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Behavioral phenotyping of young *Scn1a* haploinsufficient mice

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ABSTRACT

Dravet syndrome is a rare, severe, infancy-onset epileptic encephalopathy associated with a high premature mortality. In most patients, Dravet syndrome is caused by a heterozygous loss-of-function mutation in the *SCN1A* gene encoding the alpha 1 subunit of the sodium channel. Of the variety of *SCN1A* variants identified in patients with Dravet syndrome, *SCN1A* missense mutations occur in one-third of cases. The novel *Scn1a*-A1783V mouse model of Dravet syndrome carries the human Ala1783Val missense variant. Recently, the behavioral phenotype of *Scn1a*-A1783V haploinsufficient adult mice has been characterized, which may provide a valuable basis for assessment of novel therapeutic approaches. However, there is still limited information on the developmental course of behavioral alterations in the *Scn1a*-A1783V mouse model, which is of particular relevance for conclusions about face validity and severity classification of the model. Based on reference data from young wildtype mice, we analyzed selected behavioral parameters and fecal corticosterone metabolites in the *Scn1a*-A1783V mouse model during post-weaning development. Differences in the preference for a sweet saccharin solution between Dravet mice and wildtype mice were observed once mice reached sexual maturity. Nest building behavior was already influenced by the *Scn1a* genotype during prepubescence. Sexually mature Dravet mice showed a significantly reduced burrowing performance as compared to their wildtype littermates. In the open-field test, pronounced hyperactivity and increased thigmotactic behavior were evident in prepubescent and sexually mature Dravet mice. Analysis of Irwin scores revealed several genotype-dependent changes in handling-associated parameters during the course of adolescence. The information obtained provides insight into the age-dependence of behavioral patterns in the novel *Scn1a*-A1783V mouse model of Dravet syndrome. In addition, the dataset confirms the suitability of the applied behavioral composite measure scheme for evidence-based assessment of cumulative severity in genetic mouse lines.

1. Introduction

Dravet syndrome (DS) is a rare, severe epileptic encephalopathy with a typical onset in infancy. In 70–80 % of the cases, DS is caused by a heterozygous loss-of-function mutation in the *SCN1A* gene encoding the alpha 1 subunit of the sodium channel ($Na_v1.1$) [1,2]. Over 1,000 variants have been identified in patients with DS with the majority resulting in *SCN1A* haploinsufficiency [3]. The range of different variants may explain the diversity of *SCN1A*-associated impairments [4]. While truncating mutations occur in 50–60 % of the cases, 30–40 % of patients diagnosed with DS carry *SCN1A* missense variants [3,5]. The novel *Scn1a*-A1783V mouse model carries the human Ala1783Val missense variant [6], which has been confirmed as a loss-of-function mutation leading to impaired interneuron function [4]. From a clinical perspective, DS usually manifests in early childhood with febrile or temperature-sensitive seizures during the first year of life [2]. As the disease progresses during childhood, seizures occur spontaneously, become more frequent, and are more severe. Compared with other pediatric encephalopathies, children with DS have a 15-fold increased risk of dying from sudden unexpected death in epilepsy (SUDEP) [7]. Furthermore, DS is associated with refractory epilepsy requiring multimodal treatment strategies [8,9]. After clinical manifestation of the disease, DS is characterized by several comorbidities, including psychomotor regression, ataxia, autism-like behavior, attention deficits, circadian rhythm and sleep impairment [2,8,10]. Affected children also suffer from the conse-

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quences of severe cognitive impairment [2], with most patients of preschool age showing marked developmental delay [11]. Given these devastating influences during the sensitive phase of physical and cognitive development of young patients, surprisingly little is known about the phenotypical profile during specific developmental stages in corresponding genetic mouse models of DS [12]. Phenotyping animal models of neurodevelopmental disorders during post-weaning development can for example provide in-depth information for preclinical assessment of disease-targeting precision medicine approaches, the success of which may depend on the timing of treatment initiation [8,12,13]. Recently, we have completed a comprehensive phenotypic, molecular, and metabolic characterization of the *Scn1a*-A1783V model [14–16]. These studies comprised a behavioral analysis in adult mice [16]. Detailed information on the onset and developmental course of behavioral characteristics of the *Scn1a*-A1783V mouse model may help to further confirm the face validity of the model. Besides, phenotypical characterization of young mice may essentially contribute to the evaluation of their lifetime burden or so-called 'cumulative severity', which is the degree of distress, pain, and suffering experienced by the mice. In the European Union, genetic mouse lines are classified according to their lifetime burden, based on EU Directive 2010/63/EU [17,18]. As suggested by the German Center for the Protection of Laboratory Animals (Bf3R), the final evaluation of genetic mouse lines should include two evaluations of the litter, in which the nutritional status, body condition, and body weight of the offspring are assessed [19]. Individual animals of the line are usually investigated following puberty at an age of two months based on common clinical scoring schemes [19]. Clinical scoring, however, may not allow a sensitive assessment considering the multidimensional aspects of severity including emotional behaviors associated with pain, distress, and anxiety [20–22]. Therefore, we applied a home cage-based scheme for composite behavioral measurements in the *Scn1a*-A1783V mouse model during the developmental phases corresponding to the stages of late infancy and adolescence. Originally, the set of parameters was based on an extensive set of candidate parameters for the assessment of individual severity in adult rodent models [20,23,24], which were subjected to multivariate analysis to evaluate the informativeness and robustness of the parameters. The assembly of the test battery for determining severity in young mice was derived from reference data obtained in young C57BL/6JRj wildtype mice [25] and was subsequently validated in a genetic loss-of-function mouse model of GluA1 deficiency [26]. The information obtained provides guidance for the assessment of a mouse line-specific cumulative burden and provides a basis for the validation of the applied age-specific composite measure scheme in genetic mouse models.

2. Materials and methods

Ethical statement

All animal experiments were conducted and reported in line with the EU Directive 2010/63/EU, the German Animal Welfare Act, the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, and the Basel declaration (https://www.basel.declaration.org) including the 3R principle. All animal experiments were approved by the government of Upper Bavaria (Munich, Germany, license number ROB-55.2-2532.Vet_02-19-157).

2.2. Animals

2.2.1. Breeding

Experimental animals (n = 40) were bred in-house from parental lines B6(Cg)-*Scn1a*$^{tm1.1Dsf}$/J [6,27] (JAX stock #026133) and 129S1/Sv-*Hprt*$^{tm1(CAG-cre)Mnn}$/J [28] (JAX stock #004302). The breeding colony stock was maintained with animals originally obtained from The Jackson Laboratory (Bar Harbor, Maine, USA). To generate experimental animals, 12 female mice with an 129S1/Sv-*Hprt*$^{tm1(CAG-cre)Mnn}$/J background, heterozygous for Cre recombinase, were mated with six males of the background strain B6(Cg)-*Scn1a*$^{tm1.1Dsf}$/J, which were conditional knock-in mice with a floxed *Scn1a* gene, expressing the mutation A1783V in exon 26. From the resulting offspring animals (born alive n = 60), 53 animals survived until weaning at postnatal day (P) 19. 22 of these offspring animals (11 female, 11 male) were heterozygous *Scn1a*-A1783V mice (wildtype or heterozygous for Cre-recombinase), from which 21 mice (11 male; 10 female) survived until the end of the adolescence phase. 31 offspring mice were *Scn1a*-A1783V wildtype mice (wildtype or heterozygous for Cre recombinase). Group allocation was based on the *Scn1a*-A1783V genotype. The experimental group (n = 20, female/male 10/10), carrying the heterozygous A1783V-*Scn1a* gene mutation, are referred to as 'Dravet mice' in the following. The control group (n = 20, female/male 10/10), carrying the wildtype *Scn1a* gene, are referred to as 'wildtype mice' in the following. The genotype for Cre recombinase was not considered for group allocation as Cre had no impact on the behavioral phenotype in adult mice [16]. The genotype of the animals was determined by PCR as described previously [16]. In line with earlier reports from our group, the onset of seizures was observed at P16 [14,16].

