

RESEARCH ARTICLE

Effect of epileptogenesis on hypercapnic cardioventilatory response in kainic acid rats

Auriane Apaire^{1,2}  | Elise Collard¹  | Abigail Niyibizi¹  | Natasha N. Kumar³  | Enrique Germany Morrison^{1,2}  | Javier Chavez Cerda⁴  | Ayse S. Dereli¹  | Riem El Tahry^{1,2,5} 

¹Clinical Neuroscience, Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium

²Walloon Excellence in Life Sciences and Biotechnology Department, Walloon Excellence in Life Research Institute, Wavre, Belgium

³Department of Pharmacology, School of Biomedical Sciences, University of New South Wales, Sydney, Australia

⁴Systems & Cognitive Neuroscience, Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium

⁵Department of Neurology, Center for Refractory Epilepsy, Cliniques Universitaires Saint-Luc, Brussels, Belgium

Correspondence

Auriane Apaire, Ayse S. Dereli, and Riem El Tahry, Université Catholique de Louvain, Institute of Neuroscience, Clinical Neuroscience, Brussels, Belgium.

Email: auriane.apaire@uclouvain.be; ayse.dereli@uclouvain.be; riem.eltahry@saintluc.uclouvain.be

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Abstract

Objective: Cardioventilatory failure is the leading mechanism proposed to underlie sudden unexpected death in epilepsy (SUDEP), which occurs predominantly at night in patients with generalized tonic–clonic seizures. Interictal hypercapnic cardioventilatory responses are suggested to be involved, as they are ablated in chronically epileptic kainic acid (KA) rats, a temporal lobe epilepsy model with focal to bilateral tonic–clonic seizures. However, how this impairment emerges during epileptogenesis and whether it is influenced by day/night period remain unclear. Here, we aimed to investigate the progress of hypercapnic cardioventilatory responses through the epileptogenesis of KA rats and whether it is affected by day or night.

Methods: Ventilatory or breathing frequency (f_B) and heart rate (HR) were measured before, during, and after a 1-h exposure to acute hypercapnia (10% CO₂) using photoplethysmography in KA and healthy rats. Measurements were performed monthly for 6 months, with additional nocturnal hypercapnia recordings at month 6 to assess day/night modulation. To control for repeated CO₂ exposure, an independent cohort of age-matched KA and healthy rats underwent a single hypercapnia exposure at month 6.

Results: In healthy rats, cardioventilatory responses to hypercapnia remained stable over time, with increased f_B and reduced HR. Conversely, KA rats displayed an abrupt blunting of the f_B response at month 4, followed by blunting of the HR response at month 6. Correlation analyses revealed a loss of correlation between f_B and HR following KA injection, which reemerged when the cardioventilatory response to CO₂ was fully ablated. No association was observed between seizure severity and cardioventilatory impairment. KA rats displayed similar deficits regardless of CO₂ exposure frequency, and no day–night differences were detected in either group.

Ayse S. Dereli and Riem El Tahry are co-last authors.

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Significance: These findings indicate that cardioventilatory responses are decreased during epileptogenesis in the KA model, suggesting their potential utility for evaluating SUDEP risk.

KEYWORDS

central respiratory chemoreception, epilepsy, epileptogenesis, hypercapnic ventilatory response, kainic acid

1 | INTRODUCTION

Sudden unexpected death in epilepsy (SUDEP) is the leading cause of epilepsy-related mortality, with an incidence of .4–2/1000 in chronic epilepsy and 4–9/1000 in severe refractory cases,¹ and ranks second in years of potential life lost,² making it a major public health concern. SUDEP occurs mainly during sleep, following convulsive (tonic-clonic) seizures.³ Autonomic dysregulation, particularly respiratory dysfunction and cardiac arrhythmias, play a prominent role in the pathophysiology of SUDEP.⁴ In particular, central apnea appears to initiate a fatal sequence involving increased parasympathetic activity and subsequent cardiac signal abnormalities.⁵

