C) and WT controls showed no behavioural epileptic seizures even at high temperatures (42.5 °C). Our results suggest that seizures observed during the hyperthermia challenge in F1.Scn1a(+/−)tm1kea mice result from their sensitivity to hyperthermia rather than heat stroke. These findings are in line with studies showing that F1.Scn1a(+/−)tm1kea mice display susceptibility to febrile seizures at P18 as also often observed between 6 and 12 months of life in children with DS (Gataullina and Dulac, 2017; Hawkins et al., 2017). The duration of hyperthermia-induced seizures in F1.Scn1a(+/−)tm1kea mice has not been previously reported. For the Scn1a+/− knockout DS mouse model, hyperthermia-induced seizures last around 30 s (Kaplan et al., 2017). Here we found that hyperthermia-induced seizures in P18 F1.Scn1a
Fig. 6. Characterization of long-term neuropsychiatric comorbidities in F1.Scn1a(+/−)tm1kea mice. Long term comorbidities were investigated across a battery of behavioural assessments with male and female F1.Scn1a(+/−) (n = 19) or F1.Scn1a(+/−)tm1kea mice (n = 21/group) around ~6 months of age. In the open field, F1.Scn1a(+/−)tm1kea mice traveled more, displaying higher speed and time mobile when compared with F1.Scn1a(+/+) mice, indicating hyperactivity. F1.Scn1a(+/−)tm1kea mice displayed a higher number of stereotyped behaviour in the open field indicating autism-like behaviour. However, F1.Scn1a(+/−)tm1kea mice showed no deficits in sociability or social novelty when compared to F1.Scn1a(+/+) mice. Similarly, F1.Scn1a(+/−)tm1kea mice did not show any anxiety-like behaviour in the J. elevated plus maze and K. Light dark box or memory deficits in L. y maze spontaneous, M. y maze forced or N. in the novel object recognition test when compared to F1.Scn1a(+/+) mice. O. F1.Scn1a(+/−)tm1kea mice also showed a reduced number of fully and displaced marbles when compared to F1.Scn1a(+/+) mice indicating impaired exploratory behaviour. (A,B,C,D,F,L,N) Student’s t-test, mean ± SEM, (D,J,K,M,P,Q) Mann-Whitney test, (G,H) two-way ANOVA, Scheirer-Ray Hare test, (O), two-way repeated measures ANOVA, mean ± SEM. Whisker plots, median IQR. *p < 0.05, **p < 0.01, ***p < 0.001.
(+/−tm1kea) mice fall into two types (short ~10s and long duration ~20s) with distinct behaviour which was not influenced by the sex or temperature reached during the hyperthermia challenge. Such a distinct seizure phenotype has not been previously reported in DS mouse models indicating that it is a specific feature only observed in the Scn1a (+/−tm1kea) mouse line.

In children with Dravet, the “febrile stage” is followed by a “worsening stage” that extends up to the fifth year of life with increasing SRS frequency and severity. This has also been observed from P21 to P28/ P30 in the Scn1a+/− knock-out mouse model (Kalume et al., 2013; Gataullina and Dulac, 2017; Kaplan et al., 2017). Similarly, our results reveal that F1.Scn1a+/−tm1kea mice present a high number of severe SRS over this stage. We also observed that the frequency of SRS was higher in female than male F1.Scn1a+/−tm1kea mice. Moreover, the number of the most severe SRS featuring wild running or jumping (score 6) and hindlimb extension (score 7) were twice as high in female compared to male F1.Scn1a+/−tm1kea mice. Although such sex differences in SRS have not been previously reported in DS mouse models and patients, a previous study in F1.Scn1a+/−tm1kea mice reported higher female SUDEP rates in DS mice (Niibori et al., 2020). Thus, these findings indicate a distinct epilepsy phenotype according to the sex of F1.Scn1a+/−tm1kea mice.

There is increasing evidence that circadian rhythms affect brain excitability (Karoly et al., 2021). Previous studies showed that Scn1aR1407X/+ DS mice experience a peak of SRS ending with death between 18:00 and 19:00 (before the lights went out), (Teran et al., 2019). Here, F1.Scn1a+/−tm1kea mice also experience a peak of SRS before the lights went off (19:00-20:00) with a higher incidence of SRS ending with death at 16:00 to 06:00. Thus, the time of day may have an influence on SRS followed by SUDEP in F1.Scn1a+/−tm1kea mice. While the mechanism is unknown, it is possible that network effects of clock-related genes may interact with the deficits arising from Scn1a loss. Therefore, pre-clinical studies using F1.Scn1a+/−tm1kea mice should take account a proper monitoring of SRS, particularly if non-continuous monitoring is planned.

