A companion to the preclinical common data elements for physiologic data in rodent epilepsy models. A report of the TASK3 Physiology Working Group of the ILAE/AES Joint Translational Task Force

Gorter, J.A.; van Vliet, E.A.; Dedeurwaerdere, S.; Buchanan, G.F.; Friedman, D.; Borges, K.; Grabenstatter, H.; Lukasiuk, Katarzyna; Scharfman, H.E.; Nehlig, A.

DOI
10.1002/epi4.12261

Publication date
2018

Document Version
Final published version

Published in
Epilepsia Open

Epilepsia Open® The Open Access Journal of the International League Against Epilepsy

Open Access

SUPPLEMENT ARTICLE

A companion to the preclinical common data elements for physiologic data in rodent epilepsy models. A report of the TASK3 Physiology Working Group of the ILAE/AES Joint Translational Task Force

*Jan A. Gorter, *†Erwin A. van Vliet, ‡Stefanie Dedeurwaerdere, §Gordon F. Buchanan, ¶Daniel Friedman, #Karin Borges, **Heidi Grabenstatter, ††Katarzyna Lukasiuk, ‡‡Helen E. Scharfman, and §§Astrid Nehlig

Epilepsia Open, 3(s1):69–89, 2018
doi: 10.1002/epi4.12261

Jan Gorter is Principal Investigator specialized in experimental Epilepsy Research

Summary

The International League Against Epilepsy/American Epilepsy Society (ILAE/AES) Joint Translational Task Force created the TASK3 working groups to create *common data elements (CDEs)* for various aspects of preclinical epilepsy research studies, which could help improve standardization of experimental designs. This article concerns the parameters that can be measured to assess the physiologic condition of the animals that are used to study rodent models of epilepsy. Here we discuss CDEs for physiologic parameters measured in adult rats and mice such as general health status, temperature, cardiac and respiratory function, and blood constituents. We provide detailed CDE tables and case report forms (CRFs), and with this companion manuscript we discuss the monitoring of different aspects of physiology of the animals. The CDEs, CRFs, and companion paper are available to all researchers, and their use will benefit the harmonization and comparability of translational preclinical epilepsy research. The ultimate hope is to facilitate the development of biomarkers and new treatments for epilepsy.

KEY WORDS: **Preclinical, Common data elements, Rodent, Epilepsy, Model, Physiology.**

Accepted July 27, 2018.

*Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, Amsterdam, The Netherlands; †Amsterdam UMC, University of Amsterdam, Department of (Neuro)pathology, Amsterdam Neuroscience, Amsterdam, The Netherlands; ‡New Medicines R and D, UCB Pharma, Brussels, Belgium; §Department of Neurology, University of Iowa Carver College of Medicine, Iowa City, IA, U.S.A.; ¶Department of Neurology, NYU Langone Medical Center, New York, NY, U.S.A.; #School of Biomedical Sciences, The University of Queensland, Brisbane, Queensland, Australia; **Department of Psychology and Neuroscience, Center of Neuroscience, University of Colorado, Boulder, U.S.A.; ††Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; ‡‡The Nathan Kline Institute for Psychiatric Research and New York University Langone Medical Center, Orangeburg, NY, U.S.A.; and §§INSERM U 1129, Pediatric Neurology, Necker-Enfants Malades Hospital, University of Paris Descartes, Paris, France

Address correspondence to Astrid Nehlig, University of Paris Descartes, Inserm, Paris, France. E-mail: nehliga@unistra.fr

The purpose of this article is to provide common data elements (CDEs) for rodent (mouse and rat) epilepsy models in the area of physiology, to facilitate an understanding of the critical importance of assessing various physiologic parameters in preclinical epilepsy research. While working on these CDEs we came to realize that the measurement of physiologic data in epilepsy research in rodents is very rare (except for electroencephalography [EEG] monitoring). Although blood pressure, heart rate, and temperature measurements as indicators of general health are standard procedures in people with epilepsy, the measurement of various basic physiologic parameters that are good indicators of the general health status of the animals is rarely used in preclinical epilepsy research. We realize that implementation of these types of measurements comes with additional costs; however, we hope that the proposed experimental recommendations and forms may inspire

Key Points

- This joint ILAE/AES initiative introduces common data elements (CDEs) related to measurement of various physiologic parameters in adult rodents
- Case report forms (CRF) and a companion report discussing their use are provided for assessment of general health, temperature, heart function, blood pressure, respiration, and blood sampling and testing
- Future use of these forms may help to harmonize animal experiments and to improve and facilitate meta-analysis studies

researchers to include these types of physiologic measurements in their future experimental design when possible and to use these forms accordingly.

Here we present a companion paper to standardized data acquisition forms related to the measurements of general health and several common physiologic parameters. These are aimed at enabling consistent data collection across different experiments and different laboratories.

We aimed to collect numerous possibilities to measure various physiological aspects reflecting the health status of the animals. The provided protocols are example experimental methods for obtaining physiologic parameters in rats, mice, or both. This list is meant to help researchers to decide what type of measurement would be useful according to the type of epilepsy or seizure studied and to encourage the use of case report forms (CRFs) presented here in order to standardize future experiments that involve physiologic measurements. While assessing the general health status of the animals will always be informative and important, monitoring of respiratory or cardiovascular parameters is not specifically needed in all models studied but is strongly recommended in models of sudden unexpected death in epilepsy (SUDEP). Likewise, although body core temperature is easy to monitor and will provide useful information, brain temperature is considered similar to body core temperature and most often is not specifically needed and not measured. In addition, it must be noted that many of the techniques reported in this article are invasive and it is critical to weigh the benefit linked to any physiologic measurement versus the additional stress imposed on the animal by anesthesia and surgery.

Methods

The Physiology Working Group consisted of 8 experienced preclinical epilepsy researchers who developed CDEs for 6 physiology modules (see following paragraphs).

The CDEs are organized around the following modules: (1) General health status, (2) Temperature, (3) Respiration, (4) Heart rate, (5) Blood pressure, and (6) Blood sampling and testing.

The forms are constructed in analogy to previous preclinical CDEs by the National Institute of Neurological Disorders and Stroke (NINDS) for traumatic brain injury research.[1] CDEs generated by the EPITARGET consortium (Targets and Biomarkers for Antiepileptogenesis) served as useful templates for our TASK3 Physiology working group.[2] The proposed recommendations originate mostly from previously published methods used for rodent physiology research.

The CDEs presented here apply to adult rodents, rats or mice, and are not readily applicable to immature animals, which will need specific CDEs taking into account their size, ongoing acquisition of specific functions,[3] as well as some metabolic specificities such as the dependence on ketone bodies for their brain energy metabolism.[4]

Results

For each physiology module (A-F) we provide a rationale and an overview of the elements that are included in the corresponding CRFs. The CDE and CRF modules linked to this article can be downloaded and can be found as supplementary tables in a zip folder (Appendix S1).

Assessment of the general health status

[File names: 1 CRF Module - general health status.docx; 1 General Health Status CDE Chart.xlsx]

Rationale

General health status is assessed to monitor an animal's well-being during the experiment, including the effects of all procedures. Evaluation of the health status is an essential part of the physiology assessment. It is easy to do and provides readily available information about the general well-being of the animal to a trained observer. It will help understanding of the immediate consequences of the procedures used on the animal's basal physiology parameters and will allow researchers to rapidly decide whether the degree of suffering of the animal is acceptable. General health status is assessed by observational criteria based on the evaluation of animal behavior and appearance in the housing environment and during routine handling. The general appearance of the animals is a reliable indicator of general health and well-being. Physical examination easily provides information on body condition and the degree of hydration. With the exception of testing for pathogens, it is noninvasive. Because evaluation is based on subjective criteria this should be performed by highly trained personnel. The explanations below are provided to facilitate data acquisition using the CRF shown in Fig. 1.

Measurement of physiologic parameters for assessing general health status

All basal measurements of general health status can be performed in awake, freely moving animals.

Physiological Studies

Case Report Form: 1 CRF Module -general health status.docx

CRF module: Assessment of the General Health Status of Adult Rodents

Date at which this CRF was filled out:

Name of person filling out CRF:

Project name/Identifier:

Animal ID:

_____

CDE name	**Data collected**
Day of assessment from the procedure/trauma	_____
Age: Body Weight	____ (weeks) ___ (g)
% loss of body weight [1]	_____ (%)
Body condition score (BCS)	☐ 1; ☐ 2; ☐ 3; ☐ 4; ☐ 5
General appearance	☐Lethargy; ☐Aggressiveness; ☐Hunched posture; ☐Flat or little elevated posture; ☐Ataxia or mobility problems; ☐Self-mutilation; ☐Vocalization (crying, whimpering)[3]; ☐ No abnormalities observed
Presence of surgical scars	☐ Yes; ☐ No ; ☐ Unknown
External bleeding	☐ Mouth; ☐ Anus; ☐ At surgical site; ☐ Other injuries; site: _____ ☐ No bleeding observed
Hair; Coat and skin	☐ Hair loss; ☐ Ruffled or dirty coat; ☐ Scratching; ☐ Barbering; ☐ Rash; ☐ No abnormalities observed
Bowel and gastrointestinal function	☐ Fecal staining on the tail; ☐ Constipation; ☐ Diarrhea; ☐ Vomiting ☐ No abnormalities observed
Bowel and gastrointestinal function - number of fecal pellets	_____
Genitals	☐Testicular abnormalities; ☐ No abnormalities observed
Throat and lung	☐ Cough; ☐ Sneezing; ☐Discharge; ☐ Dyspnea[2]; ☐ No abnormalities observed

Figure 1.
Assessment of the general health status of adult rodent CRF module
Epilepsia Open © ILAE

Eyes	☐ Porphyrin staining; ☐ No abnormalities observed
Ears	☐ Circling or head tilt; ☐ No circling or head tilt;
Teeth	☐ Overgrowth; ☐ Tooth breakage; ☐ Malocclusion; ☐ No abnormalities observed
Gait	☐ Walking normally; ☐ Unsteady gait; ☐ Severely reduced mobility; ☐ Loss of balance, immobility [2]
Seizures	☐ No spontaneous seizures; ☐ Occasional focal seizures; ☐ Frequent focal seizures; ☐ Occasional generalised seizures; ☐ Frequent generalized seizures; ☐ Status epilepticus
Testing for common pathogens	☐ Sendai virus; ☐ MRM (murine respiratory mycoplasmosis); ☐ SDAV (sialodacryoadentitis virus); ☐ Pinworms; ☐Other
If testing for 'other' pathogen, specify pathogen	_____

[1] When there is weight loss, follow the institutional recommendations such as euthanasia following weight loss of a specific amount.

