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## CRITICAL REVIEW

# Are we there yet? A critical evaluation of sudden and unexpected death in epilepsy models

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## Abstract

Although animal models have helped to elaborate meaningful hypotheses about the pathophysiology of sudden and unexpected death in epilepsy (SUDEP), specific prevention strategies are still lacking, potentially reflecting the limitations of these models and the intrinsic difficulties of investigating SUDEP. The interpretation of preclinical data and their translation to diagnostic and therapeutic developments in patients thus require a high level of confidence in their relevance to model the human situation. Preclinical models of SUDEP are heterogeneous and include rodent and nonrodent species. A critical aspect is whether the animals have isolated seizures exclusively induced by a specific trigger, such as models where seizures are elicited by electrical stimulation, pharmacological intervention, or DBA mouse strains, or whether they suffer from epilepsy with spontaneous seizures, with or without spontaneous SUDEP, either of nongenetic epilepsy etiology or from genetically based developmental and epileptic encephalopathies. All these models have advantages and potential disadvantages, but it is important to be aware of these limitations to interpret data appropriately in a translational perspective. The majority of models with spontaneous seizures are of a genetic basis, whereas SUDEP cases with a genetic basis represent only a small proportion of the total number. In almost all models, cardiorespiratory arrest occurs during the course of the seizure, contrary to that in patients observed at the time of death, potentially raising the issue of whether we are studying models of SUDEP or models of periseizure death. However, some of these limitations are impossible to avoid and can in part be dependent on specific features of SUDEP, which may be difficult to model. Several preclinical tools are available to address certain gaps in SUDEP pathophysiology, which can be used to further validate current preclinical models.

## KEYWORDS

preclinical models, respiratory arrest, seizures, SUDEP

## 1 | INTRODUCTION

Sudden and unexpected death in epilepsy (SUDEP) is one of the most dangerous and distressing aspects of epilepsy for patients. Although it was noted more than a century ago,<sup>1</sup> our understanding of SUDEP pathophysiology has only recently been characterized, admittedly with a relatively small number of patients, with a common pattern of delayed cardiorespiratory collapse following a tonic-clonic seizure.<sup>2</sup> Apart from optimizing the antiseizure medication regimen, which proved to reduce the risk of SUDEP at a population level,<sup>3</sup> specific prevention strategies are still lacking. One could attribute this to specific limitations of our experimental models and the intrinsic difficulties of investigating SUDEP. More recently, with the proliferation of genetic modification techniques and a greater understanding of SUDEP pathophysiology, more valid models have been developed and are now at our disposal to further decipher the underlying pathological mechanisms of SUDEP and to test possible future medical treatments.

The characteristics of these models are very heterogeneous, including both acute induced seizures and chronic epilepsy models, with spontaneous seizures. However, the interpretation of these preclinical data and their translation to diagnostic and therapeutic developments in patients require a high level of confidence in their relevance to modeling the human situation. In recent years, numerous reviews have presented pathophysiological hypotheses and data concerning SUDEP, including dysregulation of the autonomic nervous system, impaired CO<sub>2</sub> chemoreception, or the role of adenosinergic and serotonergic control of cardiac and respiratory function.<sup>4-7</sup> The objective of the present review is not to discuss the data obtained through the use of these preclinical models, but to present and discuss their characteristics, advantages, and potential disadvantages in a translational perspective.

## 2 | ANIMAL MODELS OF SUDEP: WHAT DO WE NEED TO MODEL?

Overall, the most accurate models of SUDEP should incorporate mandatory characteristics, to be developed below, that would reproduce the core of the SUDEP definition used in patients,<sup>8</sup> with SUDEP being a sudden, unexpected, witnessed or unwitnessed, nontraumatic and nondrowning death in patients with epilepsy, excluding cases of documented status epilepticus. Death may occur with or without evidence of seizures, but unexpectedly in patients in a reasonable state of health apart from epilepsy and, when performed, postmortem examination does not reveal a toxicologic or anatomic cause of death.<sup>8</sup>

### Key Points

- Preclinical models of SUDEP models are heterogeneous.
- Several models investigate seizure-induced death in animals that do not fulfill criteria for chronic epilepsy.
- The majority of SUDEP rodent models with spontaneous seizures are of a genetic basis.
- Contrary to what is observed in patients, cardiorespiratory arrest begins during the course of the seizure in almost all animal models.
- Some limitations of models are impossible to avoid and can be related to specific features of SUDEP.

Additionally, validity of the models can be strengthened by additional aspects that would reflect some of the most robust SUDEP risk factors reported in epidemiological studies,<sup>9</sup> and a major point of the SUDEP coalition summit basic science workgroup 2020 of SUDEP research leaders was that the need to align SUDEP models with clinical findings and to create a database of available models is of paramount importance. In contrast, an accurate evaluation of the model must not be solely based upon specific aspects related to the presumptive pathophysiology of SUDEP. For instance, respiratory arrest preceded cardiac arrest in all SUDEP observed in the MORTEMUS study,<sup>2</sup> yet it is essential not to draw hasty conclusions, as relatively few SUDEP cases have been precisely monitored with cardiorespiratory data and one cannot rule out that other sequences of events might occur in some SUDEPs. Accordingly, this sequence of events (i.e., fatal apnea preceding cardiac dysfunction) might not have to be mandatory a priori. Otherwise, one might fear circular reasoning, with conclusions about the cascade of events that might lead to SUDEP potentially biased by the definition of the model itself. Following are features we propose SUDEP models should have.