The worsening stage of DS in humans is marked by a high incidence of SUDEP, typically preceded by a severe GTCS (Friedman et al., 2013; Shmuel et al., 2016). Here, EEG and video monitoring revealed that all deaths experienced by F1.Scn1a+/−tm1kea mice were preceded by a severe GTCS ending with hindlimb extension. SUDEP incidence was highest from P21 to P28, around 80%, and this had no sex bias. Interestingly, neither SUDEP nor changes in background EEG patterns were observed at the beginning of the worsening stage (P21). In contrast, SUDEP’s observed from P22 to P25 were associated with loss of EEG power during the interictal period in multiple frequency bands including beta, gamma and alpha. This matches findings in Scn1aATRSV missense mutation mice that showed low EEG power correlated with the risk of premature death during the worsening stage of DS (Fadila et al., 2020). Thus, these findings not only characterize the second stage of DS in F1.Scn1a+/−tm1kea mice but also reinforce that a severe GTCS and low EEG power during the interictal period in multiple frequency bands including hyperactivity, social impairments, anxiety and cognitive decline resulting in a poor quality of life (Gataullina and Dulac, 2017; Brown et al., 2020). We report the first comprehensive assessment of long-term behavioural phenotypes of F1.Scn1a+/−tm1kea mice during the stabilization stage of DS. Hyperactivity is one of the most consistent findings across different ages of development in individuals with DS (Brown et al., 2020). F1.Scn1a+/−tm1kea mice exhibited hyperactivity when exposed to novel environments but had normal motor coordination. This is in agreement with the higher total distance travelled reported in the open field in 8 week old male F1.Scn1a+/−tm1kea mice (Niibori et al., 2020). Autistic features including repetitive behaviour and social deficits are also observed in DS (Han et al., 2012; Ito et al., 2013; Gataullina and Dulac, 2017; Brown et al., 2020). We found that F1.Scn1a+/−tm1kea mice exhibited a prominent stereotyped behaviour in the open field arena which may be an autistic-like trait. However, F1. Scn1a+/−tm1kea mice did not exhibit social deficits in the three chamber test. Thus, the repetitive behaviours observed in F1.Scn1a+/−tm1kea mice may reflect hyperactive rather than autistic features, as concluded for Scn1aWT/A178V mice (Ricobarriza et al., 2019).

DS patients are reported to have anxiety and especially cognitive deficits (Sinoo et al., 2019; Brown et al., 2020) and anxiety-like behaviour phenotypes are present in 6–8 weeks old F1.Scn1a+/−tm1kea mice (Bahceci et al., 2020; Niibori et al., 2020; Patra et al., 2020). Similarly, 6 weeks old F1.Scn1a+/−tm1kea mice showed impaired reference and working memory in the radial arm maze (Bahceci et al., 2020). In contrast, 8 weeks old F1.Scn1a+/−tm1kea mice may display a better novel object recognition memory when compared to WT mice and just slight decline in spatial memory in the Barnes maze test (Bahceci et al., 2020). Here we consistently found that ~6 month old F1.Scn1a+/−tm1kea mice did not present any anxiogenic phenotype across the peripheral regions of the open-field, elevated plus maze or light dark box assessment. Furthermore, ~6 month old F1.Scn1a+/−tm1kea mice did not display spatial reference, working or recognition memory deficits in the forced/spontaneous Y maze or NOR assessment respectively. Together, these results suggest that F1.Scn1a+/−tm1kea mice may experience transient behaviour changes that do not persist into the later stages of life, as reported for the pathophysiology of DS in young adult F1.Scn1a+/−tm1kea mice (Favero et al., 2018).

Most DS patients experience a lifelong debilitating condition affecting their daily activities and resulting in a poor quality of life (Lagae et al., 2018). In mice, the tendency to hide objects in the marble test and nest building activity are relevant measures to compare an individual’s performance in daily life activities (Deacon, 2006). Indeed, ~6 month old F1.Scn1a+/−tm1kea mice showed impaired exploratory behaviour as indicated by a reduction in the number of buried and displaced marbles. In addition, F1.Scn1a+/−tm1kea mice displayed a poor performance in the nest building assessment. These results suggest that this epileptic encephalopathy is compromising the welfare and quality of life of F1.Scn1a+/−tm1kea mice as similarly
observed in DS patients.

In summary, the present work reports that F1.Scn1a(+/-)/Scn1a(-/-) mice reproduce the epilepsy phenotype and select comorbidities of DS, positioning this model as a reliable and versatile tool for the search for new therapies and biomarkers in future pre-clinical tests in DS. However, aspects involving sex, time of the day or application of surgical interventions may influence the phenotype. The findings also point to limitations in the use of older mice in the model to screen for corrective treatments of certain co-morbidities. These factors should be taken into consideration in future studies in Scn1a(+/-)/Scn1a(-/-) mouse line.

Authors’ contribution

RRG, CRR and DCH conceived and designed the study and DCH obtained funding. RRG established and performed the management of DS colony with the assistance of JA for genotyping tests and assessment of mice welfare parameters. CRR performed the surgeries for EEG and telemetry recordings. HB performed the EEG background analyses and RRG performed the remaining in vivo experiments (hyperthermia challenge, EEG followed by video monitoring and behaviour assessments), data analyses and wrote the initial manuscript. All authors approved the final version of the manuscript.

Conflicts of interest

None of the authors has any conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.expneurol.2022.114090.

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