[2] Consider euthanasia if lasts more than 24 h, if consistent with institutional recommendations for euthanasia criteria.

[3] Consider euthanasia if lasts more than 48 h, if consistent with institutional recommendations for euthanasia criteria.

Instructions: Please check boxes where applicable. If none of the predetermined options is appropriate use the default space to specify your answer. This form is to be filled in for one individual animal.

Figure 1.
Continued.
Epilepsia Open © ILAE

Body weight should be evaluated regularly with a frequency relevant to experimental design. Body weight can be evaluated in 2 ways: (1) Percent loss of body weight is measured as a percentage decline from initial (before procedure) weight, and (2) body weight is compared with that of untreated animals.[5] To avoid critical weight loss and dehydration, the animals are encouraged to eat by the use of moistened food pellets, gels and treats, and liquid baby food,

which can be placed on the cage floor or even given individually. In addition, subcutaneous fluids (saline or sucrose solutions) are sometimes injected after status epilepticus (SE) when animals are too weak to eat and drink.[6]

Body condition score (BCS) is an effective, noninvasive method of health assessment in rodents. It is performed by observing and palpating the flesh over the bony protuberances of the hips and lumbar spine. It is rated on the following scale: 1, emaciated; 2, under-conditioned; 3, well-conditioned; 4, over-conditioned; 5, obese.[5,7]

General appearance is evaluated by observation of animal behavior in the home cage or during handling by noting the presence or absence of the following features[5]:

- Lethargy, torpor, apathy, sluggishness
- Aggressiveness—toward other animals or experimenter, for example, biting
- Hunched posture: the animal is stooped low with the limbs pulled in close to the body and arched back, this posture is often indicative of pain
- Ataxia or mobility problems
- Mutilation—visible as open scratches or bites
- Vocalization (crying, whimpering), for example, during handling

Inspection of surgical scars is evaluated.

External bleeding is evaluated by the presence or absence of blood stains on the tongue, mouth, anus, or wounds.

Hair, coat, and skin are evaluated by the presence or absence of hair loss, ruffled or dirty coat, open scratches, or rash.[8]

Bowel and gastrointestinal function are evaluated by the presence or absence or number of fecal pellets, fecal staining on the tail, constipation, diarrhea, or recto-anal prolapse, which is an intussusception of the rectum.[9]

Genitals are evaluated by the presence of testicular or other abnormalities, such as penile prolapse.[9]

Throat and lung function is evaluated by the presence or absence of discharge and dyspnea.

Eyes are evaluated by the presence or absence of reddish porphyrin staining around the eye.

Ear dysfunction is diagnosed on the basis of circling behavior and head tilt.

Teeth problems are evaluated on the basis of teeth overgrowth, breakage, or malocclusion.

Gait is characterized by the following scoring starting from normal and ending with severe dysfunction[8,10]:

- Walking normally
- Unsteady gait
- Severely reduced mobility
- Loss of balance
- Immobility

Seizures are ideally recorded by video-EEG. If not available or not possible, seizures are recorded and counted when they occur during everyday observation phases and handling.

Testing for common pathogens is performed according to the guidelines of the local animal facility.

Additional, more detailed information is provided in Refs. 5–16.

Temperature

[File names: 2 CRF Module–temperature.docx; 2 Temperature CDE Chart.xlsx]

The following information is provided to facilitate the use of the CRF in Fig. 2 for data acquisition. The CRF may be modified according to the choices made for obtaining data, since there are options, as discussed below.

Rationale

Body temperature is a basal indicator of the animal's health. If the experimenter decides to include this physiologic parameter in the experimental design, it is recommended that the body temperature is monitored in every single animal before the onset of a procedure. This allows confirmation of the health status of the animals and helps to avoid the use of animals whose body temperature is not in the physiologic range. As an example, the induction of SE by pilocarpine leads in animals to high fever, and monitoring seizure-induced changes could help our understanding of why animals do or do not survive the SE phase, and whether mortality might be, at least in part, related to temperature elevation.[17]

Febrile seizures are common in young children aged 6 months to 6 years.[18] Most febrile seizures are benign, but complex, prolonged febrile seizures lasting over 10–20 minutes are associated with a risk of developing subsequent epilepsy.[19] Therefore, animal models for studying characteristics and consequences of febrile seizures have been developed and studied.

Body temperature can be measured in awake animals to follow changes in temperature over time, or during anesthesia (e.g., during surgery or imaging) to keep body temperature within a physiologic range.[20] Because low body temperature or head cooling can influence the extent of brain damage, it is important to report body temperature if an intervention is likely to induce changes. Moreover, failure to measure body temperature can confound the interpretation of experiments, as it may not be known whether, for example, extreme change in body temperature contributed to an outcome, or whether an intervention (e.g., an anti-inflammatory agent) had an indirect impact by changing body temperature, rather by another mechanism of action. In experimental epilepsy models, body temperature increases during SE.[17] Furthermore, the circadian rhythm of temperature is altered in epileptic rats, which is associated with regional hypothalamic neuronal loss.[21] Rats that were cooled during SE had a significantly lower body temperature compared to cooled control rats or non-SE rats,[22] indicating a disturbed temperature

Physiological Studies

Case Report Form: 2 CRF Module – temperature.docx

CRF module: Temperature

Date at which this CRF was filled out:

Name of person filling out CRF:

Project name/Identifier:

Animal ID:

_____

CDE Name	Data Collected
Anesthesia	
Anesthesia	☐ Yes; ☐ No ; ☐ Unknown
If anesthesia was administered, specify type	☐ Isoflurane; ☐ Sevoflurane; ☐ Ketamine/Xylazine; ☐ other
Isoflurane concentration induction (%)	_____ (%)
Isoflurane concentration maintenance (%)	_____ (%)
Sevoflurane concentration induction (%)	_____ (%)
Sevoflurane concentration maintenance (%)	_____ (%)
Ketamine dose (mg/Kg)	_____ (mg/kg)
Xylazine dose (mg/Kg)	_____ (mg/kg)
Methods used	
Feedback-regulated heating pad	☐ Yes; ☐ No ; ☐ Unknown
Rectal probe	☐ Yes; ☐ No ; ☐ Unknown
Infrared probe	☐ Yes; ☐ No ; ☐ Unknown
Implanted telemetric core body temperature sensor	☐ Yes; ☐ No ; ☐ Unknown
Implanted telemetric brain temperature sensor	☐ Yes; ☐ No ; ☐ Unknown
If implanted telemetric brain temperature sensor, implantation coordinates (coordinates: mm AP, ML, DV)	_____

Parameters

Time Point						
Ambient Temperature (°C)						
Humidity (%)						
Core body temperature (°C)						
Brain temperature (°C)						

Instructions: Please check boxes where applicable. If none of the predetermined options is appropriate use the default space to specify your answer. This form is to be filled in for one individual animal.

Figure 2.
Temperature CRF module
Epilepsia Open © ILAE

homeostasis during SE. Hyperthermia during an insult or injury increases the risk of developing epilepsy, as has been demonstrated with immature rodents when using the febrile seizure model.[23,24] Inducing hypothermia during SE in experimental epilepsy models had anticonvulsant and neuroprotective effects (for review see Motamedi et al.[25]). Regarding the influence of (brain) temperature on seizure outcome, it may be interesting to evaluate the potential effects of drugs on (brain) temperature.

Measurement of body temperature

All the procedures described below can be used in awake animals, but some of them necessitate a prior surgical intervention to insert the probe or the sensor that will later record the body or brain temperature.

Equipment: Rectal probe, infrared probe, or implanted radiotelemetric core body or brain temperature sensor.

Procedure: Prepare anesthesia (e.g., isoflurane anesthesia) or perform measurements in awake animals.

Temperature can be measured using one of the following more or less invasive methods:

Noninvasive methods

- *Feedback-regulated heating pad*. Indicate whether used or not.
- *Rectal probe*. Apply a bit of lubricant on the probe before inserting.[26]
- *Infrared probe* (e.g., Braintree Scientific, Braintree, MA, USA). Measure the cutaneous temperature on paw or tail.[23]

Invasive methods

- *Implanted radiotelemetric body core temperature sensor* (e.g., Data Sciences International, St Paul, MN, USA). Implant sensor under anesthesia via a small abdominal incision. Allow animals to recover for at least 4 days before further experimentation to allow circadian rhythms to normalize.[27,28]
- *Implanted radiotelemetric brain temperature sensor* (e.g., mini-mitter, Starr Life Sciences, Oakmont, PA, USA). Implant sensor under anesthesia in the brain using preselected coordinates using the Paxinos atlas. For more details on the procedure see Appendix S2. Allow animals to recover for at least 4 days before further experimentation to allow circadian rhythms to normalize (see Meyer et al.[26] and DeBow and Colbourne[28]).

Analysis and interpretation

Parameters that can be determined are core body temperature (°C) and brain temperature (°C). Normal body temperature for rats is between 37 and 38°C and for mice is between 36.5 and 38°C. Brain temperature is usually considered a "central" temperature, and in the absence of intracranial pathology, changes in brain temperature can be estimated by measuring changes in body core temperature. However, in cases of severe cerebral injury, the estimates yielded by such measurements may be inaccurate. A positive brain–body temperature gradient (brain temperature > body temperature) was observed in freely moving rats[29] and mice.[30] In anesthetized states, possibly due to the suppression of metabolic heat production by the anesthetic agent as well as effective heat exchange with the environment through the head, a negative brain–body temperature gradient (brain temperature < body temperature) has been observed in rats. In awake freely moving rats, temperature in hippocampus and piriform cortex can decrease 0.5°C over a 1 h period of sleep and quiet wakefulness, and then increase 1.5°C when the rat is actively exploring.[31]

Respiration

[File name: 3 CRF module – respiration.docx; 3 Respiration CDE chart.xlsx]

Rationale

Respiratory parameters (for data acquisition, see Fig. 3) are generally measured under anesthesia (e.g., during surgery or imaging), in spontaneously breathing animals, or ventilated animals, to monitor the physiologic range of these parameters.[20,32] Although respiratory parameters are not often measured in awake animals, it may also be important to assess respiratory parameters in experimental epilepsy models, since hypoxia may occur as a result of SE.[33] Hypoxia increases the risk of developing epilepsy, as has been demonstrated in the neonatal hypoxia model.[34,35] Furthermore, under hypoxic conditions, SE-induced neuronal damage is more severe.[36,37] In contrast, hyperoxia did not lead to additional neuronal death.[38]

Assessment of respiratory function

Equipment: Mouth mask, endotracheal tube, pulse oximeter, respiration sensor, intravenous/intra-arterial cannulas, plethysmograph, pressure sensitive catheter.