Preferred characteristics:

- Chronic epilepsy with spontaneous recurrent seizures
- Death observed after a recorded spontaneous seizure
- Fatal cardiorespiratory arrest induced by a seizure in the peri-ictal period
- Absence of progressive neurodevelopmental and/or neurological disease that can result in death independent of epilepsy
- Absence of chronic nonneurological pathology that may contribute to death, especially heart and/or lung structural abnormalities

Additional aspects:

- Clinical manifestations during the natural history of epilepsy including generalized convulsive seizures
- Spontaneous deaths are more likely to occur during or after generalized convulsive seizures
- The risk of fatal cardiorespiratory arrest follows a circadian rhythm, such as predominance during sleep or sleep transitions

It should be noted that ticking all these boxes in a single animal model is an almost insurmountable task and as such we should be aware of the limitations of the models and interpret data appropriately. Spontaneous seizures are commonly observed in SUDEP models of genetic origin, but their frequency can be low and, most importantly, the incidence of spontaneous SUDEP is overall low and age-dependent.<sup>10</sup> Although this might accurately model the incidence rate observed in patients, it creates difficulty in experimental design, such as the need for excessively large numbers of animals alongside continual long-lasting recording of electroencephalogram (EEG) and cardiorespiratory data to capture SUDEP events. As such, many studies use a combined approach of a genetic etiology alongside seizure induction protocols to elicit seizures. This reduces the validity of these models, as they do not incorporate the differentiating "unexpected" spontaneous characteristic of SUDEP. Furthermore, one can question whether these animals are experiencing status epilepticus and hence whether they are truly modeling the clinical condition. Nevertheless, these models have proven to be fundamental in uncovering pathological mechanisms leading to SUDEP and are more commonly used than those based solely on spontaneous seizures.

### 3 | RODENT MODELS OF SUDEP

The next sections, as well as [Table 1](#), provide an overview of the main rodent models used in the field of SUDEP, including brief methodology, advantages, and disadvantages. It should be noted that apart from the rat model with global knockout (KO) of *Kcnj16*,<sup>11</sup> all rodent SUDEP models are mouse models. This may be attributed to the complications of transgenic approaches in rats or possibly due to rats having a more robust cardiorespiratory system that is worth investigating. With mice and rats evolutionary similar, this highlights a possible limited translatability of using rodents for a condition afflicting humans, who are much more evolutionary distinct. Rodents share general respiratory, neurological, and cardiological characteristics with humans, yet

subtle differences are known.<sup>12,13</sup> Rodents are known to lack rare interneuron subtypes and certain species of astrocytes.<sup>14-16</sup> The largest differences are observed in the heart, with a much shorter cardiac action potential driven by varied expression and density of various cardiac potassium channels.<sup>17</sup> This needs to be considered in interpretations of results, as a more susceptible heart may account for the differences in clinical observations from rodent and human studies. Importantly, however, the brainstem, a critical brain area associated with SUDEP, is evolutionary highly conserved across vertebrates, with rodents described as excellent models for study of the brainstem.<sup>18</sup> As discussed above, a critical aspect is whether the animals have isolated seizures exclusively induced by a specific trigger or whether they suffer from epilepsy with spontaneous seizures, with or without spontaneous SUDEP. Accordingly, rodent models will be separated into two groups: (1) models of seizure-induced death when animals do not fulfill criteria for chronic epilepsy or (2) models of epilepsy-related death.

### 3.1 | Models of seizure-induced death

#### 3.1.1 | Models where seizures are elicited by electrical stimulation or pharmacological intervention

Several models of a single seizure induction by electrical or pharmacological stimulation have been used to investigate mechanisms of seizure-related death in non-epileptic animals. Overall, these models, with their ease of use and characterized mortality rate, can prove useful as a relatively high-throughput tool to test novel therapeutics or possibly identify biomarkers. For instance, maximal electroshock stimulation (MES) can be used to induce a SUDEP-like event in adult mice, with mortality up to 62.5%.<sup>19,20</sup> Tonic-clonic seizures commence with the electrical stimulation, and respiratory arrest occurs rapidly and coincides with death. Here, mice also have a greater susceptibility to death during sleep.<sup>21</sup> The rapid onset allows researchers to precisely time the SUDEP-like event and to test novel therapies, such as diaphragmatic pacing.<sup>19</sup> However, the model differs from SUDEP in that the brain is not epileptic, the seizures are not spontaneous, and the mechanisms of seizure generation are not natural.

Alternatively, kainic acid (KA) injection into the ventral hippocampus is associated with prolonged continuous seizures that >60 min, with pronounced autonomic activation and disruption of activity in medullary respiratory centers. Death results from either central apnea or obstructive apnea, the latter potentially favored by

**TABLE 1** Overview of animal models used for investigating SUDEP.

Model	Species; age of seizure onset	Description	Spontaneous seizures	Advantages	Disadvantages	Areas for future exploration	Ref
DBA/2	Mouse; >P21	Seizures induced via an audiogenic stimulus (90–110 db, 12 kHz, 60 s or until seizure initiation). Mice can be "primed" for 3 days to establish consistent susceptibility and SIRA. Can be combined with chemoconvulsants to initiate seizures and SIRA.	No	Well-characterized seizures and cardiorespiratory phenotype similar to human cases Mechanical ventilation is successful at rescuing mice Predictive validity of anticonvulsives effect of antiseizure drugs Mice more susceptible to SIRA during sleeping phase	Limited evidence of a human correlate in relation to SUDEP Relatively fragile strain, making surgery complicated Susceptible to hearing loss Lack of spontaneous mortality	N/A	25
DBA/1	Mouse; >P21	Seizures induced via an audiogenic stimulus (90–110 db, 12 kHz, 60 s or until seizure initiation). Mice can be "primed" for 3 days to establish consistent seizure susceptibility and 100% SIRA. Can be combined with chemoconvulsants to initiate seizures and SIRA.	No	Well-characterized seizure and cardiorespiratory phenotype similar to human cases Mechanical ventilation is successful at rescuing mice Longer timeframe of susceptibility than DBA/2 Predictive validity of anticonvulsives effect of antiseizure drugs Mice more susceptible to SIRA during sleeping phase	Limited evidence of a human correlate in relation to SUDEP Relatively fragile strain, making surgery complicated Susceptible to hearing loss Mice do not present with nonterminal seizures Lack of spontaneous mortality	N/A	29
<i>Scn1a</i> exon 26	Mouse; >P9 homozygous mice; >P19 heterozygous mice	Deletion of exon 26 of the <i>Scn1a</i> gene results in spontaneous seizures at P9 and premature death at P15 in homozygous mice, and spontaneous seizures and suspected SUDEP at > P19 in heterozygous mice. Susceptible to hyperthermia-induced seizures.	Yes	Good construct validity for Dravet syndrome Relatively high rate of seizures Well-characterized seizures and mortality Characterized cardiac parameters Disrupted circadian rhythms	N/A	Respiratory data needs characterizing	74
<i>Scn1a</i> R1407X	Mouse; >P19	Knockin of point mutation with 38% mortality rate by P60 in heterozygous mice. Seizures and SIRA can be induced via hyperthermia or MES.	Yes	Genetic construct validity Mice present with multiple spontaneous seizures before a fatal seizure Cardiorespiratory death obtained from SUDEP event in mice that is similar to human SUDEP Cardiac profile well characterized and similar to Dravet syndrome patient-derived cardiac myocytes Spreading depolarization observed Mechanical ventilation is successful at rescuing mice	N/A	N/A	75