2.2.2. Intense care measures

From P14-26, animals received one cup of Dietgel76A (Ssniff Spezialdiäten GmbH, Soest, Germany) per cage on a daily basis, stirred up with 5–7 ml of 10 % glucose in tap water. The days following weaning at P19 were a critical time slot regarding mortality rates [16], in the following referred to as 'period of intense care' (Fig. 1). From P19-26, offspring animals were offered wet, sweetened food pellets on the cage ground, and a cup of Dietgel76A with 5–7 ml of glucose 10 % in tap water twice a day. Since some offspring mice appeared to have difficulties with spatial orientation in the new cage after weaning, they were fed by hand with glucose 10 % and Dietgel76A, mixed and drawn up in a syringe. Feeding intervals of approximately three hours were prolonged as soon as offspring animals were observed to feed on their own. Noises and other distress provoking factors such as fixation of the animals were strictly avoided.

2.2.3. Housing

Female animals with their litters were housed individually under controlled environmental conditions (22–24 °C, 45–60 % humidity) in individually ventilated cages (Tecniplast Deutschland GmbH, Hohenpeißenberg, Germany) in a 12-hour dark-light cycle with ad libitum access to food (Ssniff Spezialdiäten GmbH, Soest, Germany) and tap water. The cages were provided with bedding material (Lignocel Select, J. Rettenmaier & Söhne GmbH & Co. KG, Rosenberg, Germany), 14 g of nesting material (Enviro Dri, Claus GmbH, Limburgerhof, Germany), a wood brick (Labodia AG, Niederglatt, Switzerland), and a triangular mouse house (Zoonlab GmbH, Castrop-Rauxel, Germany). Weaning and sampling ear biopsies for genotyping were carried out at P19 between 5 and 7 p.m. After weaning, animals were housed in sex-matched groups of four to six animals per cage in Makrolon type III cages (Ehret GmbH & Co. KG, Emmendingen, Germany), supplemented with bedding material (Lignocel Select, J. Rettenmaier & Söhne GmbH & Co. KG, Rosenberg, Germany), Enviro Dri nesting material (Enviro Dri, Claus GmbH, Limburgerhof, Germany), two nestlets (Ancare, Bellmore, New York, USA), and one square animal house (Zoonlab GmbH, Castrop-Rauxel, Germany).

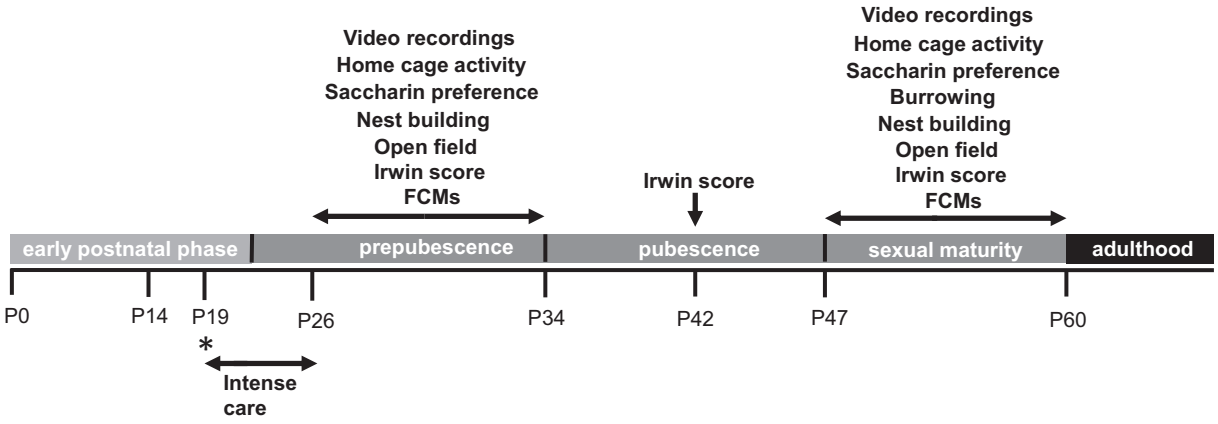

Fig. 1. Overview of the experimental design. FCMs refers to fecal corticosterone metabolites, * refers to the procedure of weaning.

From P26 onward, animals were housed in groups of two, according to their *Scn1a*-genotype and sex, as one experimental unit (n = 2). During four days each in the stages of prepubescence and sexual maturity (Fig. 1), the experimental units (n = 20) were housed in home cage systems with continuous video recording (PhenoTyper, Noldus, Wageningen, the Netherlands), combined with the video analysis tracking software EthoVision XT 15 (Noldus, Wageningen, the Netherlands, RRID:SCR_000441). Each PhenoTyper was supplemented with 200-g bedding material (Lignocel Select, J. Rettenmaier & Söhne GmbH & Co. KG, Rosenberg, Germany), two nestlets (Ancare, Bellmore, New York, USA), an infrared translucent shelter (Noldus, Wageningen, the Netherlands) and two drinking bottles (Noldus, Wageningen, the Netherlands).

2.3. Experimental design

Based on data obtained in C57BL/6JRj wildtype mice [25], experimental animals were subjected to a behavioral test battery during adolescence (Fig. 1). The developmental stage of murine adolescence can be subclassified into three narrow time windows, as described by Brust and colleagues [29]: 1) prepubescence (from P23 onward), 2) pubescence (from P35 onward), and 3) sexual maturity (from P48 onward). Adolescent mice aged 48 days and older are referred to as 'sexually mature' mice for reasons of readability in the following. Group allocation and age ranges of the mice are illustrated in Table 1. For several of the tests conducted in the home cage, the analysis was performed per experimental unit (n = 2) (Table 1). All behavioral tests carried out in the PhenoTyper home cage were video recorded.

2.4. Infrared-based litter monitoring

As an exception to the housing conditions described above, one mother with its litter, comprising six offspring animals, was transferred at P16 to a PhenoTyper home cage, supplemented as described above. The PhenoTyper home cage was combined with a system for continuous infrared video monitoring, comprising the FLIR Lepton 3.5 Micro thermal camera module (Teledyne FLIR LLC, Wilsonville, USA), and the USB webcam breakout board PureThermal 2 Smart I/O Board (Teledyne FLIR LLC, Wilsonville, USA). The Infrared camera module was controlled via the software Lepton User App for Windows (Teledyne FLIR LLC, Wilsonville, USA). Continuous infrared monitoring was carried out from P16 to P19 to obtain information about home cage group interaction, separation, and maternal neglect.

Table 1
Overview of the behavioral test battery and group allocation. FCMs refers to fecal corticosterone metabolites. Tests marked with an asterisk * were conducted in the home cage (n = 2 animals/cage) and were analyzed per experimental unit (n = 2).

Behavioral test	Female Dravet mice/ Male Dravet mice/ Female wildtype mice/Male wildtype mice/(total n = 40)	Observation period	Postnatal age range of test
Litter monitoring	Litter (2/4/0/0) and mother	Infancy	P16-P19
Saccharin preference *	10/10/10/10	Prepubescence	P27-P33
	10/10/8/6	Sexual maturity	P48-P56
Burrowing *	10/10/10/10	Sexual maturity:	P49-P55
Nest building *	10/10/10/10	Prepubescence	P27-P33
	10/10/10/10	Sexual maturity	P48-P56
Home cage activity	10/10/10/10	Prepubescence	P29-P32
	10/10/10/10	Sexual maturity	P51-P54
Open field	10/10/10/10	Prepubescence	P29-P32
	10/10/10/10	Sexual maturity	P56-P57
Irwin Score	10/10/10/10	Prepubescence	P29-P32
	10/10/10/10	Pubescence	P42
	10/10/10/10	Sexual maturity	P56-P57
FCMs	10/10/10/10	Prepubescence	P29-P32
	9/10/10/10	Sexual maturity	P56-P57
Body weight	10/10/10/10	Late infancy	P19, P21
	10/10/10/10	Prepubescence	P23, P25, P27, P30
	10/10/10/10	Pubescence	P36, P42
	10/10/10/10	Sexual maturity	P49, P55

2.5. Behavioral home cage assessment

2.5.1. Saccharin preference

With the saccharin preference test carried out during prepubescence and sexual maturity, we aimed to assess anhedonia-associated behavior as described previously [25]. During the observation period of four days each, animals had access to two water bottles filled with 200-g tap water on the first and third days to determine the daily water intake. On the second and fourth days, one water bottle was filled with 200 g of a 0.1 % saccharin solution (Aldrich Saccharin $\geq$ 98 %, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) with the other containing 200 g of tap water. The side of the bottle containing the saccharin solution in the cage

was alternated on days 2 and 4. Analysis was carried out following a protocol by Klein et al. [30]. During the investigations in sexually mature animals, four male wildtype mice and two female wildtype mice displayed stereotypical licking behavior. The animals 'sipped' from the bottles until they were empty. The embedding in the cages beneath the empty water bottles was wet, and animals were observed not to drink the amount 'sipped'. Therefore, these six animals, comprising three experimental units, were excluded from the analysis.