Procedure: Prepare anesthesia (for example, ketamine rat 40–100 mg/kg; mouse 80–120 mg/kg and xylazine rat 5–13 mg/kg; mouse 10–16 mg/kg) or perform measurements in awake animals.

Depending on the type of experiment (anesthetized vs awake animals) respiratory parameters can be measured using the following methods:

Noninvasive methods:

- *Mouth mask*. Expired CO_2 can be measured using a calibrated device that is connected to the tube.
- *Pulse oximeter.* Apply securely on the animal's hindpaw.[39]
- *Respiration sensor* (e.g., BioVet, m2 m Imaging Corp., Cleveland, OH, USA). Fix the respiration sensor under the chest of the animal to measure the respiration movement of the chest.[40] Implanted movement sensors can also be used for measuring breathing patterns such as the movement sensor 230 (Siemens) using a piezo crystal sensor.[41]
- *Unrestrained whole body plethysmography (UWBP)* can be used in epilepsy research to perform traditional measurements on pulmonary function: breath frequency, tidal volume, minute ventilation, inspiratory time, expiratory time, and so on. UWBP is an adequate technique for assessing those parameters, especially when also accounting for the animal's weight, body temperature, ambient temperature, relative humidity, atmospheric pressure, flow of gas/air into the recording chamber, flow of gas/air out of the chamber, and the activity/behavioral state (resting, moving, grooming, sniffing, eating, drinking, etc., which aid data interpretation) of the animal.[42] Despite some limitations, as discussed by Bates et al.,[43] UWBP is considered valid if performed correctly and modeled after the Drorbaugh-and-Fenn formula.[44] In this commonly used method, animals are not restrained, but movement is restricted by the use of a relatively small chamber to keep the volume small with respect to the animal's size. This allows for measurements in freely behaving animals attached to tethers for EEG, electromyography (EMG), and electrocardiography (ECG) measurements.[45,46]

Physiological Studies

Case Report Form: 3 CRF Module – respiration.docx

CRF module: Respiration

Date at which this CRF was filled out:

Name of person filling out CRF:

Project name/Identifier:

Animal ID:

CDE Name	Data Collected
Anesthesia	
Anesthesia	☐ Yes; ☐ No ; ☐ Unknown
If anesthesia was administered, specify type	☐ Isoflurane; ☐ Sevoflurane; ☐ Ketamine/Xylazine; ☐ Other ☐ No anesthesia used (awake animal)
Isoflurane concentration induction (%)	_____ (%)
Isoflurane concentration maintenance (%)	_____ (%)
Sevoflurane concentration induction (%)	_____ (%)
Sevoflurane concentration maintenance (%)	_____ (%)
Ketamine dose (mg/Kg)	_____ (mg/kg)
Xylazine dose (mg/Kg)	_____ (mg/kg)
Methods used	
Mechanically ventilated	☐ Yes; ☐ No ; ☐ Unknown
Non-ventilated	☐ Yes; ☐ No ; ☐ Unknown
Mouth mask	☐ Yes; ☐ No ; ☐ Unknown
Endotracheal tube	☐ Yes; ☐ No ; ☐ Unknown
Pulse oximeter	☐ Yes; ☐ No ; ☐ Unknown
Respiration sensor (e.g. BioVet, m2m Imaging Corp)	☐ Yes; ☐ No ; ☐ Unknown
Unrestrained plethysmography (Penh)	☐ Yes; ☐ No ; ☐ Unknown
Head-out restrained plethysmography	☐ Yes; ☐ No ; ☐ Unknown
Pressure-sensitive catheter	☐ Yes; ☐ No ; ☐ Unknown
Tracheostomy	☐ Yes; ☐ No ; ☐ Unknown
Forced oscillation technique	☐ Yes; ☐ No ; ☐ Unknown
Thermistor probe	☐ Yes; ☐ No ; ☐ Unknown
Respiratory parameters	
Respiration rate (br/min)	_____ (br/min)
Tidal volume (ml)	_____ (ml)
Respiratory minute volume (ml)	_____
Tidal mid expiratory flow (ml/s)	_____ (ml/s)
Time of inspiration (ms)	_____ (ms)
Time of expiration (ms)	_____ (ms)
Expired O_2 (mm Hg)	_____
Expired CO_2 (mm Hg)	_____
O_2 saturation (%)	_____
Blood gasses (pH, pO_2, pCO_2), see CRF module blood sampling	_____

Instructions: Please check boxes where applicable. If none of the predetermined options is appropriate use the default space to specify your answer. This form is to be filled in for one individual animal.

Figure 3.
Respiration CRF module
Epilepsia Open © ILAE

Because the data depend on measuring pressure waves, movements associated with seizures during the ictal phase might impair the breathing measurements. The method is suitable for measuring breathing during the pre- and postictal phases and allows for successful assessment of the effects of a variety of variables such as sleep state, time of day, sex, and genetic, optogenetic, and pharmacologic manipulations in a number of seizure/epilepsy models.[45–47] For more details on the procedure see Appendix S2 and Ref. 32.

- *Head-out restrained plethysmography*. Prior to the measurements, the animals are trained for 5 days during increasing time periods (from 2-3 min up to about 30 min) to get accustomed to the plethysmograph. For lung function measurements, the animals are placed in body plethysmographs while the head of each animal protrudes through a neck collar of a dental latex dam into a head exposure chamber. This can be adapted for use with seizure induction in head-fixed preparations (see Zhan et al.,[48] for example). For more details see Appendix S2 and Refs. 32, 45–48.
- *Forced oscillation technique (FOT).* Measurements are typically obtained by analyzing pressure and volume signals acquired in reaction to a predefined, small amplitude, oscillatory airflow waveform (also referred to as perturbation or input signal) applied at the subject's airway opening. In its simplest form, an FOT perturbation would be a single sinusoidal waveform at a well-defined frequency. More complex perturbations typically consist of a superposition of a selection of specific (mutually prime) frequency waveforms covering a broad spectrum. The decomposition of the multi-frequency input and output signals into their constituents using the Fourier transform allows the calculation of respiratory system input impedance (abbreviated Zrs), that is, the transfer function between the input and output signals, at every frequency included in the perturbation. Therefore, FOT permits the simultaneous assessment of respiratory mechanics over a range of frequencies in a single maneuver. Fitting advanced mathematical models (e.g., the Constant Phase Model) to the impedance data then permits a partitioning of the response into the airway (central and peripheral) and parenchymal lung tissue dependent parameters. Because many factors influencing the physiologic response (e.g., breathing frequency, tidal volume, lung volume, upper airways, spontaneous breathing efforts, and timing of measurements) are controlled and standardized by the measurement system and experimental procedures, the technique can generate precise and reproducible measurements provided that it is performed correctly.[49]

Invasive methods:

- *Chronic implantation of a thermistor probe* into the hollow space located above the anterior portion of the nasal cavity. This probe does not penetrate any soft epithelial tissues and allows recording of the respiratory rhythm in awake mice with high precision. It does not damage or irritate the nasal epithelium and is compatible with studies in freely moving animals, as in seizure or epilepsy models.[50]
- *Endotracheal tube*. When the animal is anesthetized, spray lidocaine on the endotracheal tube and intubate the animal using transillumination. For more details see Appendix S2 and Ref. 51.
- *Tracheotomy.* A small incision (1.5–2 cm) is made in the neck of the rat for tracheotomy. For more details see Appendix S2 and Ref. 52.
- *Pressure-sensitive catheter*. The pressure-sensitive catheter is surgically implanted and resides below the serosal layer of the esophagus to enable direct measurement of sub-pleural pressure. Measurements are performed by telemetry.[27]
- *Intravenous or intra-arterial cannulas*. See CRF module and forms for blood testing.

Analysis and interpretation

Respiratory parameters that can be determined:

- Respiratory rate
- Tidal volume
- Respiratory minute volume
- Tidal mid-expiratory flow
- Time of inspiration and expiration
- Expired O_2
- Expired CO_2
- O_2 saturation
- Blood gasses (pH, pO_2, pCO_2); see CRF module and forms for blood sampling

Rats: Respiration frequency ranges between 60 and 150/min in unanesthetized rats and 47-115/min in anesthetized rats. Minute volume ranges between 0.057 and 0.336 L/min in unanesthetized rats and 0.046–0.388 L/min in anesthetized rats.[53]

Mice: Respiration frequency ranges between 100 and 346/min in unanesthetized mice and 109–210/min in anesthetized mice. Minute volume ranges between 0.024 and 0.054 L/min in unanesthetized mice and 0.021–0.051L/min in anesthetized mice.[53]

Heart rate and electrocardiography (ECG)

[File names: 4 CRF Module - heart rate.docx; 4 Heart rate CDE chart.xlsx]

Rationale

There has been a renewed interest in investigating the impact of seizures and epilepsy on cardiovascular and autonomic function in preclinical models in order to try to better understand the pathophysiologic mechanisms of SUDEP.[54,55] Studies in transgenic mouse models have identified genetic defects that lead to seizures, cardiac arrhythmias, and sudden death.[56–58] Genetic defects include those

encoding for ion channels expressed in both heart and brain as well as neuronal-expressed proteins that impact vagus nerve function.[59] High rates of SUDEP are found in Dravet syndrome, a severe infantile epilepsy syndrome due to a mutation in the *SCN1A* sodium channel gene.[60] A recent study, combining simultaneous EEG and ECG monitoring in a mouse model of Dravet syndrome, demonstrated death due to seizure-related vagally mediated asystole.[61] Studies have also demonstrated acquired cardiac conduction defects in epileptic rodents, but the relationship to SUDEP is not clear.[62]

Assessment of cardiac parameters and function

Specific Methods:

- Prepare anesthesia (for example, isoflurane anesthesia) or perform measurements in awake animals.