Clinically, severe seizures are frequently accompanied by apneas leading to hypercapnia and respiratory acidosis,⁶ consistent with impaired central respiratory chemoreception.⁷ In line with this, animal models with convulsive seizures exhibit an impaired interictal hypercapnic ventilatory response (HCVR), which may increase vulnerability to seizure-related death.⁸ This deficit is accompanied by an ablated heart rate (HR) response to CO₂ and disrupted cardioventilatory correlation.⁸ Under physiological conditions, hypercapnia increases ventilation to maintain CO₂ homeostasis and modulates HR via baroreceptor mechanisms, resulting in reflex bradycardia in animals.^{8,9} In contrast, hypercapnia in humans generally increases HR, reflecting a resetting of the cardiac baroreflex toward tachycardia alongside increased ventilation.^{10,11} These findings highlight a critical role for central respiratory chemoreception in the integrated regulation of breathing and cardiovascular function. Impairments in both ventilatory and cardiac responses to CO₂ are therefore highly relevant to SUDEP, in which respiratory dysfunction is often accompanied by cardiac arrhythmias preceding death.⁵

It is well established that SUDEP risk increases with epilepsy severity, particularly in refractory epilepsy patients with a high frequency of tonic-clonic seizures.¹² Although the link between epilepsy duration and SUDEP risk in humans is debated, postmortem studies show

Key points

- Hypercapnic cardioventilatory response is abruptly ablated during epileptogenesis.
- Breathing rate response to CO₂ is ablated before heart rate in kainic acid rats.
- In the kainic acid model, the hypercapnic cardioventilatory response does not depend on age, CO₂ exposure, or time of day.
- Using hypercapnic cardioventilatory response may improve SUDEP risk prediction, as blunted responses could reflect heightened vulnerability.

that longer epilepsy duration correlates with prolonged peri-ictal oxygen desaturation.¹³ Epilepsy can manifest at various ages, and SUDEP affects both children and adults,¹⁴ indicating that rather than age, epileptogenesis could be a contributing factor. Hence, investigating hypercapnic cardioventilatory response throughout epilepsy progression in adult animal models is crucial to understand the dynamic risk of autonomic dysfunction and SUDEP.

SUDEP occurs predominantly at night in both humans and animal models.^{15–17} Circadian rhythms can influence respiratory and cardiovascular recovery during the postictal phase.¹⁸ In healthy humans, the HCVR is attenuated at night,¹⁹ whereas in healthy rats, CO₂ sensitivity is reduced during the daytime, reflecting species-specific day/night regulation.²⁰ These findings suggest that day/night modulation of central respiratory chemoreception may contribute to SUDEP vulnerability, particularly in the setting of nocturnal seizures.

Given these gaps in knowledge, we aimed to investigate hypercapnic cardioventilatory response in kainic acid (KA)-treated rats across (1) epileptogenesis and (2) the day and night cycle. We hypothesized a progressive decline in response, with greater impairment during the day (corresponding to the sleep phase in rodents), independent of repeated CO₂ exposure or age.

2 | MATERIALS AND METHODS

2.1 | Animals

The experiments were approved by the animal experimental ethical committee of Université Catholique de Louvain and the Brussels environment committee under the reference numbers 2023/UCL/MD/53 and 2024/UCL/MD/21. Male Sprague Dawley rats (Charles River RMS BENELUX, D692081301) were housed on a 12-h day/night cycle (7:00 a.m. to 7:00 p.m.) in a temperature- and humidity-controlled environment ($23 \pm 2^\circ\text{C}$ and 45%–55% humidity) with ad libitum access to food and water. Daytime experiments were conducted between 07:00 a.m. and 07:00 p.m.; nighttime experiments (7:00 p.m.–12:00 a.m.) were performed in complete darkness using infrared light (see [Supplementary Table S1](#) for per-animal timing).