2.5.2. Burrowing

We assessed burrowing performance on two consecutive days in sexually mature mice, as we previously confirmed relevant levels of burrowing activity in wildtype mice only from sexual maturity onward [25]. We assessed the amount of pellets burrowed during a two-hour light-phase session as well as the respective overnight performance on two consecutive days. Two hours prior to the dark phase, an empty water bottle (length: 20 cm, diameter of the bottleneck: 3.5 cm; Zoonlab GmbH, Castrop-Rauxel, Germany) was filled with 200 ± 1-g food pellets (Ssniff Spezialdiäten GmbH, Soest, Germany) and placed into the PhenoTyper home cage. After two hours, the weight of the bottle with the remaining pellets was measured, and the bottle was replaced for the assessment of the overnight burrowing activity. Pellets distributed on the floor of the cage from the previous two-hour test session were removed. On the next day, immediately after the dark phase, the weight of the bottle with the remaining pellets was measured again.

2.5.3. Nest building

We investigated nest building performance in the PhenoTyper home cage, assessing the complexity and shape of the nest over four days each during prepubescence and sexual maturity. On the first test day, animals were given two pressed cotton squares (nestlets, Ancare, Bellmore, New York, USA) per PhenoTyper home cage. Pictures of the nest were taken on a daily basis in the morning, including a top-down view and two side views at an angle of 90 and approximately 45 degrees. For the image-based evaluation of the nest complexity, we applied a slightly modified version of a protocol reported by Jirkof and colleagues [31]. The scoring of the images was carried out by a person who was blinded for group allocation. Detailed information about the applied scoring scheme can be found in the Supplementary file.

2.5.4. Home cage activity

We evaluated aspects of the animals' home cage-like phenotype [32], focusing on overall and zone-specific activity patterns. In the PhenoTyper home cages, we assessed the overall activity of the animals, based on the distance moved and velocity, using the tracking software Ethovision XT 15 (Noldus, Wageningen, the Netherlands, RRID:SCR_000441) For zone-specific assessment, the durations animals spent in the areas surrounding the feeder rack and the drinking bottles, as well as in the center of the cage, were analyzed, corresponding to the zones 'feeding', 'drinking', and 'center' of the home cage. Analysis was carried out approximately 30 minutes after the beginning of the dark phase, since animals showed high levels of activity then, with a tracking duration of exactly 60 minutes. Analysis was performed during prepubescence and once again during sexual maturity.

2.6. Open field

Exploratory behavior and locomotor activity were evaluated in the open-field paradigm with a total monitoring duration of 15 minutes. Male animals were tested prior to female animals. Animals were placed individually in a circular shaped open field (diameter: 60 cm; lighting: 20 lux) 10 cm away from and facing the wall. The open-field arenas were cleaned with 0.1 % acetic acid after each trial. For analysis, the entire arena was subdivided in an outer ('wall'), middle, and inner ('center') zone. The 'wall' zone was defined as the outer 17 %, and the 'center' zone as the inner 45 % of the entire arena. Open-field arenas were cleaned with 0.1 % acetic acid after each trial. Analysis was carried out using the tracking software EthoVision XT 8.5 (Noldus, Wageningen, the Netherlands, RRID:SCR_000441). Moreover, we manually assessed the number of 'rearing' positions as well as 'jumps' against the arena wall. The assessor was unaware of group allocation.

2.7. Irwin Score

We applied the traditional Irwin scoring system [33] to obtain information about general behavioral, neurological, and autonomic changes. Detailed information on the scoring system is provided in the Supplementary file. Irwin scoring was split into three consecutive parts (1. home cage, 2. open field, 3. fresh single cage), followed by rectal body temperature measurement. The assessor was unaware of group allocation.

2.8. Body weight

After weaning at P19, body weight was closely monitored during late infancy and adolescence. Detailed information on the postnatal days of weighing is provided in Table 1.

2.9. Fecal corticosterone metabolites (FCMs)

We analyzed corticosterone metabolites in fecal samples during prepubescence as well as during sexual maturity. Individual samples were collected directly after the open-field paradigm. For detailed information about processing and analysis, see the Supplementary file.

2.10. Statistics

Statistical analyses were conducted using R version 4.0.2 [34] and SPSS version 28.0.1 (IBM Corp, Armonk, New York USA). Normality of the data and equality of variances were tested based on the Shapiro–Wilk and Levene's test. Data involving both sexes were tested for significant interaction between the two main factors genotype and sex by parametric two-way analysis of variance (ANOVA) or nonparametric aligned rank transform (ART) ANOVA [35,36] with ARTool (version 2.1.2, Washington, USA). Significant results were further investigated applying Tukey's test or Bonferroni correction. In the absence of a significant sex by genotype interaction, data of female and male mice were combined for group analysis [37]. For the investigation of group differences between Dravet mice and wildtype mice, we applied parametric unpaired two-tailed t-tests or nonparametric Mann–Whitney U tests where indicated. Graphical illustration was carried out using GraphPad Prism 5.04 for Windows (GraphPad Prism Software, San Diego, USA). A p value < 0.05 was considered statistically significant in all statistical tests performed. Data analyzed with parametric statistics are presented as mean ± standard error of the mean (SEM). Data analyzed with non-parametric statistics are illustrated as median with interquartile range (IQR).

3. Results

3.1. Behavioral home cage assessment

3.1.1. Saccharin preference

During prepubescence, Dravet mice did not show a reduced preference for the sweetened solution when compared with their wildtype littermates (Fig. 2a, Mann–Whitney test: $U = 43$, $p = 0.63$). During sexual maturity, saccharin preference in wildtype mice exceeded that of age-matched Dravet mice (Fig. 2b, Mann–Whitney test: $U = 6$, $p = 0.003$).

3.1.2. Burrowing

The assessment of burrowing behavior in sexually mature animals indicated a sex-specific difference in the first overnight burrowing session (Fig. 2c, ART ANOVA: genotype: $F_{1,16} = 48.78$, $p < 0.0001$, sex: $F_{1,16} = 8.55$, $p = 0.01$, interaction sex by genotype: $F_{1,16} = 5.95$, $p = 0.03$) with male Dravet mice exhibiting a poorer burrowing performance than their male wildtype littermates (Tukey's post-hoc test: $p = 0.0002$). No significant difference was observed when comparing the respective burrowing performance between female Dravet mice and the female control group (Tukey's post-hoc test: $p = 0.20$). In the second overnight test, independent of the sex of the animals, Dravet mice burrowed a smaller amount of pellets than wildtype mice (Mann–Whitney test: $U = 0$, $p = 0.0002$).

Our findings from the two-hour light phase sessions did not indicate genotype-related differences (Day 1: Supplementary Fig. S1a: Mann–Whitney test: $U = 40$, $p = 0.47$; Day 2, Supplementary Fig. S1b: Mann–Whitney test: $U = 37$, $p = 0.34$).