Depending on the type of experiment (anesthetized vs awake animals, short term vs long term recording) heart rate can be measured using the following methods (see Ho et al.[63] for review):

Techniques usable in awake animals:

- *External heart rate sensor*. Place heart rate sensor under the chest of the anesthetized animal and position the sensor until a proper heart rate signal is obtained.[64]
- *Noninvasive platform devices* (ECGenie, mouse specifics, Framingham, MA, USA). The animal is placed on a platform with 3 paw-sized gelled electrodes (e.g., M1605A Snap, Hewlett-Packard, Andover, MA, USA) and the paws are gently positioned over the pads. The animal can be anesthetized, restrained, or acclimated to the platform. The electrodes are connected to a bioamplifier, A/D acquisition system, and analyzed as described in the following text.[65]
- *Electrocardiography (or ECG) electrodes* (e.g., BioVet, m2 m Imaging Corp., Cleveland, OH, USA). Shave and clean the skin surfaces for electrode location if needed. Subdermal needle electrodes, electrode pads, or surface electrodes (with conductance gel) can be used. Position the ECG electrodes on the skin, for example, left and right from the heart. Position the ground electrode on the hind leg. The leads are then connected to a bioamplifier or heart rate sensor.[20]
- *Implanted ECG electrodes*. Rats should be anesthetized and stainless-steel spring electrodes covered with polyurethane tubing, except for the final 5–8 mm, are implanted just caudal to the diaphragm and in the mediastinum. For additional details see Appendix S2 and Ref. 51.
- *Implanted ECG telemetry devices.* Leads are implanted similarly to above, with a telemetry device (e.g., Data Sciences International, Respironics, Mini-mitter, etc.) implanted into the peritoneum as per the manufacturer's protocol. The signals are sampled via a telemetry receiver situated under the animal's cage. Five to 7 days of recovery is recommended before recording. These systems are most suitable for chronic, long-term recordings in freely behaving animals.[66]

Qualitative, morphologic assessment of ECG rhythm:

- *Amplification, sampling, and analysis of ECG signals*. For internal or external wired ECG recordings, the ECG signals are amplified and filtered using a bioamplifier (e.g., 0.5–100 Hz bandpass, Model 12C 16OS, Grass Technologies, Quincy, MA, USA). Signals are sampled at 1000 Hz (e.g. Power Lab/8SP; AD Instruments, Melbourne, Australia). Numerous software packages exist to measure heart rate automatically in rodents (e.g., EzCG ECG analysis, mouse specifics, inc; AcqKnowledge, Biopac, Goleta, CA, USA; Chart 5, AD Instruments). It is important to note that heart rate should be measured from the interval between R peaks on the ECG. Poor signal-to-noise can complicate ECG monitoring, especially in freely behaving, epileptic rats. Data selected for heart rate variability (HRV) analysis must be artifact free and importantly, arrhythmia free. Seizure-induced arrhythmia may prevent accurate detection of the R peaks and heart rate measures. Sleep-wake states influence HRV, as do stress and seizures; thus it is critical to note the behavioral conditions during which these endpoints are measured. In addition, the signal must be assessed qualitatively for rhythm disturbances in addition to the qualitative measures of heart rate.[67,68]
- *Evaluate the QRS complex.* If the QRS complex appears of normal height and duration, then the initiating impulse originates above the A-V node. When the QRS complex appears wide and bizarre, the impulse initiating that complex originates at an ectopic pacemaker site within the ventricles.[69]
- *Evaluate the relationship between the P waves and QRS.* On a normal ECG, there should be a P wave for every QRS, with a consistent P-R interval. Prolonged P-R intervals indicate a conduction delay through the A-V node. Short P-R intervals, where the P wave is positioned very close to the QRS complex, indicate that the impulse was generated around the A-V node.[70]
- *Evaluate the T wave.* With complicated arrhythmias, it may be difficult to discern a P wave from a T wave. A T wave will always follow every QRS complex, but the same is not true for P waves, which may be buried in the complex or missing from the complex altogether.[69,71]

Parameters that can be determined:

- Frequency (heart rate): often expressed as beats/minute (bpm)
- Mean R-R interval
- Heart rate variability (in time domain):
  - SDNN: Standard deviation of R-R intervals for given epoch of time
  - RMSSD: root mean squared of successive differences
- Heart rate variability (in frequency domain):
  - Low frequency (LF, 0.3–0.75 Hz): represents sympathetic activity

  - High frequency (HF, 0.75–3 Hz): represents parasympathetic activity
  - LF/HF ratio
- Cardiac intervals such as P-R interval, QT corrected (specify correction method)
- *Measuring heart rate variability (HRV).* The ECG should be recorded (recommended) for 30–40 min to analyze HRV (a common measure of intact cardiac function). HRV is analyzed in time (standard deviation of an epoch of R-R intervals [SDNN] and root mean squared of successive differences [RMSSD]) as well as frequency (total power, low frequency power [LF], high frequency power [HF], normalized LF power [LF nu], normalized HF power [HF nu], and LF/HF ratio) using Kubios 2.0 HRV software (e.g. Kuopio University, Finland). Spectral analysis is performed using the fast Fourier transform algorithm, on 512 RR frames with 50% overlap. Suggested values of the frequency domain in rats should be 0.2–0.75 Hz for LF and 0.75–3 Hz for HF, and 0.1–1.5 Hz for LF and 1.5–5 Hz in mice. In one series of 5 unrestrained, awake Wistar rats, the mean RMSSD was 5.18 ± 1.16 msec.[72] In a study of HRV in C57BL/6J mice, the mean RMSSD was 6.1 ± 1.5 msec.[73] RMSSD is commonly reported as a natural log of the measured values (i.e., lnRMSDD).
- *Q-T interval correction.* Calculating the rate-corrected QT interval (QTc) is typically performed using Bazett's formula, $QTc = QT/RR^{1/2}$, for rodents.

Expected results for heart rate

Basal heart rate varies by species, strain, and time of day. For example, in mice, mean heart rates between 500 and 650 bpm have been reported for C57/B6, FVB, and 129Sv/J strains, respectively.[73] Resting HR in rats is 330–480 bpm.[73] In Sprague-Dawley rats, HR is higher in female animals and is influenced by stress and group/single housing.[74] Seizures, both prolonged or repeated, have been reported to prolong cardiac QT intervals and increase the susceptibility to arrhythmias.[75]

Blood pressure

[File name: 5 CRF Module - blood pressure.docx; 5 Blood pressure CDE chart.xlsx]

Rationale

There has been a renewed interest in investigating the impact of seizures and epilepsy on cardiovascular and autonomic function in preclinical models in order to understand the pathophysiologic mechanisms of SUDEP. An often overlooked aspect that is gaining considerable attraction recently is blood pressure. Blood pressure, and other autonomic function, is affected by seizures, and these effects may be associated with increased SUDEP risk.[70,76] Blood pressure is readily measurable in patients, can relatively easily be measured in rodent models, and may provide important clues for assessing the risk of SUDEP.

Measurement of blood pressure

Specific methods:

- *Tail cuff measurement in restrained animals:* Rats or mice are placed in a mechanical restraining device with the tail exposed and accessible. The tail cuff is placed around the tail and attached to a commercial tail cuff blood pressure system (e.g., Hatteras, Inc; Visitech Systems). Typically a number (commonly 10) of blood pressure readings are taken over the sampling period and the average is recorded.[77] Tail cuff measurement has the advantage of allowing sampling in noninstrumented animals. The disadvantage is that the animals need to be restrained for accurate measurements. Therefore, this is more difficult to use in animals having unpredictable spontaneous seizures but can be useful with certain acute seizure induction models.[78]
- *Telemetry in freely moving animals:* Telemetry has the advantage of allowing blood pressure sampling in awake and freely moving animals. This is especially appropriate for models in which animals are having spontaneous seizures, or in settings where animals will be subjected to recurrent, induced seizures. The disadvantage is that this requires surgical instrumentation of the animals. For measurement by telemetry, the telemeter and blood pressure leads must first be implanted. For implantation of a telemetry device in the femoral artery of a rat and into the aortic arch of a mouse, see Appendix S2 and Refs. 79, 80.

Sampling telemetry signals. Depending on the system used, animal cages are either placed directly on top of a telemetry receiver or placed near the receiver. A common approach is to sample each transmitter at 500 Hz for 10 s once every minute, and then calculate 10 min averages of blood pressure (i.e., systolic, diastolic, mean arterial pressure, and pulse pressure, etc.). A variety of software packages are available for sampling, recording, and analyzing blood pressure data.

Analysis and interpretation of the parameters that can be determined

- Systolic pressure
- Diastolic pressure
- Pulse pressure
- Mean arterial pressure
- With the telemetry methods, cardiac measures including heart rate, heart rate variability, and cardiac intervals such as P-R interval and Q-T interval can often also be obtained with the same telemetry device/receiver.

Measurement of blood pressure in anesthetized animals

For short-term experiments, isoflurane is frequently used for anesthesia in studies with mice. This volatile anesthetic compound has only moderate cardiodepressive effects compared to injectable agents. A 1.5% dose level of isoflurane was shown to yield stable blood pressure, heart rate, and

cardiac output levels comparable to those recorded in the conscious state, or to decrease only slightly.[81] When nonvolatile anesthetics such as urethane, sodium pentobarbital, or the ketamine/xylazine mixture are used, heart-function-related parameters decrease, with the greatest effects recorded for the ketamine/xylazine mixture.[82] It therefore appears preferable to use isoflurane over nonvolatile anesthetics if anesthesia is required (Figs. 4 and 5).

Blood sampling and testing

[File name: 6 CRF Module - blood testing.docx; 6 Blood Testing CDE Chart.xlsx]

It is the researcher's responsibility to select the appropriate sampling method for their goal as well as to obtain sufficient training in the technique to get valid samples. For more information see https://www.nc3rs.org.uk/our-resources/blood-sampling.[83]

Rationale

Seizure events are often associated with a wide array of physiologic changes that can be measured in blood. Blood sampling easily provides material for the analysis of the consequences of seizures such as metabolic changes, lactate accumulation, inflammation markers, and genetic analysis. Blood can be collected in various ways[83] to determine a wide variety of substances present in blood, ranging from cells, proteins, blood gases, small RNAs, and the concentration of antiepileptic drugs (AEDs) or test compounds. Furthermore, biomarker discovery research can be performed in experimental epilepsy models using blood, plasma, or serum[84]. See Fig. 6 for advantages and disadvantages of different methods. The following information is provided to facilitate the use of the CRF in Fig. 7 for data acquisition.

Equipment: Needles, collection tubes.