2.2 | Electroencephalographic electrode implantation

Rats were anesthetized with ketamine (100 mg/kg ip) and xylazine (7 mg/kg ip) and monitored via hind-paw pinch reflex for maintenance of anesthesia. Core body temperature was maintained at 37.5°C using rectal probe-integrated heating pad. The rat was head-fixed on a stereotaxic frame (David Kopf Instruments). Custom-made epidural stainless-steel screw electrodes (4.8 mm, 0–80 $\times$ 3/16, Bilaney) were implanted at the following coordinates relative to bregma, as previously used^{8,21}: two frontal electrodes (+) at anteroposterior (AP) +2 mm, mediolateral (ML) $\pm$ 3 mm; one reference electrode at AP +6 mm, ML 0 mm; and one ground electrode at AP –5 mm, ML +3 mm.²² Differential electroencephalographic (EEG) recordings were acquired between the frontal (+) and reference electrodes. During the same surgical session, a guiding cannula was implanted using the stereotaxic frame at coordinates AP –5.6 mm, ML –4.5 mm, dorsoventral –4.5 mm for subsequent intrahippocampal injections. An ultraviolet-cured resin headcap (CompoFlow, R&S) was constructed to secure and protect the electrodes and the cannula. Postoperatively, analgesia (Tramadol 5 mg/kg and Ketofen 5 mg/kg sc) was given twice daily for 3 days. Sunflower seeds and enriched pellets were provided to support recovery.

2.3 | Induction of epilepsy

KA (.4 μg in .2 μL saline, Hello Bio) was injected into the right hippocampus of 22 rats between 0 and 7 days post-surgery via a guiding cannula using a 25-gauge Hamilton

microsyringe.²³ Following intrahippocampal KA injection, rats were continuously monitored via video-EEG for 24 h in dedicated recording cages. Status epilepticus (SE) onset occurred within 5–30 min after KA injection, with seizures of at least stage 3 persisting for 3 h. To avoid introducing variability, no benzodiazepines were administered to abort SE, allowing natural progression of epileptogenesis across all animals. Fourteen control healthy rats received the same volume of intrahippocampal saline injection (.2 μL) during a sham surgery.

2.4 | Experimental procedure

2.4.1 | Cardioventilatory measurements during hypercapnia challenge

Acute hypercapnia (AH) challenges were conducted in a custom-built, semisealed plexiglass video-EEG-photoplethysmography (PPG) hypercapnia chamber.⁸ Each challenge session lasted 3 h and consisted of three consecutive phases: 1 h of baseline room air exposure, 1 h of 10% CO_2 exposure (AH, balanced with room air), and 1 h of post-AH room air recovery. The 10% CO_2 concentration ($10\% \pm .3\%$) was achieved rapidly (within 1 min) and maintained throughout the exposure period using a Biospherix gas controller. Atmospheric oxygen levels were continuously monitored with a Voltcraft OM-100 oxygen detector to ensure normoxic conditions ($18\% \pm .5\% \text{O}_2$).

Cardioventilatory parameters were recorded noninvasively using photoplethysmography (PPG) with the MouseOx Plus 2.0 system (Starr Life Sciences Corp., BIOSEB) as performed previously.⁸ In short, PPG signals were acquired via a neck-mounted MouseOx Plus collar sensor, positioned near the base of the ear. The system enabled continuous monitoring of respiratory and cardiac-modulated blood oxygenation, thereby providing continuous measurement of ventilatory or breathing frequency (f_B), HR, and arterial oxygen saturation. To confirm the absence of seizures during AH, PPG recordings were performed in conjunction with video-EEG monitoring.