TABLE 1 (Continued)

Model	Species; age of seizure onset	Description	Spontaneous seizures	Advantages	Disadvantages	Areas for future exploration	Ref
<i>Scn1a</i> R1648H	Mouse; >P16 homozygous mice; >P19 heterozygous mice	Knockin of missense mutation into exon 26 results in a lifespan in homozygous mice of 16–26 days. Heterozygous mice have a rare incidence of recorded spontaneous seizures. Heterozygous mice are susceptible to induced seizures and then develop spontaneous recurrent seizures.	Yes	Genetic construct validity Confirmed predictive validity with ASDs With flurothyl induction similar seizures induced in both WT and mutants, allowing for investigation of mutation irrespective of seizures Well-characterized seizure profile and subsequent later life behavioral comorbidities Circadian rhythms well characterized	No spontaneous SUDEP in heterozygous mice	Cardiorespiratory and seizure recording in homozygous mice to capture the SUDEP event would be beneficial	89
<i>Scn1a</i> E1099X	Mouse; >P12	Knockin point mutation in homozygous mice results in spontaneous seizures at P12 and 100% mortality by P17. Heterozygous mice have spontaneous seizures at 3–4 weeks and up to 60% mortality by 5 weeks. Seizures can be hyperthermia-induced.	Yes	Genetic construct validity Predictive validity, as ASDs reduce seizures	N/A	Cardiac and respiratory data need exploring Further characterization of seizure pathology needed	93
<i>Scn1a</i> exon 1 (Scn1atmKea)	Mouse; >P21	Targeted deletion of exon1 of <i>Scn1a</i> gene. Multiple daily spontaneous seizures during 3rd postnatal week and 50% mortality by the 4th week in mice heterozygous for mutation.	Yes	Genetic construct validity Relatively high rate of seizures Well-characterized seizures and mortality	N/A	Cardiac and respiratory data need characterizing	97
<i>Scn1a</i> A1783V	Mouse; >P12 homozygous mice; >P19 heterozygous mice	Conditional knockin of missense mutation into GABAergic neurons. Mortality in homozygous mice observed at P14 and 100% by P23 following seizures. Heterozygous mice have 70% mortality by 8 weeks. Susceptibility to hyperthermia seizures at >P12.	Yes	Genetic construct validity Characterized respiratory data Behavioral phenotype well characterized Brainstem neuronal dynamics characterized	N/A	Cardiac data need characterizing Capturing respiratory data around the SUDEP event would be beneficial	92
<i>Scn1a</i> R613X	Mouse; >P14 homozygous mice; >P19 heterozygous mice	Point mutation in homozygous mice results in 100% mortality by P16, and heterozygous mice having 60% mortality by P60. Heterozygous mice susceptible to heat-induced seizures.	Yes	Genetic construct validity Relatively high rate of mortality for ease of studies	N/A	As a novel model, seizure and cardiorespiratory data currently not well characterized Capturing electroencephalogram data of the fatal event would be beneficial	95

(Continues)

TABLE 1 (Continued)

Model	Species; age of seizure onset	Description	Spontaneous seizures	Advantages	Disadvantages	Areas for future exploration	Ref
<i>Scn8a</i> NI 768D	Mouse; >P18	Gain-of-function missense mutation results in single terminal seizure in homozygous mice at approximately P18. Multiple seizures per day in heterozygous mice commencing at 8 weeks in 50%. Mortality occurs 1 month later. Single copy of mutated allele and WT allele KO creates a severe phenotype of 25 seizures per day. Seizures can be induced via electrical stimulation or with an audiogenic stimulus.	Yes	Genetic construct validity Multiple daily seizures Characterized electrophysiological and cardiorespiratory data Mechanical respiration can rescue animals from SUDEP	Relatively long timeframe of possible SUDEP occurrence	N/A	99
<i>Kcna1</i> -null mice	Mouse; >P14	Targeted deletion of <i>Kcna1</i> gene reading frame on chromosome 6. In homozygous mice, KO results in frequent seizures (20 per day) that commence at P14–P21. Mortality follows seizures, with a mean survival of P42.8. Can be combined with inducible seizures in heterozygous mice.	Yes	Well-characterized lifespan allowing for studies monitoring end points Extensively characterized cardiac phenotype following seizures that mimics the human condition (AV conduction blocks, bradycardia, and asystole) with absence of structural heart defects Early onset seizures Spreading depolarization observed	Relatively few data on a link of <i>Kcna1</i> mutation with SUDEP in patients	Further characterization of respiratory data is needed	104
<i>Ryr2</i> –R176Q	Mouse; P18–P25	Mice heterozygous for the missense mutation and seizures induced with topical cortical application of 4-AP, leads to 71% mortality (not observed in WT mice).	Yes	Mutation of <i>Ryr2</i> associated with SUDEP Spreading depolarization well characterized correlating with human condition Cardiac response to seizures characterized	Rare spontaneous seizures in absence of 4-AP	Characterization of respiratory data is needed	54
CACNA1A-S218L	Mouse; adult	Knockin of missense mutation. In homozygous mice results in spontaneous seizures and SUDEP. SUDEP-like event induced with cortical stimulation.	Yes	Shared cardiorespiratory sequence of SUDEP and lung edema observed postmortem Well characterized spreading depolarization	High mortality rate after surgery Spontaneous mortality occurs mainly in adulthood, adding difficulty to study	Further exploration of spontaneous seizure pathology is needed	115
Maximal electroshock seizures	Mouse; C57BL/6 10 weeks (adult)	Single electroshock stimulation delivered to mice via ear clip electrodes (50 mA, 2 s, 60 Hz), induces GTCs with mortality up to 62.5%. Is normally used in combination with genetic models to induce seizures.	No	Timing of respiratory arrest can be precisely controlled and examined by the experimenter Good predictive validity, as ASDs counter seizures	Lack of construct validity Respiratory arrest commences immediately after stimulation, in contrast to the human condition	N/A	19
<i>Lmx1b</i> <sup>fl/fl</sup>	Mouse; adult	Conditional KO of serotonergic neurons across the brain. Seizures induced via pilocarpine or MES and SIRA up to 100%, dependent on severity of stimulus.	No	Well-characterized respiratory data under baseline and during seizures Confirmed PGES	Limited construct validity Lack of spontaneous seizures and mortality	Further characterization of cardiac data is needed	40