Procedure:

General laboratory animal guidelines include (see also Ref. 83):

- Too much blood collected at any single time may cause hypovolemic shock, physiologic stress, and even death. If smaller volumes are collected too frequently, anemia may result.
- As a general rule, 10% of the total blood volume can be collected at one time every 2–4 weeks or 1% at intervals of 24 h or more. Total blood volume can be calculated as approximately 7.5% of body weight.
- The estimated volume at exsanguination is approximately half of the total blood volume.
- For repeated blood sampling, use aseptic techniques.
- To achieve vasodilation effects in rodents, it is helpful to warm the entire animal and/or to put the tail in warm water (38°C for 0.5–2 min) when blood withdrawal from the tail vein is planned.
- The choice of anesthetics is an important consideration when collecting blood from rodents due to the potential effects of the anesthetic agent on blood constituents, such as metabolites.

General guidelines for blood collection in the rat (see also Ref. 82):

- The approximate blood volume of a rat is 64 ml/kg. For a 400 g rat this is equivalent to 25.6 ml.
- Single sampling: Without fluid replacement, the maximum blood volume that can be safely removed for a single-time sample is 10% of the total blood volume or ~64 ml/kg. For a 400 g rat, this is equivalent to 2.5 ml.
- Multiple sampling: If it is necessary to take multiple samples, smaller blood volumes should be drawn, maximum <1% of the total blood volume (= 0.25 ml) in 24 h. For repeated blood collection, fluid replacement does not allow for a larger blood volume or more frequent blood collection.
- Exsanguination: Approximately half of the total blood volume can be collected at exsanguination. This is equivalent to 32 ml/kg or about 13 ml for a 400 g rat.

General guidelines for blood collection in the mouse (see also Ref. 83):

- The approximate blood volume of a mouse is 77–80 µl/g. For a 25 g mouse this is equivalent to 1.9–2.0 ml.
- Single sampling: Without fluid replacement, the maximum blood volume that can be safely removed for a single-time sample is 10% of the total blood volume or ~8 µl/g. For a 25 g mouse, this is equivalent to ~200 µl. With fluid replacement, up to 15% of the total blood volume or 12 µl/g can be removed, that is, 290–300 µl. Generally, the fluid used as replacement should be warmed and given subcutaneously.
- Multiple sampling: If it is necessary to take multiple samples, smaller blood volumes should be drawn. The maximum blood volume that may be drawn per 24 h is less than 1% of the total blood volume, or 10 µl.
- Exsanguination: Approximately half of the total blood volume can be collected at exsanguination. This is equivalent to 40 µl/g or about 1 ml for a 25 g mouse.

If the animal will be killed immediately before or after the blood collection:

- *Trunk blood* (to collect up to 2–6 ml of whole blood for a rat, up to 1 ml for a mouse). Collect blood directly from the trunk, after decapitation, without touching the animal with the collection tube. This approach allows the collection of large amounts of whole blood, but blood may be mixed with tissue fluids.[83]
- *Intracardiac withdrawal* (to collect up to 2–6 ml of whole blood for a rat, up to 1 ml for a mouse). The blood will be collected from the heart. For additional technical details, see Appendix S2 and Ref. 83.

If the animal will not be killed after the blood collection:

- Lateral saphenous vein withdrawal (no anesthesia required).

  Rat: Up to 0.2 ml may be taken for a single sample, which can usually be repeated at 2-week intervals

Physiological Studies

Case Report Form: 4 CRF Module - heart rate.docx

CRF module: Heart Rate

Date at which this CRF was filled out:

Name of person filling out CRF:

Project name/Identifier:

Animal ID:

_____

CDE Name	Data Collected
Anesthesia	
Anesthesia	☐ Yes; ☐ No ; ☐ Unknown
If anesthesia was administered, specify type	☐ Isoflurane; ☐ Sevoflurane; ☐ Ketamine/Xylazine; Other ☐
Isoflurane concentration induction (%)	_____ (%)
Isoflurane concentration maintenance (%)	_____ (%)
Sevoflurane concentration induction (%)	_____ (%)
Sevoflurane concentration maintenance (%)	_____ (%)
Ketamine dose (mg/Kg)	_____ (mg/kg)
Xylazine dose (mg/Kg)	_____ (mg/kg)
Methods	
Methods Used	☐ Electrocardiograhy (ECG) electrodes; ☐ External heart rate sensor; ☐ Implanted telemetry heart rate sensor ☐ Noninvasive platform device ☐ Implanted ECG electrodes ☐ Implanted ECG telemetry device
If implanted telemetry heart rate sensor, brand name of telemeter	_____

Parameters

Time Point							
Ambient Temperature (˚C)							
Humidity (%)							
Heart Rate(BPM) (beats/min)							
R-R Interval (ms)							
HRV SDNN (ms)							
HRV RMSSD (ms)							
HRV LF (Hz)							
HRV HF (Hz)							
P-R interval (ms)							
QRS interval (ms)							
QTc interval* (ms)							
Arrhythmia(Y or N)							
Sampling rate (Hz)							
DurationMonitoring (hrs:min:sec)							

*Note correction method: _____

The P wave is generated by the activation of the atria.

The PR segment represents the duration of the atrioventricular (AV) conduction.

The QRS complex is produced by activation of both ventricles.

The ST-T wave reflects ventricular recovery.

Instructions: Please check boxes where applicable. If none of the predetermined options is appropriate use the default space to specify your answer.

This form is to be filled in for one individual animal.

Figure 4.
Heart rate CRF module
Epilepsia Open © ILAE

Physiological Studies

Case Report Form: 5 CRF Module - blood pressure.docx

CRF module: Blood Pressure

Date at which this CRF was filled out:

Name of person filling out CRF:

Project name/Identifier:

Animal ID:

CDE Name	Data Collected
Anesthesia	
Anesthesia	☐ Yes; ☐ No ; ☐ Unknown
If anesthesia was administered, specify type	☐ Isoflurane; ☐ Sevoflurane; ☐ Ketamine/Xylazine; ☐ Other
Isoflurane concentration induction (%)	_____ (%)
Isoflurane concentration maintenance (%)	_____ (%)
Sevoflurane concentration induction (%)	_____ (%)
Sevoflurane concentration maintenance (%)	_____ (%)
Ketamine dose (mg/Kg)	_____ (mg/kg)
Xylazine dose (mg/Kg)	_____ (mg/kg)
Methods used	
Telemetry in freely-moving animal	☐ Yes; ☐ No ; ☐ Unknown
If telemetry was used, telemeter brand	_____
If telemetry was used, telemeter number	_____
If telemetry was used, date calibrated	_____ (MM/DD/YY)
If telemetry was used, date implanted	_____ (MM/DD/YY)
Tail cuff measurement in restrained animal	☐ Yes; ☐ No

Parameters

Time Point										
Ambient Temperature (˚C)										
Humidity (%)										
Systolic Pressure (SBP; mm Hg)										
Diastolic Pressure (DBP; mm Hg)										
Mean Arterial Pressure (MAP; mm Hg)										
Pulse Pressure (PP; mm Hg)										
Sampling Rate (Hz)										
Duration of testing (hrs:min:sec)										

General instructions

Please check mark with a cross where applicable. If none of the predetermined options is appropriate use the default space to specify your answer.

The form is to be filled in for one individual animal.

Figure 5.
Blood pressure CRF module
Epilepsia Open © ILAE

without disturbance of the hematologic status. Alternatively, multiple smaller samples (e.g., 0.02 ml daily) may be obtained, taking into account the limits on total sample volume.[85]

Mouse: Up to 0.15 ml for a single sample; this can usually be repeated at 2-week intervals without disturbance to the hematologic status. Alternatively, multiple smaller samples (e.g., 0.01 ml daily) can be withdrawn, taking into account limits on sample volume.[85]

There should not be more than 3 attempts to collect blood. Continuous sampling should be avoided and collecting more than 4 samples in a day (24-h period) is not advised.

Shave the back of the hind leg with an electric trimmer until the saphenous vein is visible. Hair removal cream can also be used. Restrain the animal manually or use a suitable animal restrainer. Immobilize the hind leg and apply slight pressure above the knee joint. Puncture the vein using a 20 gauge needle and collect blood with a capillary tube or a needle attached to a syringe. Compress the punctured site to stop the bleeding. A local anesthetic cream may be applied at the collection site.[85]

Collection Site	Advantages	Disadvantages
Trunk blood **Cardiac Puncture**	• Maximum volume of blood can be collected	• Requires deep anesthesia. • Non-survival procedure only
Saphenous Sampling (medial or lateral approach)	• Anesthesia not required • Excellent technique for serial blood sampling • Moderate volume of blood can be collected	• Requires some specialized training • Specialized equipment required
Lateral Tail Vein or Ventral/Dorsal Artery Sampling	• Anesthesia not required • Vein is easily accessed	• Must be securely restrained • Yields only small quantities • Some specialized equipment needed
Vessel cannulation	• Anesthesia not required • Repeated blood sampling • Multiple needle entries prevented	• Requires surgery • Risk of adverse effects (infection, hemorrhage, coagulation and blockage of cannula)
Retro-orbital sinus	• High quality aseptic sample	• Anesthesia is required • This technique should rather be avoided • Risk of injury to the eye and surrounding structures
Submandibular Sampling	• Alternative blood collection method • Maximum allowable sample volume with minimal trauma	• Must be securely restrained • Anesthesia recommended • Yields a large sample so it should not be used for frequent small sampling

Figure 6.
Comparison of blood collection methods
Epilepsia Open © ILAE

- Tail vein withdrawal
  Rat: collect 0.1–2 ml of whole blood repeatedly with long intervals (hours-days-weeks). No more than 8 blood samples should be taken per session and in any 24-h period.
  Mouse: collect 50–200 μl of whole blood. One or 2 blood samples can be taken per session and in any 24-h period, depending on sample volume.
  Restrain the animal in a cylinder or anesthetize the animal. Warm up the tail with a heating lamp or in warm water to dilate the blood vessels. Visualize a sampling site on the lateral tail vein in the distal third of the tail. While extending the tail, insert a 20 gauge needle with syringe and collect the blood.[85]
- Cannula withdrawal
  Rat: Usually 0.1–0.2 ml can be taken per sample, and depending on the sample volume and scientific justification, up to 6 samples over a 2 h period or up to 20 samples over a 24-h period may be taken.
  Mouse: 0.01–0.02 ml of blood can be taken and, depending on the sample volume and scientific justification, up to 6 samples may be taken in a 24-h period.
  For additional details, see Appendix S2 and Refs. 86, 87.
  This procedure can be used for venous or arterial blood withdrawal only,[86,87] but can also be used in combination with venous drug administration to determine pharmacokinetics of AEDs.[88]
- Orbital plexus
  This method should rather be used in anesthetized animals, especially in a seizure or epilepsy model, since it generates a large amount of stress that might itself generate seizures. In case animals are not anesthetized, usually before sampling a local anesthetic is dropped into the eye (e.g., 2% tetracaine).
  Rat: Up to 4 ml blood can be collected with recovery; 4–10 ml nonrecovery. It is recommended that only one sample be taken.
  Mouse: Up to 0.2 ml blood can be collected with recovery; up to 0.5 ml nonrecovery. It is recommended that only one sample be taken.
  For additional details, see Appendix S2 and Ref. 85.
  Lateral canthus: Pick up the animal and restrain it in one hand. Insert a small diameter glass capillary tube or Pasteur pipette into the lateral canthus. The tube should be at about a 30 degrees angle to the side of the head.