3.1.3. Nest building

When comparing scores from individual days, genotype-related differences were observed in prepubescent and sexually mature mice. In prepubescent mice, a genotype-related difference was observed only on the fourth day following the offer of new nesting material with Dravet mice achieving lower nest complexity scores than their wildtype littermates (Fig. 3a, Mann–Whitney test: $U = 13.5$, $p = 0.005$). The observation of nest complexity in sexually mature mice indicated a genotype-dependent reduction of nest complexity on the first (Fig. 3b, Mann–Whitney test: $U = 17$, $p = 0.009$) and the third observation day (Fig. 3b, Mann–Whitney test: $U = 25$, $p = 0.04$). On day 2, the analysis of nest complexity in sexually mature mice revealed a relevant sex-specific difference (Fig. 3b, ART ANOVA: genotype: $F_{1,16} = 6.37$, $p = 0.02$, sex: $F_{1,16} = 5.44$, $p = 0.03$, interaction sex by genotype: $F_{1,16} = 5.44$, $p = 0.03$): while female Dravet mice achieved lower complexity scores than female wildtype mice (Tukey's post-hoc test: $p = 0.02$), the respective comparison for male animals indicated no significant genotype-dependent difference (Tukey's post-hoc test: $p = 1.0$). Analysis of nest complexity on the fourth day in sexually mature mice indicated no significant impact of the *Scn1a* genotype (Fig. 3b, Mann–Whitney test: $U = 48.5$, $p = 0.94$). When comparing sum scores, calculated by adding the scores of the individual days, we did not confirm a difference related to the *Scn1a* genotype in prepubescent mice, but observed a respective trend ($p < 0.1$) with Dravet mice reaching lower sum scores than their wildtype littermates (Fig. 3c, Mann–Whitney test: $U = 25$, $p = 0.06$). Comparison of sum scores in sexually mature mice, however, demonstrated a relevant sex-specific difference (Fig. 3d, ART ANOVA: genotype: $F_{1,16} = 5.98$, $p = 0.03$, sex: $F_{1,16} = 0.03$, interaction sex by genotype: $F_{1,16} = 4.52$, $p = 0.049$) with female Dravet mice reaching lower sum scores than female wildtype littermates (Tukey's post-hoc test: $p = 0.03$). Comparison of sum scores from sexually mature male mice failed to show a significant genotype-related difference (Tukey's post-hoc test: $p = 0.98$).

3.1.4. Home cage activity

Analysis of the overall home cage activity (Supplementary Fig. S2) revealed that prepubescent Dravet mice moved with increased speed (Mann–Whitney test: $U = 110$, $p = 0.02$) as compared to their wildtype littermates, whereas the analysis of the distance moved by the prepubescent mice did not reveal significant group differences (Mann-Whitney test: $U = 143$, $p = 0.13$). Once sexually mature, the findings indicate that wildtype mice moved a greater distance (Mann–Whitney test: $U = 54$, $p < 0.0001$) with increased velocity (Mann–Whitney test: $U = 86$, $p = 0.002$) in comparison to Dravet mice.

Analysis of home cage activity per zone (Supplementary Fig. S3) demonstrated a sex-specific difference considering the durations prepubescent mice spent in the zones 'feeding' and 'drinking' (ART ANOVA: zone 'feeding', genotype: $F_{1,36} = 4.66$, $p = 0.04$, sex: $F_{1,36} = 1.12$, $p = 0.30$, interaction sex by genotype: $F_{1,36} = 10.11$, $p = 0.003$, zone 'drinking', genotype: $F_{1,36} = 4.60$, $p = 0.04$, sex: $F_{1,36} = 0.06$, $p = 0.81$, interaction sex by genotype $F_{1,36} = 10.78$, $p = 0.002$): male wildtype mice spent significantly more time in the zone 'feeding' (Tukey's post-hoc test: $p = 0.004$) and in the zone 'drinking' (Tukey's post-hoc test: $p = 0.01$) than male Dravet mice during prepubescence. Independent of sex, sexually mature wildtypes spent significantly more time in the zone 'feeding' (Mann-Whitney test: $U = 31$, $p < 0.0001$) and in the zone 'drinking' (Mann–Whitney test: $U = 5$, $p < 0.0001$) as compared to age-matched Dravet mice. Considering the duration the animals spent in the zone 'center', we detected no significant difference during prepubescence (Mann–Whitney test: $U = 158$, $p = 0.53$). In contrast, we found a significant sex-specific difference in sexually mature mice (ART ANOVA: genotype: $F_{1,36} = 23.57$, $p < 0.0001$, sex: $F_{1,36} = 6.95$, $p = 0.01$, interaction sex by genotype: $F_{1,36} = 13.40$, $p = 0.0008$) with female wildtype mice spending significantly more time in the zone 'center' than female Dravet mice (Tukey's post-hoc test: $p < 0.0001$).

3.2. Open field

During the total recording duration of 15 minutes, Dravet mice moved a greater distance and with increased velocity as compared to the wildtype control group during prepubescence (Fig. 4a, distance, Mann–Whitney test: $U = 21$, $p < 0.0001$; Supplementary Fig. S4a, velocity, Mann–Whitney test: $U = 21$, $p < 0.0001$) as well as during sexual maturity (Fig. 4b, distance, Mann–Whitney test: $U = 2$, $p < 0.0001$ Supplementary Fig. S4b, velocity, Mann–Whitney test: $U = 2$, $p < 0.0001$). Dravet mice spent significantly more time in the zone 'center' during prepubescence (Supplementary Fig. S4c, Mann–Whitney test: $U = 65$, $p = 0.0003$) as compared to their wildtype littermates. The respective analysis in sexually mature mice demonstrated a sex-specific difference (Supplementary Fig. S4d, ART ANOVA: genotype: $F_{1,36} = 13.34$, $p = 0.0008$, sex: $F_{1,36} = 0.71$, $p = 0.41$, interaction sex by genotype: $F_{1,36} = 5.08$, $p = 0.03$) with male Dravet mice spending less time in the center than male wildtype mice (Tukey's post-hoc test: $p = 0.009$). Dravet mice spent significantly more time in the zone 'wall' than the wildtype control group in both age phases, i.e. during prepubescence (Fig. 4c, Mann–Whitney test: $U = 40$, $p < 0.0001$) and sexual maturity (Fig. 4d, Mann–Whitney test: $U = 64$, $p = 0.0002$). Independent of the sex of the animals, rearing frequency was significantly increased in Dravet mice during prepubescence (Fig. 4e, Mann–Whitney test: $U = 20$, $p < 0.0001$) as well as once having reached sexual maturity (Fig. 4f, Mann–Whitney test: $U = 5$, $p < 0.0001$). Dravet mice showed significantly more 'jumps' against the wall of the open field than their wildtype littermates in prepubescence