TABLE 1 (Continued)

Model	Species; age of seizure onset	Description	Spontaneous seizures	Advantages	Disadvantages	Areas for future exploration	Ref
Adenosine kinase knockdown mice	Mouse; adult	I.H. kainic acid to induce chronic epilepsy and subsequent I.P. kainic acid to induce SUDEP. Mortality 8% in WT, 34% in mice with heterozygous KO of adenosine kinase.	Yes	Chronic epilepsy phenotype Delayed postictal death	Poor construct validity Lack of spontaneous mortality	Further characterization of cardiorespiratory data is needed	65
Epileptic baboon	Captive mixed strain baboon colony	Spontaneous seizures in 26% of animals. Epileptic baboons have half the lifespan (5.6 years) of nonepileptic baboons.	Yes	Greatest translatability to humans Extensive cardiorespiratory and neuroimaging data from interictal periods that correlate well with human patients	Ethical considerations Only available at one facility in Texas Studies can take years and difficulty in recording data around the spontaneous SUDEP event	N/A	119
<i>Drosophila para</i>	<i>Drosophila melanogaster</i>	KO of <i>para</i> gene or knockin of human <i>scn1a</i> mutations present with seizures. Reduced lifespan. Seizures also elicited via submersion in water bath (40°C).	Yes	Well-characterized and shared electrophysiological properties with mouse models and humans Low-cost high-throughput screening tool	Vastly different interspecies genetics, neurobiology, and cardiorespiratory physiology Uncertainty around the translatability of the <i>para</i> gene to the human <i>SCN1A</i> gene	N/A	124

Abbreviations: 4-AP, 4-aminopyridine; ASD, antiseizure drug; AV, atrioventricular; GABAergic,  $\gamma$ -aminobutyric acidergic; GTCS, generalized tonic-clonic seizures; I.H., intrahippocampal; I.P., intraperitoneal; KO, knockout; MES, maximal electroshock; N/A, not applicable; P, postnatal day; PGES, generalized postictal electroencephalographic background suppression; Ref, reference; RyR2, ryanodine receptor 2; SIRA, seizure-induced respiratory arrest; SUDEP, sudden and unexpected death in epilepsy; WT, wild type.

acid reflux-induced laryngospasm.<sup>22,23</sup> Although this model has great ease of use, it models death due to status epilepticus in which there are massive derangements of metabolism, cellular energetics, ion homeostasis, and systemic physiology that develop after prolonged seizures.<sup>24</sup> In contrast, SUDEP occurs after short seizures that by themselves would not be expected to be fatal. Neither MES nor KA injection would translate well to clinical SUDEP.

### 3.1.2 | DBA mouse strains with audiogenically induced seizures

The DBA mouse strains are one of the most utilized models for SUDEP studies. These mouse strains, both DBA/1 and DBA/2, undergo audiogenically induced seizures. The protocol of induced seizures in DBA mice has not changed significantly since its inception<sup>25</sup>: a mouse (~postnatal day 21 [P21]) is placed into an enclosed arena and exposed to a loud noise (90–122 db) for 60 s maximum or until the seizure occurs. Immediately after the sound presentation, the mouse presents with stereotyped behaviors progressing to tonic-clonic seizures, then extension of the limbs toward the tail, in many cases succumbing to death.<sup>26</sup>

The DBA/2 strain was predominantly used in SUDEP studies, as a single audiogenic stimulus elicited reliable seizures in the majority of animals, with mortality following the first seizure being approximately 60%–75%.<sup>27,28</sup> However, the susceptibility to seizures and seizure-induced death decreases with age, with consistent induction lost at >P28. The DBA/1 mouse was initially less explored, due to a low number of mice (10%–30%) presenting with audiogenic seizures compared with DBA/2. DBA/1 mice do, however, have a reliable 100% mortality following a seizure.<sup>27,29</sup> Both strains have a well-characterized cardiorespiratory sequence of death, with a seizure-induced respiratory arrest (SIRA) always commencing during the seizure and terminal asystole following shortly after.<sup>26,28–30</sup> The use of the DBA/1 mice increased dramatically following the finding by Faingold et al.<sup>29</sup> that DBA/1 mice become more susceptible to seizures and subsequent respiratory arrest after a priming protocol. Here, an audiogenic stimulus on consecutive days acts as a kind of kindling, whereby on the 3rd day 100% of mice are susceptible to SIRA. Mice that present with SIRA have to be resuscitated via a ventilator (within 5 s) to continue with priming.<sup>28,29</sup>

Certain disadvantages of DBA models exist. Neither strain shows spontaneous seizures, and there is no human correlate of audiogenic seizures. The anatomical localization of audiogenic seizures in DBA mice is also different from human epilepsy, as the former is thought to originate



in brainstem structures,<sup>31</sup> and the data collected may not be relevant to human SUDEP. This strain is notorious for its fragile nature, making surgical procedures less viable,<sup>27,32</sup> while also being susceptible to hearing loss,<sup>27,33</sup> reducing likelihood of seizure induction and adding a potentially undiagnosed conflicting variable. Additionally, as fatal apnea in DBA mice is observed during the tonic phase of the seizure, any evaluation of novel pharmacological interventions affecting SIRA risk should also consider that a potential effect on seizure severity may ultimately be responsible. Although several antiseizure medications can reduce audiogenic seizures, adding validity to the models,<sup>34</sup> other drugs, including selective serotonin reuptake inhibitors, demonstrate a significant effect on seizure severity and SIRA in DBA mice.<sup>28,35</sup> Many studies have outlined an inherent dysfunction of the serotonergic system in DBA mice that might predispose mice to peri-ictal respiratory arrest.<sup>36,37</sup> Although this is observed in SUDEP cases and has suggested the possibility that serotonergically acting drugs can target SUDEP, the latter have no validated effect in epilepsy patients,<sup>38</sup> other than a small decrease in O<sub>2</sub> saturation.<sup>39</sup> To summarize, audiogenic seizures induced in DBA mice allow the experimenter to precisely time and reliably observe SIRA. This has led to widespread use in the field of this model. However, we should be cautious about its translatability to the human condition for the reasons described above.