Physiological Studies

Case Report Form: 6 CRF Module - blood testling.docx

CRF module: Blood Testing

Date at which this CRF was filled out:

Name of person filling out CRF:

Project name/Identifier:

Animal ID:

_____

CDE Name	Data Collected
Body weight (g)	_____ (g)
Anesthesia	
Anesthesia	☐ Yes; ☐ No ; ☐ Unknown
If anesthesia was administered, specify type	☐ Isoflurane; ☐ Sevoflurane; ☐ Ketamine/Xylazine; ☐ CO_2; ☐ Pentobarbitone; ☐ Other
Isoflurane concentration induction (%)	_____ (%)
Isoflurane concentration maintenance (%)	_____ (%)
Sevoflurane concentration induction (%)	_____ (%)
Sevoflurane concentration maintenance (%)	_____ (%)
Ketamine dose (mg/Kg)	_____ (mg/kg)
Xylazine dose (mg/Kg)	_____ (mg/kg)
Pentobarbitone dose (mg/Kg)	_____ (mg/kg)
Animal sacrifice	☐ Decapitation; ☐ Overdose; ☐ Not sacrificed (tail vein or cannula withdrawal)
Blood collection	
Collection modality	☐ Trunk; ☐ Intracardiac withdrawal; ☐ Lateral saphenous vein withdrawal; ☐ Tail vein withdrawal; ☐ Cannula withdrawal; ☐ Orbital plexus; ☐ Retromandibular venous plexus
If cannula withdrawal, specify blood vessel	_____
If trunk blood collected, volume (ml) collected	_____ (ml)
If intracardiac withdrawal, volume (ml) collected	_____ (ml)
If lateral saphenous vein withdrawal, volume (ml) collected	_____ (ml)
If tail vein withdrawal, volume (ml) collected	_____ (ml)
If cannula withdrawal, volume (ml) collected	_____ (ml)
If orbital plexus blood collected, volume (ml) collected	_____ (ml)
If retromandibular plexus blood collected, volume (ml) collected	_____ (ml)
Collection container	☐ Becton Dickinson Tubes; ☐ Other manufacturer: _____; ☐ Custom made tubes
Volume of tube (ml)	_____ (ml)
Anticoagulant used	☐ EDTA[1]; ☐ Heparin; ☐ Citrate
If EDTA, concentration $(mM)^2$	_____ $(mM)^2$
If Heparin, concentration $(U/ml)^2$	_____$(U/ml)^2$
If Citrate, Concentration $(mM)^2$	_____$(mM)^2$

Figure 7.
Blood testing CRF module
Epilepsia Open © ILAE

Plasma separation	
Number of centrifugation steps	☐ 1; ☐ 2
Centrifugation speed (g) step 1	_____ (g)
Centrifugation time step 1	_____
Centrifugation temperature (°C) step 1	_____(°C)
Centrifugation speed (g) step 2	_____ (g)
Centrifugation time step 2	_____
Centrifugation temperature (°C) step 2	_____(°C)
Delay between plasma collection and centrifugation	
Samples kept on ice before centrifugation?	☐ Yes; ☐ No ; Unknown
Delay between centrifugation and storage (min)	_____
Plasma storage	☐-70°C; ☐-80°C; ☐Liquid nitrogen
Plasma storage - volume per aliquot (µl)	_____(µl)
Serum separation	
Clotting time	_____
Clotting temperature (°C)	_____(°C)
Centrifugation speed (g)	_____ (g)
Centrifugation time	_____
Centrifugation temperature (°C)	_____(°C)
Serum storage	☐-70°C; ☐-80°C; ☐Liquid nitrogen
Serum storage - volume per aliquot (µl)	_____(µl)
Blood tests	
Blood tests	☐Arterial blood gas analysis; ☐Blood cell analysis; ☐Glucose; ☐Ketones; ☐Western Blot assay; ☐ELISA assay; ☐Metabolite analysis; ☐Hormone analysis; ☐RNA/protein; ☐Other
If arterial blood gas analysis, specify O_2/CO_2/pH	_____
If blood cell analysis, specify red blood cells/leukocytes/Ht[3]/MCV[4]	_____
If metabolite analysis, specify metabolite(s)	_____
If hormone analysis, specify hormone(s)	_____

Parameters

Time Point						

_____

[1] EDTA, K_2 Ethylenediaminetetraacetic acid, is recommended for RNA analysis.

[1] Indicate final concentration in blood (recommended EDTA about 5 mM)

[3] Ht, hematocrit, the volume percentage (vol%) of red blood cells in blood

[4] MCV, Mean corpuscular volume, the average volume of a red blood cell

Instructions: Please check boxes where applicable. If none of the predetermined options is appropriate use the default space to specify your answer.

This form is to be filled in for each individual animal.

Figure 7.
(Continued)
Epilepsia Open © ILAE

Medial canthus: Place the animal on a table or cage lid on its side. The body of the animal is restrained against the table with the palm of the hand. The thumb and forefingers of the same hand restrain the animal and gently open the eyelids to expose the eye. Insert the tube into the medial canthus and hold it at a 30 degrees angle to the nose.

- Retromandibular venous plexus
  This procedure allows collecting up to 300 µl of whole blood in mice, and care needs to be taken to limit as much

as possible the volume to the allowed amounts described above. Please note that it can be difficult to restrict the blood flow, especially if the mouse is conscious, and if more than 500 µl of blood is withdrawn in a mouse, euthanasia must be considered.

For additional details, see Appendix S2 and Ref. 89 plus Ref. 90 indicating the level of stress generated in animals by these repeated samplings and other laboratory routines.

Analysis and interpretation

Samples can be used for various blood tests. Select the appropriate method to handle the sample according to the test that will be used. Use whole blood as soon as possible for blood gas analysis or hematology. For RNA/protein analysis, centrifuge to separate plasma from whole blood within 1 h after whole blood collection using the following recommended parameters: 1300–1500 *g* for 10 min without a break at 4°C. For serum collection, let blood clot at room temperature for 1 h and centrifuge at 1300–1500 *g* for 10 min without the break at 4°C. Transfer the upper-phase into a new tube. To prevent platelet contamination, a second centrifuge step is needed (3000 *g*, 10 min, 4°C) before freezing. Freeze samples on dry ice and store aliquots at −70 or −80°C.

For additional reading, see Refs. 91–93.

Discussion

The purpose of this discussion is not to paraphrase all the critical aspects of physiology that have been detailed in the guidelines and CRF forms presented in this article. We chose to focus on a few points that are related to the topic of physiology but that were not developed in the previous sections.

The forms developed here are aimed at research in adult rodent epilepsy models but they can also be used for research in other rodent disease models. The forms that are presented in this article have been established for adult male rodents, which are most often used in experimental studies. However, some studies require the use of female animals, and it is critical to remember that although all physiologic parameters suggested in research on male rodents should also be measured in female rodents, some will differ with gender, especially with the estrous cycle in female rodents.[94]

In most studies, the basal physiology of the animals is not considered by researchers, who rather focus on the validity and reproducibility of their models. In this paper, we developed forms for monitoring the physiologic status of animals when inducing epilepsy and during the course of the disease. Characterizing the physiologic status of animals that have undergone various procedures leading to the chronic pathology is critical. Mainly, the general status of the animals that have become epileptic should be assessed to establish valid criteria allowing inclusion of the animals in the groups studied, to increase homogeneity, but also to prevent unnecessary suffering of the animals.

An issue faced in animal research at the outset of a study the consideration of homogeneity of the groups, because the insertion of already sick animals (hypertensive or else) might strongly influence the outcome of the whole experimental group and introduce a bias in the experiment. Indeed, it is not unusual to find, for example, borderline diabetic or hypertensive animals in a given group of animals (AN, personal observations). It is clear that different genetic backgrounds will generate differences in sensitivity to and consequences of the epilepsy-generating insult.

The CDEs described in the previous sections were prepared for research in adult animals and are not fully valid for immature animals. Although the measurement of physiologic parameters listed here applies to immature animals as well, the equipment needed for assessing these parameters in immature rodents will need miniaturization, which at this point is not necessarily available for all types of measurements. Immature rodents are different from adult rodents, not only a miniature form of adult ones, and this will affect their responsiveness to seizures. It has been reported that immature rats are more or less sensitive than adult rats to convulsive agents, depending on the convulsant.[95,96] In addition, at the same time, seizure spread is limited by brain maturation, especially in the limbic system,[97] and hence the consequences of seizures are also different in adult compared to immature rodents.[97,98] There are periods of transient susceptibility to some types of seizures as in humans. This is the case in febrile seizures[99] or epileptic encephalopathies.[100] In addition, suckling immature rodents are in a state of natural ketosis, which might influence their sensitivity to seizures.[4] At this point, forms need to be developed for immature rodents with an adaptation to the size and immaturity.

The physiology CRFs and CDEs are also presented for other areas (pharmacology, EEG, and behavior; see other recent supplement articles), and we hope that researchers using the translational approach to epilepsy research will find them useful. Researchers are encouraged to use these forms as often as possible but, as stated in the introduction, depending on the type of model of seizure or epilepsy studied, the physiology, CDEs, and measurements detailed in the present manuscript will not all need to be used in every single experiment. The success of standardization of translational research will depend on the willingness of individual researchers to fill in the forms. The final aim of this whole process, that is, collecting all CDEs of the different approaches, is the hope of better comparison of the studies and performing homogeneous meta-analyses in order to reach stronger evidence of seizure- or treatment-induced changes that cannot always be concluded from individual studies. The ultimate hope is indeed to try to develop

biomarkers and to find new treatments for epilepsy. Of note, the EPITARGET consortium has developed CDEs for other modules and has started to use a Research Electronic Data Capture (REDCap) database, in which actual data from preclinical studies can be registered online.[2,101] Finally, to be able to develop these more standardized approaches, funds should be made available to publish and use interactive forms, maintain databases, and to take care that unpublished data are protected.