A total of 41 rats were used; nine rats died before completion of the experiments due to surgery, KA injection overdose/complications, or undetermined causes. To assess longitudinal cardioventilatory responses during epileptogenesis and normal aging, two age-matched groups of rats underwent monthly hypercapnia challenges for 6 months; one group received a single KA injection ($n = 10$, hereafter referred to as KA-repeated), and the other consisted of healthy controls saline-injected ($n = 14$, hereafter referred to as healthy-repeated). Challenges began at 3 months of age. At the end of the 6-month period, both groups were additionally exposed to hypercapnia during

the dark phase to evaluate potential day/night modulation of the cardioventilatory response. Separate cohorts of KA-single ($n=3$) and noninjected healthy-single ($n=5$) rats received a single hypercapnia challenge at 9 months of age to assess the effects of single versus repeated hypercapnic exposure.

2.4.2 | Seizure severity

Video-EEG measurements were performed 1 week per month either before or after each challenge session to monitor the progression of epilepsy throughout the 6-month experimental period. This allowed for longitudinal assessment of seizure severity and the evolution of epileptogenesis over time.

2.5 | Data analysis

2.5.1 | Video-EEG

EEG signals were preprocessed using second-order Butterworth digital filters (.5–70 Hz) applied with zero-phase forward and reverse filtering to preserve the temporal integrity of waveform features, using a custom-developed application in MATLAB R2023a (MathWorks). Status epilepticus and spontaneous seizures were classified according to the modified Racine scale (0–5) based on synchronized video recordings as follows^{24,25}:

- Stage 0: Normal behavior with no seizure activity
- Stage 1: Immobilization or freezing with mouth clonus
- Stage 2: Head nodding, neck jerks, or whisker movements
- Stage 3: Unilateral forelimb clonus
- Stage 4: Rearing with bilateral clonic seizures and falling onto the side
- Stage 5: Wild running/jumping with tonic-clonic seizures and falling onto the back

For analytical purposes, spontaneous seizures were grouped as stage 0–2 or stage 3–5. To validate behavioral observations, seizures were also defined based on the EEG. For this, EEG signals were preprocessed using second-order Butterworth digital filters (.5–70 Hz) applied with zero-phase forward and reverse filtering to preserve the temporal integrity of waveform features, using a custom-developed application in MATLAB R2023a (MathWorks). Seizures were identified on EEG as rhythmic spike-and-wave discharges with high amplitude $\geq 3 \times$ baseline and elevated frequency (>5 Hz) sustained for at least 10 s, as previously defined.²⁶ Stage 1–2 seizures

were characterized by high-frequency, high-amplitude discharges without clear evolution or postictal depression on EEG, accompanied by behavioral arrest with head and whisker movements on video. Stage 3–5 seizures exhibited a progressive increase in spike frequency and amplitude followed by pronounced postictal suppression marking seizure termination on EEG, and unilateral clonus progressing to bilateral tonic-clonic movements on video. When EEG recordings were unavailable due to headcap detachment, seizure assessment relied exclusively on video monitoring, and only stage 3–5 seizures with overt behavioral manifestations were scored.

2.5.2 | Photoplethysmography

PPG signals were acquired at 300 Hz and downsampled to 15 Hz for analysis as previously described.⁸ f_B and HR were extracted using a 1-min sliding window (no overlap) and averaged into 5-min bins. To account for interanimal variability, mean f_B and HR values were normalized to each animal's mean baseline (room air) value and expressed as percentage change from baseline. Data are presented as time-course plots with SEM indicated by error bars, except in Figures 1–2, where SEM was omitted for clarity and included only in single/repeated measures (Figure 3) and day/night comparisons (Figure 5). Examples of raw PPG traces, along with detailed derivation of cardiorespiratory parameters (f_B , HR, and signal processing steps) are provided in the supplementary data of Dereli et al.⁸

2.6 | Statistical analysis

Statistical analyses were performed using R (version 2024.04.2 + 764) or JASP (version .95.1.0), and graphs were plotted on GraphPad Prism (version 10.1.2). For animals tested repeatedly over several months, f_B and HR during the AH period were analyzed using linear mixed-effects models with time relative to epilepsy onset (pre-KA and months 1–6 post-KA) and their interaction as fixed effects. Random intercepts for each animal accounted for repeated measures within subjects. Pairwise comparisons of estimated marginal means were Sidak corrected with only significant differences relative to the pre-KA baseline ($p < .05$) interpreted and indicated on violin and box plots, where individual data points during AH are grouped together. The same approach was used to compare responses between rats with stage 0–2 and stage 3–5 seizures. Day-night comparisons were assessed using paired t -tests and single versus repeated AH challenges using unpaired t -tests. Bonferroni correction was performed on all analyses for multiple comparisons adjustment.