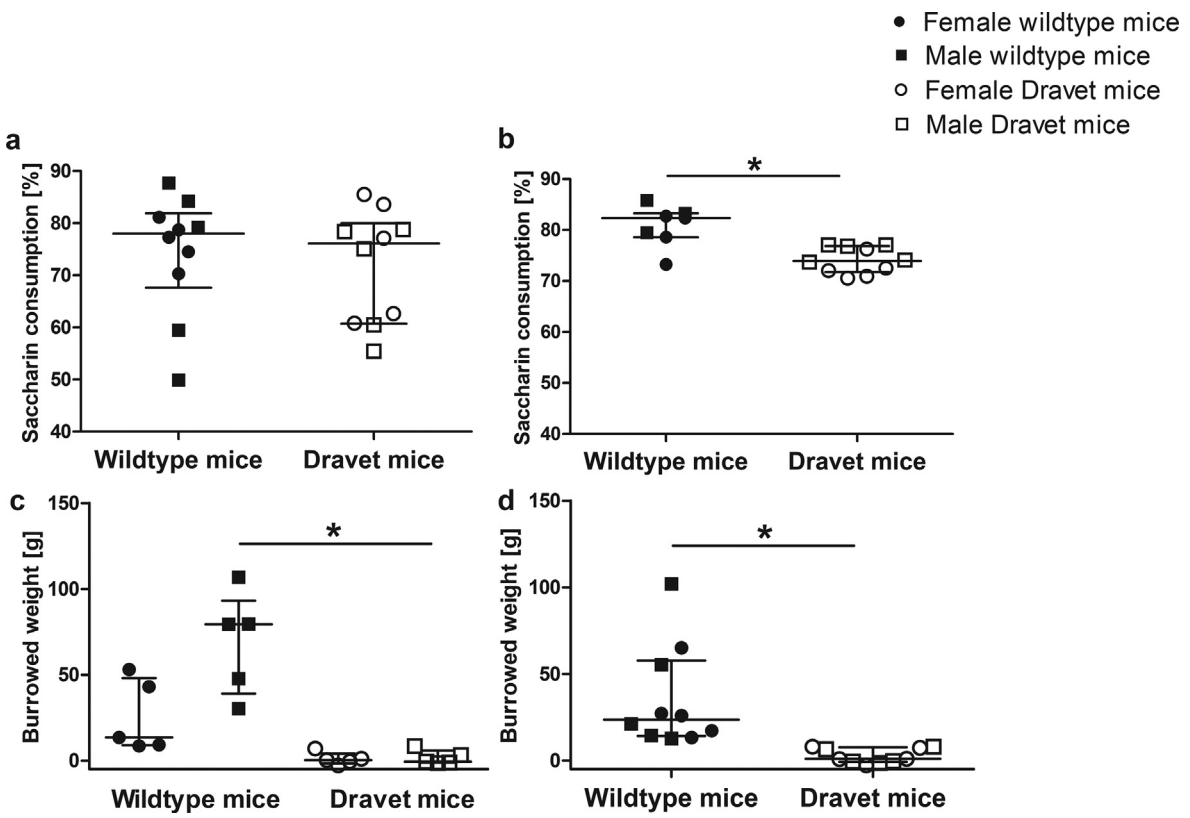

Fig. 2. Saccharin preference and burrowing performance. The assessment of the preference for a saccharin solution in prepubescent animals (a) did not indicate differences between Dravet mice and wildtype mice (Mann–Whitney test). Once sexually mature (b), wildtype mice showed a significantly stronger preference for saccharin than age-matched Dravet mice (Mann–Whitney test). The overnight burrowing performance indicated a sex-specific difference during the first test (c) with male wildtype mice burrowing a larger amount of pellets than male Dravet mice (ART ANOVA, followed by Tukey's post-hoc test). In the second overnight test (d), independent of the sex of the animals, Dravet mice burrowed a smaller amount of food pellets than wildtype littermates (Mann–Whitney test). * $p < 0.05$, median (IQR).

(Supplementary Fig. S4e, Mann–Whitney test: $U = 45.5$, $p < 0.0001$) and once sexually mature (Supplementary Fig. S4f, Mann–Whitney test: $U = 50$, $p < 0.0001$).

Analysis of the first five minutes in the open field reveals information about exploratory behavior of the animals. During the first five minutes, Dravet mice moved a greater distance and with increased speed as compared to the wildtype control group during prepubescence (Supplementary Fig. S5a, distance, Mann–Whitney test: $U = 13$, $p < 0.0001$; Supplementary Fig. S5b, velocity, Mann–Whitney test: $U = 1$, $p < 0.0001$) and when sexually mature (Supplementary Fig. S5c, distance, Mann–Whitney test: $U = 1$, $p < 0.0001$; Supplementary Fig. S5d, velocity, Mann–Whitney test: $U = 1$, $p < 0.0001$). The duration spent in the zone 'wall' was significantly increased in prepubescent Dravet mice (Supplementary Fig. S5e, Mann–Whitney test: $U = 1$, $p < 0.0001$) as compared to age-matched wildtype mice. In sexually mature mice, the duration spent in the zone 'wall' indicated no significant group difference (Supplementary Fig. S5f, Mann–Whitney test: $U = 135$, $p = 0.08$). Concerning the duration mice spent in the zone 'center' during the first five minutes, significant group differences were neither found in prepubescent mice (Supplementary Fig. S5g, Mann–Whitney test, $U = 161$, $p = 0.30$) nor in sexually mature mice (Supplementary Fig. S5h, Mann–Whitney test, $U = 174$, $p = 0.49$).

3.3. Irwin Score

The analysis of Irwin sum sores indicated a genotype-related increase in Dravet mice as compared to wildtype littermates for the three age brackets (Fig. 5a, prepubescence, Mann–Whitney test: $U = 121$, $p = 0.03$; Fig. 5b, pubescence, Mann–Whitney test: $U = 71.5$, $p = 0.0005$, Fig. 5c, sexual maturity, Mann–Whitney test: $U = 100$, $p = 0.006$). The increase of sum scores is calculated based on the changes in single parameters, in particular touch response, vocalization, irritability, and urination. Our findings indicate that, independent of the sex of the animals, Dravet mice showed increased responsivity to light touch by the observer during the three developmental stages (Fig. 5d, prepubescence, Mann–Whitney test: $U = 124$, $p = 0.007$; Fig. 5e, pubescence, Mann–Whitney test: $U = 35.5$, $p < 0.0001$, Fig. 5f, sexual maturity, Mann–Whitney test: $U = 75$, $p = 0.0001$). Regarding handling-associated irritability (bite propensity), we found no group difference in prepubescent mice (Supplementary Fig. S6a, Mann–Whitney test: $U = 199.5$, $p = 1.0$), whereas the respective analysis in pubescent mice indicated a sex-specific difference (Supplementary Fig. S6b, ART ANOVA: genotype: $F_{1,36} = 11.97$, $p = 0.001$, sex: $F_{1,36} = 7.21$, $p = 0.01$, interaction sex by genotype: $F_{1,36} = 7.20$, $p = 0.01$) with female Dravet mice showing increased levels of irritability toward handling by the observer (Tukey's post-hoc test: $p = 0.007$). Independent of sex, sexually mature Dravet mice reached higher irritability scores than wildtype littermates (Supplementary Fig. S6c, Mann–Whitney test: $U = 150$, $p = 0.02$). We detected a sex-specific difference of handling-associated vocalization in prepubescent mice (Supplementary Fig. S6d, ART ANOVA: genotype: $F_{1,36} = 6.22$, $p = 0.02$, sex: $F_{1,36} = 7.28$, $p = 0.01$, interaction sex by genotype: $F_{1,36} = 4.80$, $p = 0.04$) with female Dravet mice reaching lower scores than age- and sex-matched wildtypes (Tukey's post-hoc test: $p = 0.03$). We further confirmed sex-specific differences of handling-associated urination in prepubescent mice (Supplementary Fig. S6e, ART ANOVA: genotype: $F_{1,36} = 7.21$, $p = 0.01$, sex: $F_{1,36} = 11.74$, $p = 0.002$, interaction sex by genotype: $F_{1,36} = 4.27$, $p = 0.046$) and sexually mature mice (Supplementary Fig. S6f, ART ANOVA: genotype: $F_{1,36} = 25.92$, $p < 0.0001$, sex:

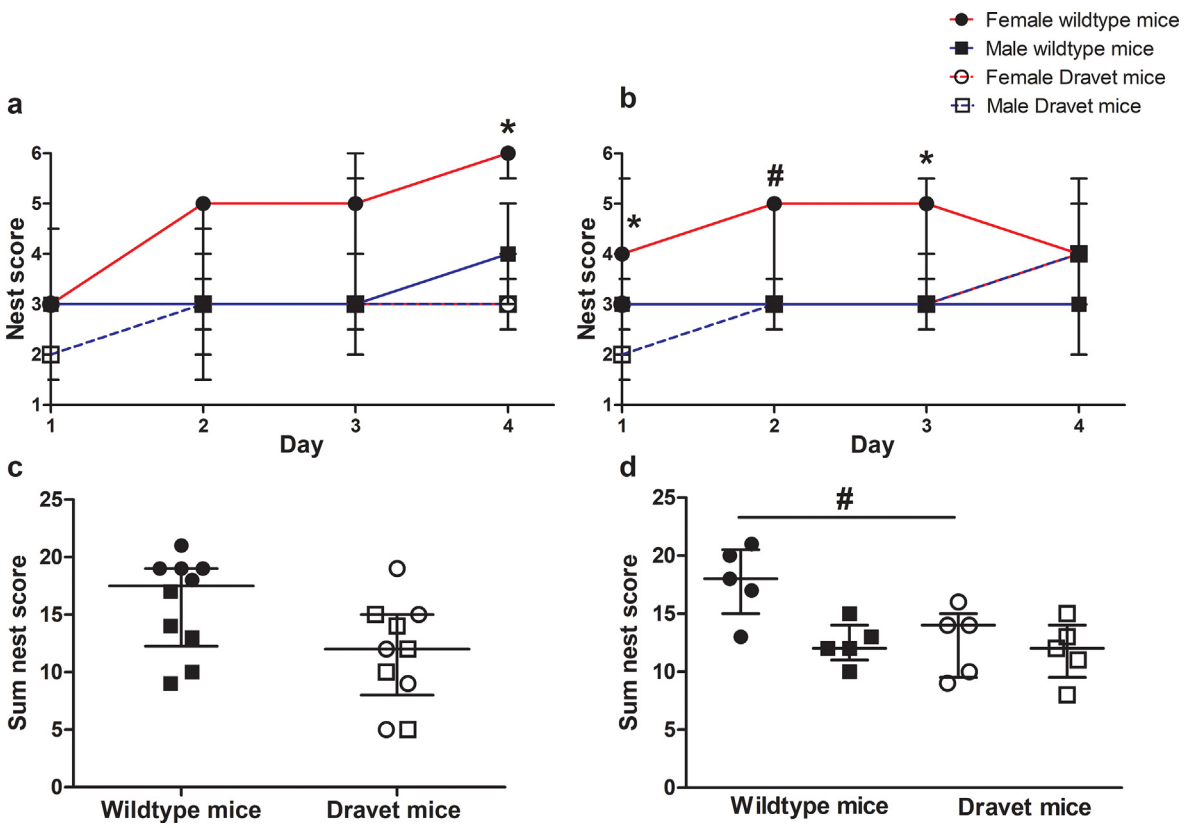

Fig. 3. Nest building performance and nest complexity. During prepubescence (a), the analysis of nest scores per day revealed a difference between Dravet mice and their wildtype littermates only on the last day before offer of new nesting material (Mann–Whitney test). In sexually mature mice (b), nest scores per day significantly differed between Dravet mice and wildtype mice on the first and third days following the offer of new nesting material (Mann-Whitney tests). On day 2, we observed a sex-specific difference with female Dravet mice reaching lower scores than female wildtypes (ART ANOVA, followed by Tukey's post-hoc test). Sum nest scores (c) from the total observation period of four days did not significantly differ between genotypes during prepubescence (Mann–Whitney test). Sum nest scores of sexually mature mice (d) demonstrated a sex-specific decrease in female Dravet mice as compared to female wildtype littermates (ART ANOVA, followed by Tukey's post hoc test). * $p < 0.05$ (both sexes), # $p < 0.05$ (females); median (IQR).

$F_{1,36} = 25.92$, $p < 0.0001$, interaction sex by genotype: $F_{1,36} = 17.55$, $p = 0.0002$): male Dravet mice reached lower scores for handling-associated urination than male wildtype littermates during prepubescence and sexual maturity (Tukey's post-hoc test: $p = 0.04$ and $p = 0.0001$, respectively).

Body temperatures in Dravet mice were decreased as compared to wildtype littermates during prepubescence and sexual maturity, whereas the respective analysis in pubescent mice did not indicate a significant group difference (Supplementary Fig. S7a, prepubescence, Mann–Whitney test: $U = 93.5$, $p = 0.01$; Supplementary Fig. S7b, pubescence, Mann–Whitney test: $U = 142.5$, $p = 0.12$; Supplementary Fig. S7c, sexual maturity, Mann–Whitney test: $U = 71$, $p = 0.0005$).

3.4. Body weight

We illustrated body weight data based on measurements at certain postnatal days from weaning at P19 onward during late infancy, early-, mid-, and late-adolescence (Supplementary Fig. S8). Independent of the sex of the animals, body weight data from weaning at P19 and from P21 demonstrated significantly lower body weights in Dravet mice when compared to wildtype littermates (P19, unpaired t-test, $t_{38} = 8.89$, $p < 0.0001$; P21, unpaired t-test, $t_{38} = 5.75$, $p < 0.0001$). Interestingly, the analysis of body weight measurements from certain postnatal days indicated sex-specific differences from P23 onward. In male Dravet mice, we observed lower body weights when compared to sex-matched wildtype littermates from P25 onward during the entire observation period (Bonferroni post hoc tests: P23: $p < 0.0001$; P25: $p < 0.0001$; P27: $p < 0.0001$; P30: $p < 0.0001$; P36: $p < 0.0001$; P42: $p < 0.01$; P49: $p < 0.05$; P55: $p < 0.05$). In female animals, genotype-related differences were observed only from P23 until P27 (Bonferroni post hoc tests: P23: $p < 0.05$; P25: $p < 0.0001$; P27: $p < 0.01$). Detailed descriptions of postnatal day-specific results are provided in the Supplementary file.

3.5. Litter monitoring

The continuous infra-red based home cage video monitoring of one litter with mother was carried out from P16 until P19 (Supplementary Fig. S9). We hereby observed a clear separation of some offspring animals from their littermates, especially after the occurrence of seizures. The separation of animals became more and more evident over time. The dam initially appeared to show interest in the separated young mice, but was then mostly observed staying with the rest of the litter in the feeding zone and home zone, while some severely affected young mice lay separated from the group. Especially directly after recovery from seizures, offspring were observed eating from the special diet offered. Offspring with a high seizure burden were rarely observed eating together with their littermates.

3.6. Fecal corticosterone metabolites (FCMs)

The analysis of FCMs confirmed a genotype-related difference in prepubescent mice with decreased fecal corticosterone metabolite

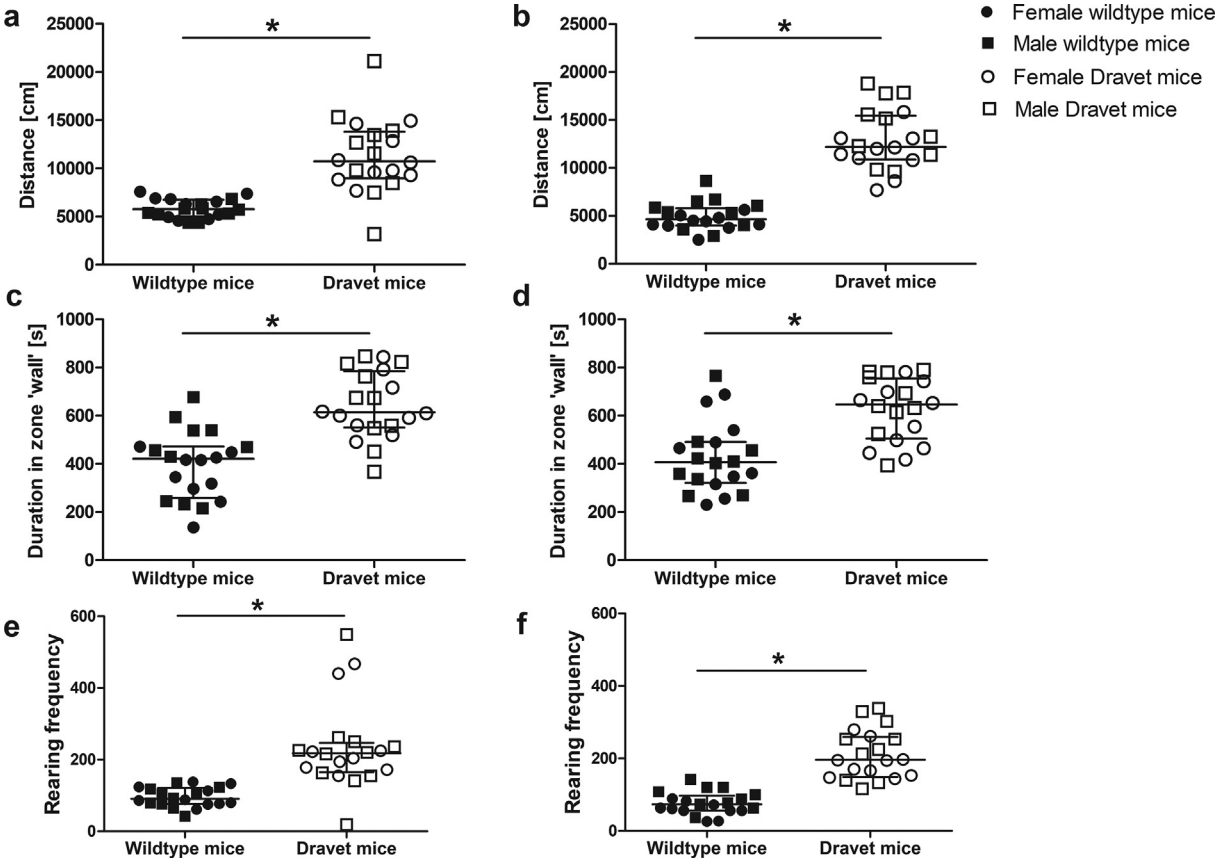

Fig. 4. Open field. Analysis of the total observation period of 15 minutes revealed that Dravet mice moved a greater distance than wildtype mice during prepubescence (a) as well as during sexual maturity (b). Dravet mice spent significantly more time in the wall zone than wildtype mice during prepubescence (c) and when having reached sexual maturity (d). Analysis of the posture 'rearing' revealed a significantly increased total rearing frequency in Dravet mice as compared to wildtype littermates during prepubescence (e) and when animals had reached sexual maturity (f). Mann–Whitney tests. * $p < 0.05$, median (IQR).

levels in Dravet mice as compared to wildtype littermates (Supplementary Fig. S10a, Mann–Whitney test: $U = 108$, $p = 0.01$). The respective analysis of FCMs in sexually mature mice did not indicate a significant genotype-dependent difference (Supplementary Fig. S10b, Mann–Whitney test: $U = 136.5$, $p = 0.14$).