Acknowledgments

This report was written by experts selected by the International League Against Epilepsy (ILAE) and the American Epilepsy Society (AES) and was approved for publication by the ILAE and the AES. The experts formed a Physiology working group of TASK3 of the ILAE/AES Joint Translational Task Force. Opinions expressed by the authors, however, do not represent the policy or position of the ILAE or the AES. We are also grateful to the AES, ILAE, and the National Institute of Neurological Disorders and Stroke (NINDS) for their financial support of the activities of TASK3 working groups. The authors are grateful to the invaluable help of Dr Lauren Harte-Hargrove, project manager of the ILAE/AES Joint Translational Task Force, in helping with the CDEs and CRFs discussed in this article, including the formatting, editing for consistency, and generation of the CDE Excel charts.

Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

1. Smith DH, Hicks RR, Johnson VE, et al. Pre-clinical traumatic brain injury common data elements: toward a common language across laboratories. *J Neurotrauma* 2015;32:1725–1735.
2. Lapinlampi N, Melin E, Aronica E, et al. Common data elements and data management: Remedy to cure underpowered preclinical studies. *Epilepsy Res* 2017;129:87–90.
3. Nehlig A, Pereira de Vasconcelos A, Boyet S. Quantitative autoradiographic measurement of local cerebral glucose utilization in freely moving rats during postnatal development. *J Neurosci* 1988;8:2321–2333.
4. Nehlig A, Pereira de Vasconcelos A. Glucose and ketone body utilization by the brain of neonatal rats. *Prog Neurobiol* 1993;40:163–221.
5. Hickman DL, Swan M. Use of a body condition score technique to assess health status in a rat model of polycystic kidney disease. *J Am Assoc Lab Anim Sci* 2010;49:155–159.
6. Lidster K, Jefferys JG, Blümcke I, et al. Opportunities for improving animal welfare in rodent models of epilepsy and seizures. *J Neurosci Methods* 2016;260:2–25.
7. Ullman-Cullere MH, Foltz CJ. Body condition scoring: a rapid and accurate method for assessing health status in mice. *Lab Anim Sci* 1999;49:319–323.
8. Burkholder T, Foltz C, Karlsson E, et al. Health evaluation of experimental laboratory mice. *Curr Protoc Mouse Biol* 2012;2:145–165.
9. Bogdanske JJ, Hubbard-Van Stelle S, Rankin-Riley MR, et al. Laboratory Mouse and laboratory rat procedural Rat. In Bogdanske JJ, Hubbard-Van Stelle S, Rlley MR, et al. (Eds) *Procedural Techniques*. Boca Raton, FL: CRC Press, Taylor and Francis Group, 2014.
10. Jacobs BY, Kloefkorn HE, Allen KD. Gait analysis methods for rodent models of osteoarthritis. *Curr Pain Headache Rep* 2014;18:456.
11. Chen H, Du J, Zhang Y, et al. Establishing a reliable gait evaluation method for rodent studies. *J Neurosci Methods* 2017;283:92–100.
12. Hubrecht R, Kirkwell J. *The UFAW handbook on the care and management of laboratory and other research animals*. 8th Ed. Oxford: Wiley-Blackwell; 2010.
13. Latham N. Brief introduction to welfare assessment: A 'toolbox' of techniques. In Hubrecht R, Kirkwell J (Eds) *The UFAW handbook on the care and management of laboratory and other research animals*. 8th Ed. Oxford: Wiley-Blackwell; 2010:76–91.
14. Baumans V. The Laboratory Mouse. In Hubrecht R, Kirkwell J (Eds) *The UFAW handbook on the care and management of laboratory and other research animals*. 8th Ed. Oxford: Wiley-Blackwell; 2010:276–310.
15. Koolhaas JM. The laboratory rat. In Hubrecht R, Kirkwell J (Eds) *The UFAW handbook on the care and management of laboratory and other research animals*. 8th Ed. Oxford: Wiley-Blackwell; 2010:311–326.
16. Foltz CH, Ullman-Cullere MH. Guidelines for assessing the health and condition of mice. *Lab Anim* 1999;28:28–32.
17. Schmitt FC, Matzen J, Buchheim K, et al. Limbic self-sustaining status epilepticus in rats is not associated with hyperthermia. *Epilepsia* 2005;46:188–192.
18. Hauser WA. The prevalence and incidence of convulsive disorders in children. *Epilepsia* 1994;35(Suppl 2):S1–S6.
19. Mathern GW, Pretorius JK, Babb TL. Influence of the type of initial precipitating injury and at what age it occurs on course and outcome in patients with temporal lobe seizures. *J Neurosurg* 1995;82:220–227.
20. Tremoleda JL, Kerton A, Gsell W. Anaesthesia and physiological monitoring during in vivo imaging of laboratory rodents: considerations on experimental outcomes and animal welfare. *EJNMMI Res* 2012;2:44.
21. Quigg M, Clayburn H, Straume M, et al. Hypothalamic neuronal loss and altered circadian rhythm of temperature in a rat model of mesial temporal lobe epilepsy. *Epilepsia* 1999;40:1688–1696.
22. Holtkamp M, Schmitt FC, Buchheim K, et al. Temperature regulation is compromised in experimental limbic status epilepticus. *Brain Res* 2007;1127:76–79.
23. Baram TZ, Gerth A, Schultz L. Febrile seizures: an appropriate-aged model suitable for long-term studies. *Brain Res Dev Brain Res* 1997;98:265–270.
24. Dubé C, Chen K, Eghbal-Ahmadi M, et al. Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. *Ann Neurol* 2000;47:336–344.
25. Motamedi GK, Lesser RP, Vicini S. Therapeutic brain hypothermia, its mechanisms of action, and its prospects as a treatment for epilepsy. *Epilepsia* 2013;54:959–970.
26. Meyer CW, Ootsuka Y, Romanovsky AA. Body temperature measurements for metabolic phenotyping in mice. *Front Physiol* 2017;8:520.
27. Lundt A, Wormuth C, Siwek ME, et al. EEG radiotelemetry in small laboratory rodents: a powerful state-of-the art approach in neuropsychiatric, neurodegenerative, and epilepsy research. *Neural Plast* 2016;2016:8213878.
28. DeBow S, Colbourne F. Brain temperature measurement and regulation in awake and freely moving rodents. *Methods* 2003;30:167–171.
29. Kiyatkin EA, Brown PL, Wise RA. Brain temperature fluctuation: a reflection of functional neural activation. *Eur J Neurosci* 2002;16:164–168.
30. Wang H, Wang B, Normoyle KP, et al. Brain temperature and its fundamental properties: a review for clinical neuroscientists. *Front Neurosci* 2014;8:307.
31. Andersen P, Moser EI. Brain temperature and hippocampal function. *Hippocampus* 1995;5:491–498.
32. Hoymann HG. Lung function measurements in rodents in safety pharmacology studies. *Front Pharmacol* 2012;3:156.
33. Lucchi C, Vinet J, Meletti S, et al. Ischemic-hypoxic mechanisms leading to hippocampal dysfunction as a consequence of status epilepticus. *Epilepsy Behav* 2015;49:47–54.
34. Mrozek S, Vardon F, Geeraerts T. Brain temperature: physiology and pathophysiology after brain injury. *Anesthesiol Res Pract* 2012;2012:989487.