3 | RESULTS

3.1 | Repeated acute hypercapnia challenges over 6 months in KA rats

f_B and HR responses to hypercapnia were assessed before intrahippocampal KA injection and then monthly for 6 months. Each month, cardioventilatory responses to AH were recorded over three consecutive periods: 1-h

baseline (room air), 1-h AH (10% CO_2) and 1-h post-AH recovery (room air; Figure 1 A,B). Baseline raw values are provided in Supplementary Table 2 and raw data for the AH challenge in Supplementary Table 3.

Before KA injection, AH increased f_B and decreased HR (Figure 1A,B, red traces). The mean f_B response during AH was preserved for the first 3 months post-KA (violin plots in Figure 1A), although time-course analysis revealed attenuation toward the end of the 60-min AH period. From

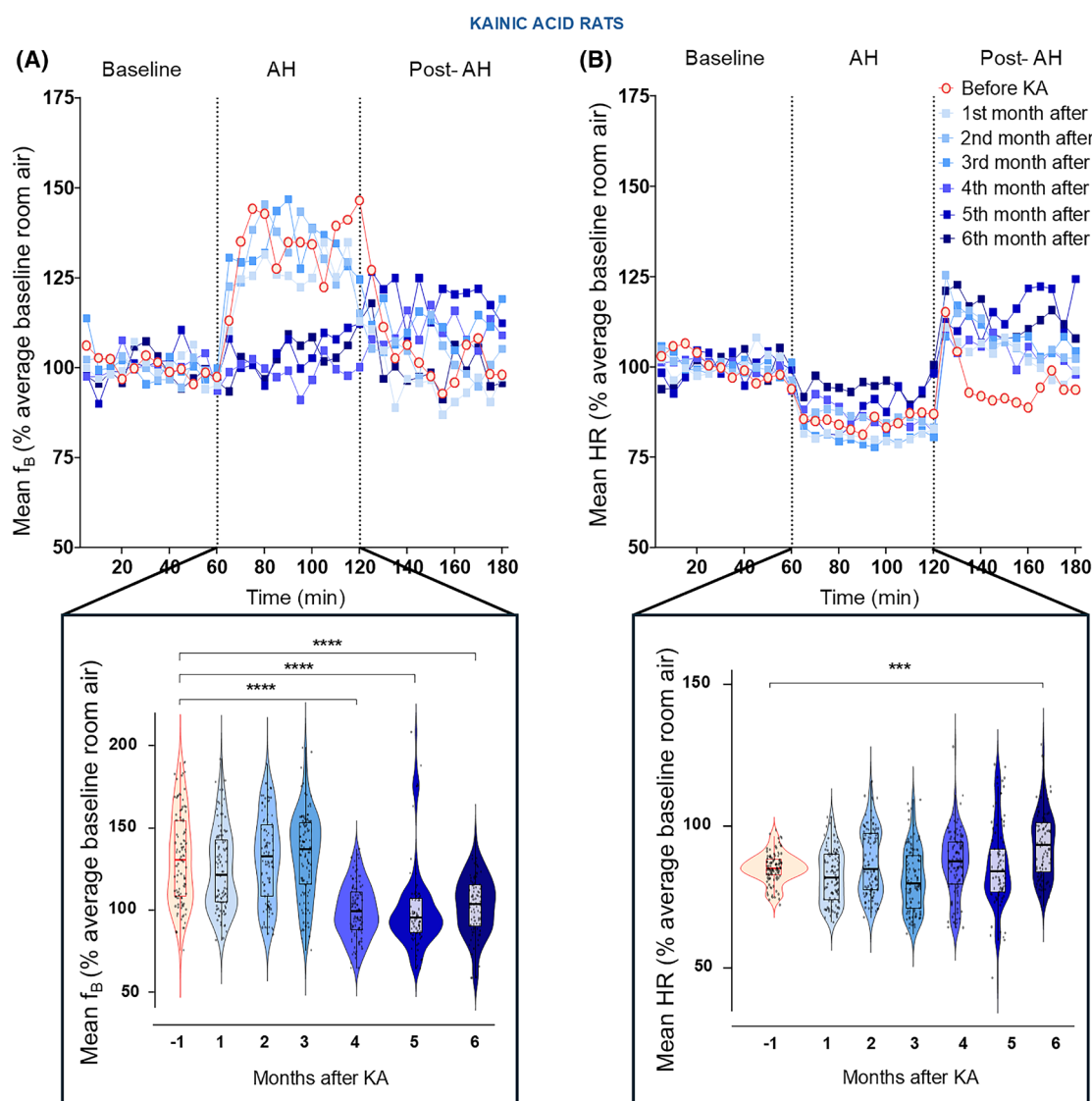


FIGURE 1 The effect of epileptogenesis on hypercapnic cardioventilatory response in kainic acid (KA) rats, showing time course of (A) breathing rate (f_B) and (B) heart rate (HR) as a percentage of the average baseline during 1 h room air (RA), 1 h acute hypercapnia (AH; 10% CO_2), and 1 h post-AH RA before KA injection and each month after KA injection. Violin plots illustrate the mean of all individual data points (% average baseline), each representing the average of a 5-min interval during the AH period (12 points per rat and 10 rats in total make 120 points per month). Red circles denote healthy rats prior to KA injection. Squares in shades of blue indicate time since KA injection, from light (1 month) to dark (6 months). *** $p < .001$, **** $p < .0001$, significantly different from before KA by linear mixed model with Sidak correction for multiple comparisons.