### 3.1.3 | Combination of genetic susceptibility to epilepsy with seizure induction

As discussed below, electrical or pharmacological stimulation can also be used in several models of chronic epilepsy, either to increase the risk of death or to precisely time the SUDEP-like event. This approach can also be used in genetic models that are not associated with spontaneous seizures, but whose neurobiological alterations could participate in the pathophysiology of SUDEP.

*Lmx1b*<sup>f/f/p</sup> mice were generated using Cre recombinase technology to conditionally knock out LIM homeobox transcription factor 1  $\beta$  (*Lmx1b*) from serotonergic (*Pet1*-positive) neurons, resulting in an absence of serotonergic neurons across the brain.<sup>40</sup> Surprisingly, mice survive to adulthood, appear healthy, and do not show spontaneous seizures or recorded epileptiform activity.<sup>41,42</sup> However, they show respiratory abnormalities, a hypercapnic ventilatory response decreased by 50%, and a lack of CO<sub>2</sub>-mediated arousal from sleep.<sup>43,44</sup> Induction of seizures with multiple pilocarpine injections or MES at 50 mA is fatal in all male mice, whereas lower stimulus intensities result in approximately 40% survival, allowing for various experimental designs.<sup>20,42</sup> Respiratory arrest in these mice

occurs with the fatal seizure, with postictal generalized EEG suppression (PGES) without recovery and cardiac activity continuing for approximately 9 min.<sup>42</sup> However, one should enforce a high degree of caution in interpreting these data, as this model does not fulfill many of the outlined SUDEP criteria, especially an epileptic phenotype. In addition, although studies from SUDEP patients and various animal models demonstrate a reduction in serotonergic tone,<sup>45,46</sup> a complete lack of serotonergic neurons is not representative of patients. However, this model, which might implement an exaggeration of SUDEP pathophysiology, has helped understanding of the role of serotonin in SUDEP.

Similarly, spontaneous seizures are rare in a mouse model with abnormal ryanodine receptor 2 (RyR2) protein expression, yet RyR2 mutations have been associated with SUDEP.<sup>47,48</sup> The RyR2 mainly acts to regulate intracellular calcium and neurotransmitter release, and it is ubiquitously expressed, particularly in the heart and brain.<sup>49,50</sup> In mice with the R176Q RyR2 mutation, topical cortical administration of the potassium channel blocker 4-aminopyridine (4-AP) induces seizure-related death within 2 h. 4-AP administration was associated with spreading depolarization in the dorsal medulla, subsequent cardiac arrhythmias and agonal breathing, and finally cardiac arrest 5 min later.<sup>51,52</sup> According to these data collected in RyR2 mutated mice, alongside *CACNA1A*<sup>53</sup> and *Kcna1*<sup>54</sup> mutated mice described later in this review, it has been suggested that spreading depolarization in the brainstem might play a specific role in postictal respiratory dysfunction and SUDEP,<sup>53,54</sup> in line with the relation between PGES and hypoxemia observed in patients<sup>55,56</sup> and with the relation between PGES and long-term risk of SUDEP,<sup>57</sup> although this specific association remains a matter of debate.<sup>9</sup>

These models again provide a reliable induction of a SUDEP-like event for ease of experimental design and have helped to shed light on certain pathogenic mechanisms of SUDEP. However, the translatability of these models is limited to modeling specific aspects of SUDEP pathophysiology.

## 3.2 | Rodent models of chronic epilepsy with spontaneous seizures

### 3.2.1 | Models of nongenetic epilepsy etiology

Only a limited number of SUDEP studies have used models of nongenetic epilepsy. This probably reflects the lack of spontaneous mortality in the chronic phase of most rodent models of structural epilepsy, including amygdala

kindling, intrahippocampal injection of KA, and intraperitoneal pilocarpine or KA.<sup>58</sup> Despite that models of post-status epilepticus epilepsy present with spontaneous focal seizures that can evolve to generalized clonic, tonic, or tonic-clonic seizures (seizure types generally associated with seizure-related mortality), death is minimal or not reported, especially in rats.<sup>59</sup> The reasons underlying this are unknown, and perhaps it is just inherent in ethical and efficient experimental animal model designs that limit mortality as much as possible. Alternatively, these methods are usually used in rats, and as discussed above, one cannot exclude that the robustness of the respiratory network might differ in mice and rats, making the latter more resistant to seizure-related death.

Shen et al. in 2022<sup>65</sup> generated a novel mouse model that might elegantly overcome this intrinsic limitation of rodent models of structural epilepsy. Their previous work formed the basis of the adenosine hypothesis of SUDEP,<sup>60</sup> where they pharmacologically inhibited adenosine clearance via adenosine kinase (ADK) in acute seizures. Despite a potent anticonvulsive effect, seizure-related mortality was elevated. As reviewed recently in detail,<sup>4</sup> adenosine signaling may facilitate sudden respiratory collapse in patients with epilepsy. Extracellular adenosine results from dephosphorylation of adenosine triphosphate (ATP), adenosine diphosphate, and adenosine monophosphate, with levels varying in respect to ATP consumption,<sup>61</sup> with a large transient increase induced by seizure.<sup>62</sup> As activation of the adenosine receptor A1 decreased neuronal excitability,<sup>61</sup> this seizure-induced surge in extracellular adenosine could contribute to seizure termination. However, adenosine is also implicated in the regulation of several functions, including central respiratory control; adenosine signaling inhibits both respiration rhythm generation and chemosensitive neurons.<sup>4</sup> The nonspecific adenosine receptor antagonist caffeine can thus lower the risk of seizure-induced respiratory dysfunction both in patients<sup>63</sup> and in animal models.<sup>64</sup> Expanding on the adenosine hypothesis, Shen et al. recently validated a two-hit approach, with a single intrahippocampal KA dose establishing chronic seizures in wild-type and ADK genetic knockdown adult mice, with no spontaneous death observed 6–8 weeks later, and then a subsequent low dose of intraperitoneal KA triggering either death immediately following induced seizures or a delayed postictal death approximately 13 min after the last seizure. Interestingly, this delayed postictal death might fulfill criteria for SUDEP, adding greater validity to the model, with a mortality rate of 8% in wild-type mice and of 34% in ADK knockdown mice<sup>65</sup>; however, cardiorespiratory parameters are yet to be investigated, so it cannot be ruled out that prolonged low-level cardiorespiratory dysfunction may begin immediately after the seizure ends and lead to death later. This

two-hit SUDEP model fulfills many criteria needed for valid SUDEP research, yet caution is required, as adding a conflicting specific variable limits interpretation of results, similar to that of *Lmx1b<sup>f/f/p</sup>* mice, and SUDEP is not spontaneous, instead requiring induction by KA injection. Nevertheless, these studies have helped in further dissecting the adenosine hypothesis of SUDEP, which closely matches some clinical features of SUDEP.