4. Discussion

The findings demonstrate several relevant alterations in the behavioral profile of the genetic *Scn1a*-A1783V mouse model during the post-weaning phase. Considering the complex clinical presentation of DS, which is associated with a variety of cognitive and behavioral impairments, we identified several phenotypic traits that could mimic some of the clinical symptoms during disease progression. Our findings suggest that Dravet mice exhibit age-related behavioral changes to varying degrees, allowing conclusions about the extent of distress the animals experience during distinct stages of development.

Anhedonia, which can be defined as hyposensitivity to pleasure, is considered a classical symptom of depression [30,38]. Because of their high predictive power combined with good practical applicability, sucrose and saccharin consumption tests have been widely used to assess anhedonia-associated traits in rodent models. Previously, we had already demonstrated that saccharin preference can serve as a valuable parameter for evidence-based severity assessment in adult rodents [39–43]. Considering sweetness preference in adolescent mice, Eltokhi and colleagues have reported relevant differences between adolescent mice of the three wildtype inbred strains C57BL/6N, DBA/2, and FVB/N [44]. Based on a protocol developed by Klein and colleagues [30], we have recently evaluated the suitability of the saccharin consumption test in adolescent and young adult wildtype mice. Our recent findings suggested that this paradigm is suitable for application in mice from prepubescence throughout the entire adolescence phase [25]. In the *Scn1a*-A1783V model, our group has already observed a reduced preference for saccharin in animals aged seven to ten weeks [16]. In the present study, we confirmed a reduced preference for sweetness only in the later stage of adolescence, when animals had reached sexual maturity. At first sight, the lack of differences in the early post-weaning phase is surprising, especially in light of the high seizure burden experienced by such young animals. However, young Dravet mice with reduced body weight and pronounced hyperactivity [16] might try to compensate their high loss of energy by drinking the sweet solution. In addition, anhedonia-associated traits might emerge as a consequence of multiple stressors experienced by the animal over time in terms of a cumulative burden.

Non-maternal nest building and burrowing both represent non-essential activity patterns, so-called 'luxury behaviors' [45]. They are among the first to be reduced when animals' well-being is compromised, thereby representing an 'early warning signal' and a highly sensitive indicator of well-being in mice [45]. Furthermore, these complex home cage behaviors, which require performance of a precise sequence of tasks in the daily routine of laboratory rodents, may be suitable models for daily self-care and routine self-management in humans [45]. Protocols have been established for the evaluation of basic daily-routine-activities in human patients, so-called 'activities of daily living' (ADL), and more com-

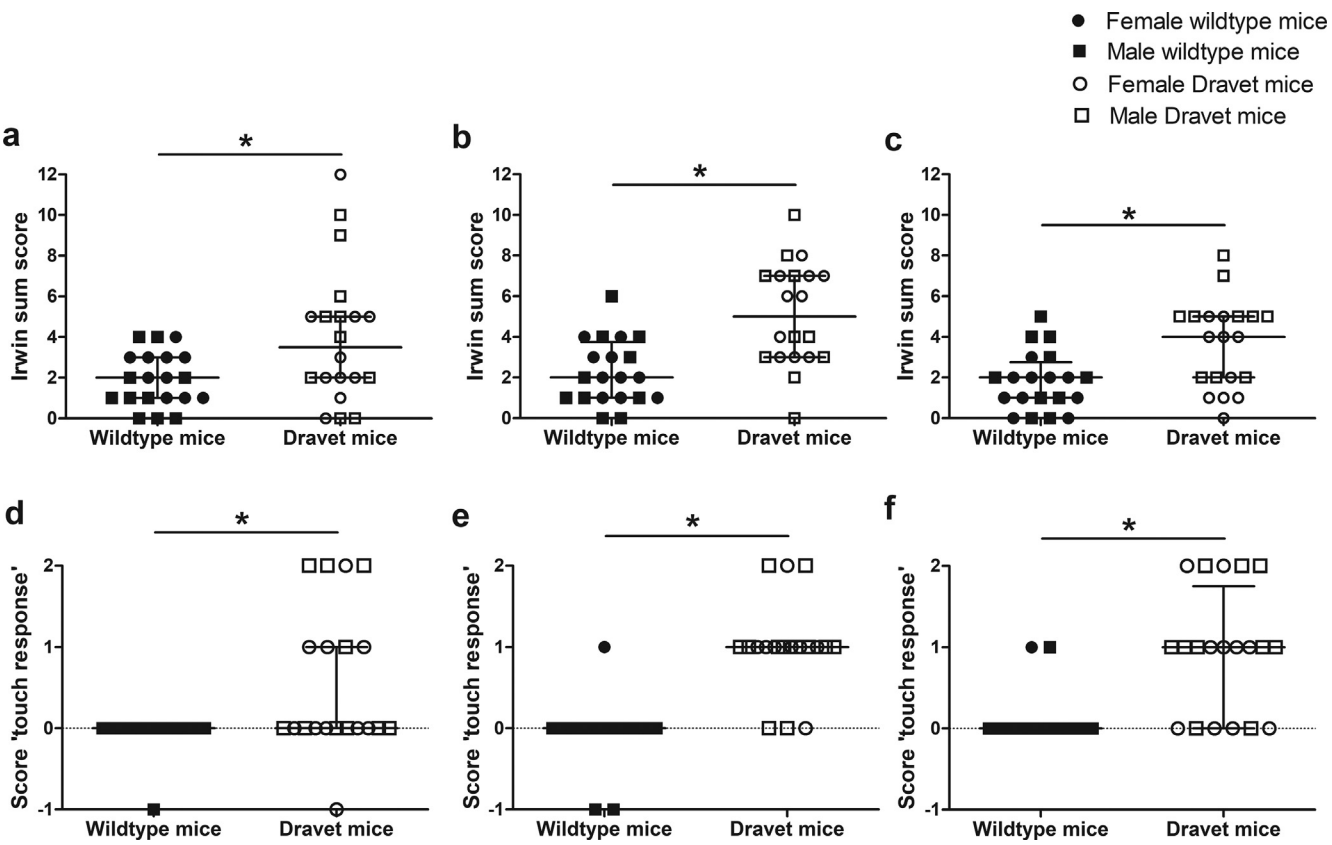

Fig. 5. Irwin Score. The analysis of Irwin sum scores revealed genotype-dependent differences during prepubescence (a), pubescence (b), and sexual maturity (c). Responsivity to light touch by the observer was significantly increased in Dravet mice during prepubescence (d), pubescence (e) and sexual maturity (f). Mann–Whitney tests. * $p < 0.05$, median (IQR).

plex daily-routine-activities, so-called 'instrumental ADL', to detect early-onset symptoms and anticipated declines in quality of life in geriatric patients and in patients with (mild) cognitive impairment [46,47].

The construction of complex nests requires a certain degree of focused concentration, combined with perseverance toward fulfilling the task, which requires a successful interplay of coordinated behaviors such as pulling, carrying, fraying, pushing, digging, sorting, and fluffing [45,48]. Nest building performance evaluated based on complexity and shape of the nest, is known to be affected by a range of biological, environmental, and social factors, including body weight, strain, housing conditions, and nesting material [49–52]. In the present study, a comparison of individual days after the offer of new nesting material revealed significantly poorer nest-building performance in Dravet mice as early as during prepuberty. We have previously observed relevant levels of nest building activity in adolescent wildtype mice [25]. Moy and colleagues [53] have already described relevant nest building activity in mice of different strains aged three to four weeks. With respect to the *Scn1a*-A1783V model, previous studies by our group in Dravet mice at seven to ten weeks of age showed an influence of the *Scn1a* genotype on nest complexity throughout the observation period [16], indicating its particular suitability as a non-intrusive assessment parameter for longer study periods.