35. Jensen FE, Applegate CD, Holtzman D, et al. Epileptogenic effect of hypoxia in the immature rodent brain. *Ann Neurol* 1991;29:629–637.
36. Rakhade SN, Klein PM, Huynh T, et al. Development of later life spontaneous seizures in a rodent model of hypoxia-induced neonatal seizures. *Epilepsia* 2011;52:753–765.
37. Mathern GW, Price G, Rosales C, et al. Anoxia during kainate status epilepticus shortens behavioral convulsions but generates hippocampal neuron loss and supragranular mossy fiber sprouting. *Epilepsy Res* 1998;30:133–151.
38. Söderfeldt B, Blennow G, Kalimo H, et al. Influence of systemic factors on experimental epileptic brain injury. Structural changes accompanying bicuculline-induced seizures in rats following manipulations of tissue oxygenation or alpha-tocopherol levels. *Acta Neuropathol* 1983;60:81–91.
39. Decker MJ, Conrad KP, Strohl KP. Noninvasive oximetry in the rat. *Biomed Instrum Technol* 1989;23:222–228.
40. Waisman D, Lev-Tov L, Levy C, et al. Real-time detection, classification, and quantification of apneic episodes using miniature surface motion sensors in rats. *Pediatr Res* 2015;78:63–70.
41. Schuchmann S, Schmitz D, Rivera C, et al. Experimental febrile seizures are precipitated by a hyperthermia-induced respiratory alkalosis. *Nat Med* 2006;12:817–823.
42. Lim R, Zavou MJ, Milton PL, et al. Measuring respiratory function in mice using unrestrained whole-body plethysmography. *J Vis Exp* 2014;90:e51755.
43. Bates J, Irvin C, Brusasco V, et al. The use and misuse of Penh in animal models of lung disease. *Am J Respir Cell Mol Biol* 2004;31:373–374.
44. Drorbaugh JE, Fenn WO. A barometric method for measuring ventilation in newborn infants. *Pediatrics* 1955;16:81–87.
45. Buchanan GF, Murray NM, Hajek MA, et al. Serotonin neurones have anti-convulsant effects and reduce seizure-induced mortality. *J Physiol* 2014;592:4395–4410.
46. Smith HR, Leibold NK, Rappoport DA, et al. Dorsal raphe serotonin neurons mediate CO2-induced arousal from sleep. *J Neurosci* 2018;38:1915–1925.
47. Goldman AM, Buchanan GF, Aiba A, et al. Animal models of sudden unexpected death in epilepsy. In Pitkänen A, Buckmaster PS, Galanopoulou AS, et al. (Eds) *Models of seizures and epilepsy*. 2nd Ed. London: Academic Press; 2017:1007–1018.
48. Zhan Q, Buchanan GF, Motelow JE, et al. Impaired serotonergic brainstem function during and after Seizures. *J Neurosci* 2016;36:2711–2722.
49. McGovern TK, Robichaud A, Fereydoonzad L, et al. Evaluation of respiratory system mechanics in mice using the forced oscillation technique. *J Vis Exp* 2013;75:e50172.
50. McAfee SS, Ogg MC, Ross JM, et al. Minimally invasive highly precise monitoring of respiratory rhythm in the mouse using an epithelial temperature probe. *J Neurosci Methods* 2016;263:89–94.
51. Lizio R, Westhof A, Lehr CM, et al. Oral endotracheal intubation of rats for intratracheal instillation and aerosol drug delivery. *Lab Anim* 2001;35:257–260.
52. Ghali MGZ. Microsurgical technique for tracheostomy in the rat. *MethodsX* 2017;5:61–67.
53. Arms AD, Travis CC. *Reference physiological parameters in pharmacokinetic modeling*. Washington DC: Environmental Protection Agency; 1988.
54. Lhatoo S, Noebels J, Whittemore V. The NINDS Center for SUDEP Research. Sudden unexpected death in epilepsy: Identifying risk and preventing mortality. *Epilepsia* 2015;56:1700–1706.
55. Tomson T, Surges R, Delamont R, et al. Who to target in sudden unexpected death in epilepsy prevention and how? Risk factors, biomarkers, and intervention study designs. *Epilepsia* 2016;57:4–16.
56. Goldman AM, Glasscock E, Yoo J, et al. Arrhythmia in heart and brain: KCNQ1 mutations link epilepsy and sudden unexplained death. *Sci Transl Med* 2009;1:2ra6–2ra6.
57. Glasscock E, Yoo JW, Chen TT, et al. Kv1.1 potassium channel deficiency reveals brain-driven cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. *J Neurosci* 2010;30:5167–5175.
58. Powell KL, Jones NC, Kennard JT, et al. HCN channelopathy and cardiac electrophysiologic dysfunction in genetic and acquired rat epilepsy models. *Epilepsia* 2014;55:609–620.
59. Glasscock E. Genomic biomarkers of SUDEP in brain and heart. *Epilepsy Behav* 2014;38:172–179.
60. Auerbach DS, Jones J, Clawson BC, et al. Altered Cardiac Electrophysiology and SUDEP in a Model of Dravet Syndrome. *PLoSOne* 2013;8:e77843.
61. Kalume F, Westenbroek RE, Cheah CS, et al. Sudden unexpected death in a mouse model of Dravet syndrome. *J Clin Invest* 2013;123:1798–1808.
62. Biet M, Morin N, Lessard-Beaudoin M, et al. Prolongation of action potential duration and QT interval during epilepsy linked to increased contribution of neuronal sodium channels to cardiac late Na+ current: potential mechanism for sudden death in epilepsy. *Circ Arrhythm Electrophysiol* 2015;8:912–920.
63. Ho D, Zhao X, Gao S, et al. Heart rate and electrocardiography monitoring in mice. *Curr Protoc Mouse Biol* 2011;1:123–139.
64. González-Sánchez C, Fraile JC, Pérez-Turiel J, et al. Capacitive sensing for non-invasive breathing and heart monitoring in non-restrained, non-sedated laboratory mice. *Sensors (Basel)* 2016;16(7):E1052.
65. Chu V, Otero JM, Lopez O, et al. Method for non-invasively recording electrocardiograms in conscious mice. *BMC Physiol* 2001;1:6.
66. Sgoifo A, Stilli D, Medici D, et al. Electrode positioning for reliable telemetry ECG recordings during social stress in unrestrained rats. *Physiol Behav* 1996;60:1397–1401.
67. Arini PD, Liberczuk S, Mendieta JG, et al. Electrocardiogram delineation in a Wistar rat experimental model. *Comput Math Methods Med* 2018;2018:2185378.
68. Cheng X, Waghulde H, Mell B, et al. Positional cloning of quantitative trait nucleotides for blood pressure and cardiac QT-interval by targeted CRISPR/Cas9 editing of a novel long non-coding RNA. *PLoS Genet* 2017;13:e1006961.
69. Jin H, Welzig CM, Aronovitz M, et al. QRS/T-wave and calcium alternans in a type I diabetic mouse model for spontaneous postmyocardial infarction ventricular tachycardia: A mechanism for the antiarrhythmic effect of statins. *Heart Rhythm* 2017;14:1406–1416.
70. Damasceno DD, Savergnini SQ, Gomes ER, et al. Cardiac dysfunction in rats prone to audiogenic epileptic seizures. *Seizure* 2013;22:259–266.
71. Boehm M, Lawrie A, Wilhelm J, et al. Maintained right ventricular pressure overload induces ventricular-arterial decoupling in mice. *Exp Physiol* 2017;102:180–189.
72. André E, Ramaekers D, Beckers F, et al. The analysis of heart rate variability in unrestrained rats. Validation of method and results. *Comput Methods Programs Biomed* 1999;60:197–213.
73. Gehrmann J, Hammer PE, Maguire CT, et al. Phenotypic screening for heart rate variability in the mouse. *Am J Physiol Heart Circ Physiol* 2000;279:H733–H740.
74. Azar T, Sharp J, Lawson D. Heart rates of male and female Sprague-Dawley and spontaneously hypertensive rats housed singly or in groups. *J Am Assoc Lab Anim Sci* 2011;50:175–184.
75. Bealer SL. Little JG Seizures following hippocampal kindling induce QT interval prolongation and increased susceptibility to arrhythmias in rats. *Epilepsy Res* 2013;105:216–219.
76. Bozorgi A, Chung S, Kaffashi F, et al. Significant postictal hypotension: expanding the spectrum of seizure-induced autonomic dysregulation. *Epilepsia* 2013;54:e127–e130.
77. Kubota Y, Umegaki K, Kagota S, et al. Evaluation of blood pressure measured by tail-cuff methods (without heating) in spontaneously hypertensive rats. *Biol Pharm Bull* 2006;29:1756–1758.
78. Nayate A, Moore SA, Weiss R, et al. Cardiac damage after lesions of the nucleus tractus solitarii. *Am J Physiol Regul Integr Comp Physiol* 2009;296:R272–R279.
79. Gupte M, Boustany-Kari CM, Bharadwaj K. ACE2 is expressed in mouse adipocytes and regulated by a high-fat diet. *Am J Physiol Regul Integr Comp Physiol* 2008;295:R781–R788.
80. Koriyama H, Nakagami H, Nakagami F, et al. Long-term reduction of high blood pressure by angiotensin II DNA vaccine in Spontaneously Hypertensive Rats. *Hypertension* 2015;66:167–174.
81. Constantinides C, Mean R, Janssen BJ. Effects of isoflurane anesthesia on the cardiovascular function of the C57BL/6 mouse. *ILAR J* 2011;52:e21–e31.
82. Janssen BJ, De Celle T, Debets JJ, et al. Effects of anesthetics on systemic hemodynamics in mice. *Am J Physiol Heart Circ Physiol* 2004;287:H1618–H1624.

83. https://www.nc3rs.org.uk/our-resources/blood-sampling
84. van Vliet EA, Puhakka N, Mills JD, et al. Standardization procedure for plasma biomarker analysis in rat models of epileptogenesis: Focus on circulating microRNAs. *Epilepsia* 2017;58:2013–2024.
85. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* 2010;1:87–93.
86. Feng J, Fitz Y, Li Y, et al. Catheterization of the carotid artery and jugular vein to perform hemodynamic measures, infusions and blood sampling in a conscious rat model. *J Vis Exp* 2015;95. https://doi.org/10.3791/51881
87. Thrivikraman KV, Huot RL, Plotsky PM. Jugular vein catheterization for repeated blood sampling in the unrestrained conscious rat. *Brain Res Brain Res Protoc* 2002;10:84–94.
88. van Vliet EA, van Schaik R, Edelbroek PM, et al. Region-specific overexpression of P-glycoprotein at the blood-brain barrier affects brain uptake of phenytoin in epileptic rats. *J Pharmacol Exp Ther* 2007;322:141–147.
89. Golde WT, Gollobin P, Rodriguez LL. A rapid, simple, and humane method for submandibular bleeding of mice using a lancet. *Lab Anim (NY)* 2005;34:39–43.
90. Balcombe JP, Barnard ND, Sandusky C. Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci* 2004;43:42–51.
91. Guidelines for the Survival Bleeding of Mice and Rats. Animal Research Advisory Committee Guidelines, NIH. 2015;https://oacu.oir.nih.gov/animal-research-advisory-committee-guidelines/rodent_bleeding.pdf
92. McGuill MW, Rowan AN. Biological effects of blood loss: implications for sampling volumes and techniques. *ILAR News* 1989;31:5–20.
93. Mitruka BM, Rawnsley HM (Eds). *Clinical, biochemical and haematological reference values in normal experimental animals and normal humans*. New York: Masson Publishing;1981:413.
94. Harkness JE, Wagner JE. Biology and husbandry. In Harkness JE, Turner PV, VandeWoude S, et al. (Eds) *Harkness and Wagner's biology and medicine of rabbits and rodents*. 5th Ed. Oxford: Wiley Blackwell; 2010:23–105.
95. Kubová H, Folbergrová J, Mares P. Seizures induced by homocysteine in rats during ontogenesis. *Epilepsia* 1995;36:750–756.
96. Haas KZ, Sperber EF, Opanashuk LA, et al. Resistance of immature hippocampus to morphologic and physiologic alterations following status epilepticus or kindling. *Hippocampus* 2001;11:615–625.
97. Tremblay E, Nitecka L, Berger ML, et al. Maturation of kainic acid seizure-brain damage syndrome in the rat. I. Clinical, electrographic and metabolic observations. *Neuroscience* 1984;13:1051–1072.
98. Sankar R, Shin DH, Liu H, et al. Patterns of status epilepticus-induced neuronal injury during development and long-term consequences. *J Neurosci* 1998;18:8382–8393.
99. Dubé CM, McClelland S, Choy MK, et al. Fever, febrile seizures and epileptogenesis. In Noebels JL, Avoli M, Rogawski MA,et al. (Eds) *Jasper's basic mechanisms of the epilepsies [Internet]*. 4th Ed. Bethesda (MD): National Center for Biotechnology Information (US); 2012.
100. Jarvis PR, Holmes GL. Models of epileptic encephalopathies. In Pitkänen A, Buckmaster PS, Galanopoulou AS, et al. (Eds) *Models of seizures and epilepsy*. 2nd Ed. London: Academic Press; 2017:995–1005.
101. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377–381.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Physiology CRF and CDE files. The CDE and CRF modules linked to this article can be found and downloaded as a zip folder.

Appendix S2. Procedures for monitoring cardiorespiratory parameters or temperature and blood sampling. Additional technical information on relevant procedures can be found and downloaded as a word document.