### 3.2.2 | Genetically based developmental and epileptic encephalopathies

Developmental and epileptic encephalopathies (DEEs) are a heterogeneous group of neurological diseases often caused by genetic mutations; they are characterized by severe early onset epileptic seizures, developmental impairment, motor/gait abnormalities, and high risk of SUDEP,<sup>66</sup> features that are reproduced by gene-targeted mouse models.<sup>67</sup> Here, we will focus on those that have been studied in more detail regarding SUDEP features.

#### 3.2.2.1 | Models of mutated voltage-gated sodium channels

- *Mutation of Scn1a* gene and models of Dravet syndrome

Dravet syndrome (DS) is a rare genetic epileptic encephalopathy that usually manifests in the first year of life. Approximately 5%–10% of SUDEP cases were diagnosed with DS, making them the cohort of epilepsy patients at highest risk for SUDEP.<sup>68,69</sup> Loss-of-function mutations in the gene *Scn1a*, encoding the Na<sub>v</sub>1.1 voltage-gated sodium channel, are found in >80% of DS patients, with >1000 different mutations currently known.<sup>70</sup> The Na<sub>v</sub>1.1 voltage-gated sodium channel is mainly expressed in  $\gamma$ -aminobutyric acidergic (GABAergic) interneurons in the brain, with mutations impairing these sodium currents and thus action potential firing causing an imbalance of excitation/inhibition neurotransmission that can lead to seizures.<sup>71–74</sup>

Studies of homozygous *Scn1a* KO mice have shown seizures starting at P9 and premature death by P15, with no gross abnormalities on postmortem examination.<sup>74</sup> Heterozygous *Scn1a*-KO mice present with spontaneous seizures after P21, and 40% of them experience premature death between the 3rd and 4th postnatal week.<sup>74,75</sup> These mice have well-characterized cardiac dysfunction, with reduced heart-rate variability and frequent episodes of atrioventricular (AV) block in interictal periods, and present with bradycardia immediately preceding a SUDEP event,<sup>76,77</sup> with respiratory data relatively unexplored. These heterozygous *Scn1a* KO mouse models have been further characterized in relation to SUDEP, susceptibility to hyperthermia-induced seizures, age-dependent severity

of spontaneous seizures, sleep and circadian rhythm dysregulation, and premature deaths following seizures, which are frequently observed.<sup>76,78–80</sup> Rates of premature death can vary; survival rate ranges from 42% to 67% between studies, possibly due to various background strains being utilized.<sup>76,81</sup> This is not restricted to *Scn1a* KO mice, in which distinct background strains are well known to have vastly different mortality rates and pathological consequences in epilepsy studies, and should be taken into consideration in interpreting data across studies.<sup>82</sup> Age and rate of mortality can be increased with specific deletion of *Scn1a* in forebrain interneurons.<sup>77</sup> The severe phenotype induced by the complete KO of this gene in homozygous mice is less relatable to patients, who are heterozygotes.

Heterozygous mice carrying the R1407X *Scn1a* truncating mutation have been used for in-depth SUDEP studies. They show recurrent spontaneous seizures commencing from P16 and mortality rate of 38% by P60 always following a tonic hindlimb extension seizure.<sup>10</sup> Respiration was normal at baseline, but sudden terminal apnea preceded cardiac arrest and flat EEG was observed with the fatal seizure,<sup>10</sup> with death occurring predominately at night.<sup>83</sup> Bradycardia and arrhythmias were also observed leading up to the terminal seizure.<sup>84</sup> Interestingly, cardiac myocytes obtained from these mice demonstrate similar arrhythmic properties to those from DS patient-derived induced pluripotent stem cell cardiac myocytes with similar *Scn1a* mutations, such as paradoxical increased myocyte sodium current perhaps due to an overcompensation of other sodium channels.<sup>84,85</sup> Apart from the timing of the fatal apnea onset, which occurred during the course of the seizure and not in the postictal period, the cardiorespiratory sequence in this model was close to the one observed in the MORTEMUS study.<sup>2</sup> The difficulties of SUDEP investigations in this model were highlighted in the study by Kim et al.,<sup>10</sup> in which only two SUDEP events were observed during 4680 h of concurrent video-EEG and cardiorespiratory recording. Although the low incidence of a fatal event resembles human SUDEP, it does impede the ability to obtain experimental data. However, capturing one SUDEP-like event is likely if three mice are monitored for 1 month. Alternatively, when seizures were induced via hyperthermia, MES, or 4-AP, similar cardiorespiratory features were observed at the time of death as with spontaneous events, alongside an increased susceptibility to spreading depolarization.<sup>10,54</sup> It can be argued that fatal seizures induced by hyperthermia replicate the human condition, as SUDEP occurs in some DS cases due to hyperthermia.<sup>86</sup>

Approximately 40% of DS patients carry missense mutations, and more recent studies have generated

preclinical models of missense *Scn1a* mutations. One of the first *Scn1a* mutations identified, the missense mutation R1648H,<sup>87</sup> was associated with genetic epilepsy with febrile seizure plus, which shares a common pathophysiology with DS. Recordings of sodium currents in neurons dissociated from transgenic mice expressing R1648H showed that the mutation causes loss of function because of modifications of gating properties of Na<sub>v</sub>1.1.<sup>88,89</sup> Subsequently, knockin R1648H mice have been generated and recordings from their dissociated cortical neurons and brain slices showed similar loss-of-function effects on sodium currents in GABAergic neurons, leading to a selective hypoexcitability of GABAergic neurons and widespread reduced GABAergic inhibition and network hyperexcitability in numerous brain areas.<sup>71</sup> Homozygous knockin R1648H mice show spontaneous seizures occurring from P16 and a well-characterized shortened lifespan of 18 days on average.<sup>89</sup> Cardiorespiratory parameters have not been investigated yet, possibly due to the difficulty of recording in very young animals. Heterozygous mice exhibit rare spontaneous seizures, and their lifespan is unaffected; as such, heterozygous mice are commonly used with induced seizure methods, generally for studies investigating seizure dynamics rather than SUDEP.<sup>89,90</sup> However, if short seizures are induced on 10 consecutive days in heterozygous R1648H mice, with either hyperthermia or flurothyl, the phenotype becomes DS-like and seizure-induced mortality rate reaches approximately 30%.<sup>91</sup>