Burrowing behavior represents another innate, non-essential, evolutionarily preserved behavior in mice [54,55]. Burrowing performance in rodents has been successfully used in preclinical pain models to validate the efficacy of inflammation-associated therapeutic approaches [56–58]. Previous assessments of burrowing behavior in young mice revealed that even adolescent animals display burrowing activity to some degree [59–61]. Based on the results of our previous study, in which we could not detect a relevant level of burrowing activity until sexual maturity [25], we applied the burrowing paradigm only to late adolescent mice. Dravet mice performed significantly worse than their wild-type littermates in both overnight testing sessions. Our data from the first overnight session indicated a sex-specific reduction of nest complexity related to the *Scn1a* genotype. The reduction or loss of activities based on high levels of intrinsic motivation indicates a corresponding reduction in the ability to experience pleasure, which, as highlighted above, is a hallmark of anhedonia-associated behavior.

The open-field paradigm was originally introduced as a tool to measure emotional behavior in rats, using the situation of exposure to a new environment as an emotional stimulus [62]. The test was then further developed as a means to assess locomotor activity and anxiety-related behavior in rodents [63,64]. In addition to test variants for recording anxiety-related behavior, such as those described and evaluated by Carola and colleagues [65], the open-field test provides data on exploratory behavior and locomotor activity. Regarding the use in juvenile mice, there are controversial data on the corresponding activity patterns in the open field [29]. Our findings during the course of adolescence in wildtype mice confirmed a progressive increase of activity patterns with age in both the familiar home cage environment and in the open-field arena [25]. In the *Scn1a*-A1783V model, our group previously observed pronounced hyperactivity, particularly hyperlocomotion, increased rearing frequency, and increased levels of thigmotaxis in mice aged seven to ten weeks [16]. Interestingly, Bahceci and colleagues [12] observed normal locomotor activity and increased thigmotactic behavior in mice aged 34–37 days in the *Scn1a*^{tm1Kea} model of DS. The analysis of locomotor activity in the *Scn1a*-

A1783V model in the present study revealed pronounced hyperactivity in Dravet mice during prepubescence and sexual maturity. It has to be considered that hyperlocomotion can mask other (coordinated) behaviors. Activity was observed with 'horizontal' hyperactivity, measured by total distance moved and mean velocity, and 'vertical' hyperactivity, assessed by manual scoring of the posture 'rearing'. Increased levels of thigmotaxis in adolescent *Scn1a*$^{+/-}$ mice could be due to deficits in goal-directed exploratory activity, as previously discussed by Bahceci and colleagues [12]. Originally, thigmotaxis, the tendency to stay close to the arena walls, was found to gradually decrease during the first minutes of exploration, and the degree of decrease was thought to represent the level or index of anxiety [66]. However, in the present study, we applied dimmed light conditions with constant low lux values. Therefore, the increased levels of thigmotaxis do not necessarily reflect anxiety-associated behavior. Increased times spent in the wall zone could be due to the pronounced hyperactivity that the young Dravet mice exhibited here when they continuously ran along the arena wall and showed a pronounced delay in exploring the inner circle. Interestingly, prepubescent and sexually mature Dravet mice exhibited significantly more 'jumps' against the wall of the arena than wildtype mice. The increased jumping frequency might on one hand reflect hyperactivity, but 'jumps' could also be interpreted as attempts to escape from the arena. However, as it turned out that the exploratory behavior is reduced, 'jumps' against the arena wall could also be a consequence of an unbalanced risk assessment.

The Irwin observation test, originally developed by Irwin to evaluate and specify drug-induced changes in animal behavior [33], has become widely used as a neurobehavioral test and is widely used in central nervous system safety pharmacology [67]. The significant increases in total sum scores in Dravet mice detected during all three stages of adolescence confirmed continuous genotype-dependent differences independent of age. However, the increased sum scores must be interpreted in light of the underlying discrepancies in individual parameters. Of note, regardless of the sex of the animals, responsiveness to light touch was increased at all three ages. Higher levels of observer-induced reactivity could mimic increased anxiety-associated traits in Dravet mice. Sex differences between genotypes were observed in irritability, particularly biting tendencies toward the observer, and urination associated with handling.

Given the clinical presentation of DS, there is limited information on gender-specific behavioral disorders in young patients. In a survey targeting parents and caregivers of patients with DS, language impairments, autism, and ADHD were reported more frequently by caregivers of male patients [68]. In another survey, caregivers observed irritability, aggression and a lack of social interaction significantly more often in male children and adolescents [69]. However, one has to keep in mind that the progression and presentation of the syndrome in patients with a de-novo *Scn1a* deficiency is shaped by a number of genetic, epigenetic, social, and pharmacological variables, which essentially complicate the prediction of phenotypical manifestations. The pathogenic variant results in early-onset seizures with their devastating structural, cellular, and molecular effects on the highly sensitive developing brain. The phenotypical impact and interaction of these factors are still unclear. This inevitably leads to a significant limitation of any approach aimed at validating animal models of DS.

Interestingly, sex-specific differences in body weights between Dravet mice and their wildtype littermates could be detected from P23 onward. Significantly reduced body weights in mice harboring the genetic *Scn1a*-A1783V deficiency have previously been described by our group [14,16]. Compared to sex-matched littermates, body weights of female Dravet mice were higher toward the end of the adolescence phase. In rats, excessive weight gain over an extended phase of kindling with repeated seizure induction has already been described [70]. Considering that the hypothalamus is known as a central control element for food intake, fluctuations in the neuroendocrine system could have caused the corresponding weight gain.

In addition to close monitoring of body weight, measurement of adrenocortical activity can be used as a sensitive tool for monitoring distress levels [71] and can also reflect metabolic needs [72]. However, the courses of corticosterone metabolite levels during postweaning development in mice have rarely been studied [71,73]. We have previously demonstrated elevated fecal corticosterone metabolite concentrations in prepubescent female and male wildtype mice compared with sex-matched adult controls [25]. With respect to genetic mouse models, Ambrée and colleagues [74] have demonstrated genotype-dependent differences in adrenocortical activity with increasing age.

In terms of refinement, litter monitoring including an analysis of the social interaction with littermates and with the mother may provide essential additional information for rodent models with early-onset symptoms [75]. In the *Scn1a*-A1783V model, the onset of seizures in mice is described at 16 days of age [16]. Our pilot data suggest that high-resolution infrared thermography could allow semi-automatic monitoring of maternal neglect as well as home cage group interaction and animal separation before and after weaning. This could provide valuable additional monitoring information related to the 3Rs principle. Regarding refinement and reduction in terms of the 3Rs principle, it is of interest that our group previously reported an overall mortality rate of 40 % in the *Scn1a*-A1783V model [16]. Through consequent implementation of intense care measures, we succeeded in further reducing the overall mortality rate to 18 % (preweaning mortality: 12 %, postweaning mortality: 6 %).

With respect to the conclusions drawn from our findings, several limitations must be considered. Direct interaction between the observer and the prey animal mouse, or even frequent handling, as applied here during the sensitive developmental phase of the animals, could substantially affect the outcome of behavioral tests resulting in an increased risk of bias. Although home cage assessments allow observer-independent measurements in a familiar environment during the natural circadian rhythm of the mice, new objects have to be introduced in the home cage environment for some of the tests. In particular, mice with impaired spatial memory and elevated anxiety-related traits are easily distracted by the presentation of multiple novel objects within a relatively short period of time. In turn, features of hyperactivity in response to novel stimuli could substantially affect other outcome parameters. In addition, it should be taken into account that the animals were repeatedly exposed to multiple behavioral tests, sometimes in parallel. While the use of one cohort substantially reduced the number of experimental and breeding animals needed in the present study, the sequential testing situation must be considered as a potential confounding factor.

In light of the complex clinical presentation and progression of the disease, it is of particular importance that the behavioral patterns of young *Scn1a*-A1783V haploinsufficient mice recapitulate the phenotypic changes during postweaning development. The information obtained supports the general validity of the *Scn1a*-A1783V model and reveals relevant age-specific phenotypic features of the syndrome. The latter is of particular relevance for the design of future studies comparing the consequences of early versus later disease-targeting approaches in the Dravet mouse model. On the other hand, the results are also of interest for laboratory animal science, evidence-based severity assessment, and refinement. The readout parameters and their temporal application along the developmental trajectory were confirmed as a basis for a

sensitive composite measurement scheme to assess cumulative severity in young genetically modified mice.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yebeh.2022.108903.

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