Several other mouse models carrying a human DS mutation of the *Scn1a* gene have also been generated, including A1783V *Scn1a* missense mutation conditionally expressed in GABAergic neurons,<sup>92</sup> the E1099X *Scn1a* truncating mutation,<sup>93</sup> the K1270T missense mutation,<sup>94</sup> the R613X truncating mutation,<sup>95</sup> and targeted deletion of *Scn1a* exon 1 (*Scn1a*<sup>tm1Kea</sup> mice).<sup>96,97</sup> As for other *Scn1a* models, these mice show spontaneous seizures and a mortality rate of >30% after P30. However, to date, cardiorespiratory data have not been examined in these models.

*Scn1a*-deficient models align well with many criteria needed in SUDEP models, particularly an etiology of SUDEP similar to that of patients, well-characterized mortality rates, spontaneous seizures, and for ease of use, seizures can also be induced with a stimulus, hyperthermia, known to induce seizures in patients. Although shared cardiorespiratory features of death with patients have only been demonstrated in R1407X mice, we can assume this is shared across loss-of-function *Scn1a* models.

- Mutations of the *Scn8a* gene

The *Scn8a* gene encodes the Na<sub>v</sub>1.6 sodium channel, which is expressed at high levels in glutamatergic neurons. Gain-of-function mutations of *Scn8a* cause a DEE

and high incidence of SUDEP ( $\approx 5\%$ ),<sup>98</sup> due to the overactivity of Nav1.6, leading to hyperexcitability of glutamatergic neurons.<sup>73</sup> Homozygous mice carrying the *Scn8a* missense mutation N1768D appear normal until approximately P18, when motor deficits appear and are shortly followed by a single terminal seizure in all animals.<sup>99</sup> Approximately 50% of heterozygous mice show tonic-clonic seizures commencing at 8 weeks with 100% mortality within 1 month. These mice have no gross brain malformations, baseline bradycardia and cardiac arrhythmias, ictal bradycardia, postictal tachycardia, and apnea in the tonic phase of seizures.<sup>99–102</sup> Isolated myocytes from N1768D mice exhibit abnormal firing properties resulting in lower contraction rates, presumably driven by hyperexcitable parasympathetic innervation.<sup>100</sup> Mutant mice can be rescued by mechanical respiration.<sup>103</sup> Audiogenically induced seizures in heterozygous mice at P20–P21 or via electrical hippocampal stimulation at 8 weeks result in similar seizure semiology and respiratory sequence leading to death as with spontaneous seizures, but with an age-dependent more elevated mortality rate (approximately 85%).<sup>102,103</sup> This mouse model has validity according to the defined criteria, yet caution may be needed in interpretation of results in relation to the global patient population at risk of SUDEP, as *Scn8a* mutations represent only a small subset of SUDEP patients.

### 3.2.2.2 | *Kcna1*-null mice

The *Kcna1* gene encodes the alpha subunit of the  $K_v1.1$  channel that encodes the delayed rectifier potassium current, which plays an important role in several physiological functions, including action potential shaping, neurotransmitter release, synaptic integration, and network oscillatory behavior. Human mutations of *Kcna1* have been associated with episodic ataxia type 1 as well as DEEs.<sup>67</sup> However, there has only been one reported case of a patient with a *Kcna1* mutation succumbing to SUDEP.<sup>48</sup>

*Kcna1*-null mice are a popular SUDEP model. Mice do not show embryonic lethality and appear healthy until the 3rd postnatal week. However, by this time, they develop spontaneous recurrent seizures ( $\approx 20$  per day), and 50% of mice succumb to SUDEP by the 5th week of life.<sup>104</sup> Terminal apnea occurs during the fatal seizure, followed by cessation of brain activity, and terminal asystole approximately 2 min later.<sup>105</sup> These mice also present with basal respiratory dysfunction such as hyperventilation, ataxic breathing, hypopnea, bradypnea, and apnea, which increases in severity with age.<sup>106,107</sup> In ictal periods, the majority of *Kcna1* null mice experience bradycardia and AV conduction block, as seen in a subset of approximately 21% of epilepsy patients.<sup>105,108</sup> Loss of the  $K_v1.1$  protein also increases the mice's susceptibility to spreading depolarization

in the brainstem with cessation of respiration and death,<sup>54</sup> potentially modeling the relation between postictal respiratory dysfunction and postictal EEG suppression observed in patients.<sup>56,109</sup> Additionally, *Kcna1*-null mice have disrupted circadian rhythm and sleep architecture.<sup>110</sup> However, in this model SUDEP generally occurs during the active period in these mice,<sup>111</sup> as with most murine SUDEP models yet in contrast to patients. The validity of this model is reinforced by the observation that clinically used anticonvulsants are effective in the majority of mice, with some remaining refractory.<sup>110</sup> As such, *Kcna1*-null mice have many desirable qualities for SUDEP studies, resulting in their popularity. However, *Kcna1* KO mice may not truly represent the global SUDEP population, with limited evidence of this mutation expressed in SUDEP cases.<sup>112</sup>

### 3.2.2.3 | *CACNA1A* mutation models

Mutations in the *CACNA1A* gene, which encodes the alpha-1A subunit of the  $Ca_v2.1$  channel responsible for high-voltage-activated P/Q type  $Ca^{2+}$  currents, cause enhanced excitatory neurotransmitter release and are associated with epilepsy and SUDEP.<sup>113,114</sup> Mice carrying the missense mutation S218L, primarily used to model familial hemiplegic migraine type 1, demonstrate spontaneous seizures and elevated mortality in adulthood.<sup>53,115,116</sup> Lung edema is found in postmortem examinations of early mortality cases. Behavioral seizures immediately precede death, and video inspection revealed respiratory movements indicative of gasps following seizures.<sup>53</sup> Seizures also induced apnea, bradypnea, brainstem hypoxia, postictal increase of heart rate variability, and skipped heart beats; resuscitation can prevent death.<sup>53,117</sup> An enhanced predisposition to brainstem spreading depolarization has been well characterized and has been proposed as responsible for seizure-related mortality.<sup>53,117,118</sup> In fatal seizures, spreading depolarization was always observed, with bradycardia preceding respiratory arrest and finally cardiac arrest. These mice helped identify the pathophysiology of brainstem spreading depolarization in SUDEP. However, their use is perhaps limited by the observation that surgery itself increases the likelihood of SUDEP-like events in these mice.<sup>53</sup>

## 4 | NONRODENT MODELS

Few nonrodent SUDEP models have been reported, and they may add weight to current findings.

The epileptic baboon pedigree at the Southwest National Primate Research facility in Texas is likely the most translational model of SUDEP. Approximately 26% of baboons become epileptic, with well-characterized EEG-confirmed spontaneous myoclonic, absence, and generalized tonic-clonic seizures, particularly nocturnally and

upon awakening.<sup>119</sup> Many baboons are found dead in their habitats and have half the lifespan (5.6 years) of nonepileptic baboons.<sup>120</sup> However, an accurate SUDEP rate is difficult to determine, as many baboons are euthanized once epileptic.<sup>120</sup> SUDEP animals present with potential cardiac biomarkers at postmortem analysis, as well as pulmonary edema, aligning with humans.<sup>120,121</sup> Whole genome sequencing identified a genetic etiology of RBFOX1, which regulates splicing of many epilepsy candidate genes and is found in epilepsy patients.<sup>122,123</sup> Finally, SUDEP animals were found to have no underlying health issues, aside from epilepsy.<sup>120</sup>

These epileptic baboons possess the greatest translational ability to human epilepsy patients; however, they exhibit the main drawback of clinical SUDEP studies: difficulty in recording the SUDEP event due to the relatively long lifespan of animals. This explains the lack of characterization of respiratory parameters, because continuous long-term recording of respiration is difficult. Second, ethical issues arise using nonhuman primates, limiting more in-depth and invasive investigation of SUDEP. Nevertheless, with an elevated epilepsy and SUDEP rate in a shorter timeframe than humans, this baboon population could be very useful in identifying novel biomarkers and testing possible seizure-detecting devices or novel noninvasive treatments in the future.

On the other hand, *Drosophila* models carrying KO or mutations of paralytic (or *para*), their sole voltage-gated sodium channel, can present spontaneous seizures ( $\approx 20\%$  of animals) and related mortality in a period of weeks rather than years.<sup>124–126</sup> Although human DS mutations have been incorporated into the *para* gene,<sup>124,126,127</sup> certainly adding validity to these studies, we must question similarities of the *para* gene to the human *Scn1a* gene. The *para* gene is a representation of all mammalian voltage-gated sodium channels that have various roles in the brain,<sup>125</sup> the *para* gene is three times smaller than *Scn1a* gene, and also the *para* gene is known to be spliced into 60 isoforms, which all can impede interpretation of data.<sup>128</sup> Despite this, sodium currents in mutant animals show a similar reduction compared to rodent models of DS.<sup>124–126</sup> *Drosophila* can be manipulated easily, presenting a low-cost, high-throughput method to screen novel compounds to target SUDEP and epilepsy. However, the differences in the genetics, neurophysiology, and cardiorespiratory system of insects may strongly limit the translational value of these models.

## 5 | CONCLUSIONS

Despite models of SUDEP being available for >20 years, they are currently underutilized in the testing of novel

therapeutics. This perhaps reflects a current focus on characterizing and refining our models to gain greater understanding of SUDEP pathophysiology. The use of animal models has helped to elaborate meaningful hypotheses about the anatomical and molecular basis of SUDEP, including the serotonergic and/or adenosinergic hypotheses, and also helped develop our understanding of pathogenic mechanisms such as spreading depolarization and PGES. Generally, the majority of the above animal models present with many common hallmarks of SUDEP pathophysiology and a shared cardiorespiratory sequence of death.

Despite these successes, these preclinical models have several limitations. First, the majority of models with spontaneous seizures are of a genetic basis, and in many cases fatal seizures can be rare. This complicates investigation of seizure-related mortality, with large animal numbers required. Some models present with seizures and mortality mainly at early age, when experiments are more difficult. As such, researchers have focused on induced-seizure models, perhaps limiting the translatability of data to patients. Second, there are a plethora of genetic models of SUDEP that target a specific facet of SUDEP, whether this be  $Na_v1.1$  or  $K_v1.1$  channels. However, SUDEP has a vastly heterogeneous genetic makeup, with mutations that are not generally conserved across all SUDEP patients. Furthermore, SUDEP cases with a genetic basis only comprise a small proportion of SUDEP patients. Hence, results may not be translatable to many patients at risk of SUDEP. With the relative ease of genetic engineering, there has been a recent proliferation of genetic models; however, in many cases their SUDEP parameters, aside from seizures and reduced lifespans, have not been well characterized. Finally, in almost all models, when recorded, death begins during the course of the seizure, especially during the tonic phase, and not in the postictal period, contrary to that observed in SUDEP patients. Although this might be due to differences in the characteristics of the respiratory and cardiac network between mice and humans, which are unable to reproduce identical temporal features, it still raises the question of the interpretation of the observed sequences; are we studying models of SUDEP or models of periseizure death, potentially closer to cardiorespiratory arrest during status epilepticus? In the perspective of developing interventions to prevent SUDEP, this open question might be critical.

Some of these limitations are, however, impossible to avoid and can in part be dependent on specific features of SUDEP, which may be difficult to model. We do have many available preclinical tools to investigate certain gaps in our understanding of SUDEP pathophysiology; however, it is necessary to work in tandem with clinical studies to realize the full picture of SUDEP. To accelerate the development of SUDEP therapeutics from bench to the clinic, it is imperative to fully validate the models.

## AUTHOR CONTRIBUTIONS

Jonathon Smith and Sylvain Rheims designed the study and wrote the first draft. All authors contributed to the final version of the manuscript.

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