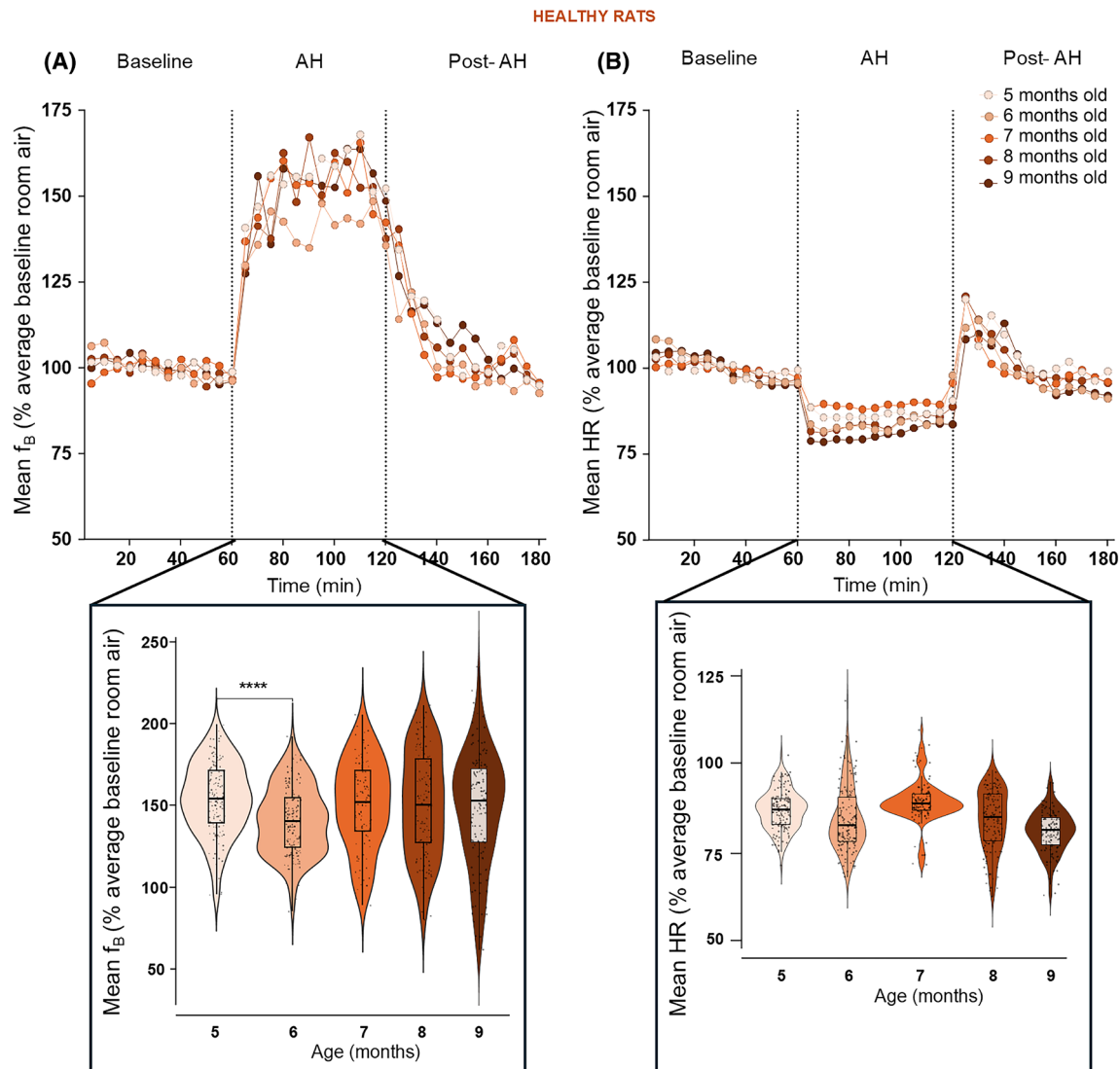


FIGURE 2 The effect of age on hypercapnic cardioventilatory response in healthy rats, showing time course of (A) breathing rate (f_B), and (B) heart rate (HR) as a percentage of the average baseline during 1 h room air (RA), 1 h acute hypercapnia (AH; 10% CO_2), and 1 h post-AH RA of 5-month-old healthy rats and each month after a single intracranial saline injection. Violin plots illustrate the mean of all individual data points (% average baseline), each representing the average of a 5-min interval during the AH period (12 points per rat and 14 rats in total make 168 points per month). Colors indicate age, from light cream (5 months old) to dark brown (9 months old). **** $p < .0001$, significantly different from 5 months old by linear mixed model with Sidak correction for multiple comparisons.

the 4th month onward, the hypercapnia-induced increase in f_B was abolished and remained absent at months 5 and 6 (all $p < .0001$; Figure 1A).

In contrast, HR responses to AH were preserved through month 4 after KA injection. HR responses showed a nonsignificant reduction at month 5 and were abolished by month 6 ($p < .001$; Figure 1B). During post-AH recovery, f_B and HR returned to baseline within 15 min, with f_B recovering more slowly than HR. Following KA, baseline recovery was impaired for both variables. By 6 months post-KA, f_B remained at baseline throughout the protocol.

Correlation analyses of normalized f_B and HR responses revealed a significant association before KA ($p < .05$, Pearson correlation analysis), which was lost after

KA injection and remained absent until month 6. Notably, at 6 months post-KA, the correlation between f_B and HR reemerged despite the absence of cardioventilatory responses to CO_2 , (Supplementary Table 4; $p = .01$, Pearson correlation analysis).

3.2 | Repeated acute hypercapnia challenges over 6 months in healthy rats

Age-matched intrahippocampally saline-injected rats underwent the same experimental timeline as the KA group to control for age-related effects (see Supplementary Table 2 for baseline raw values and

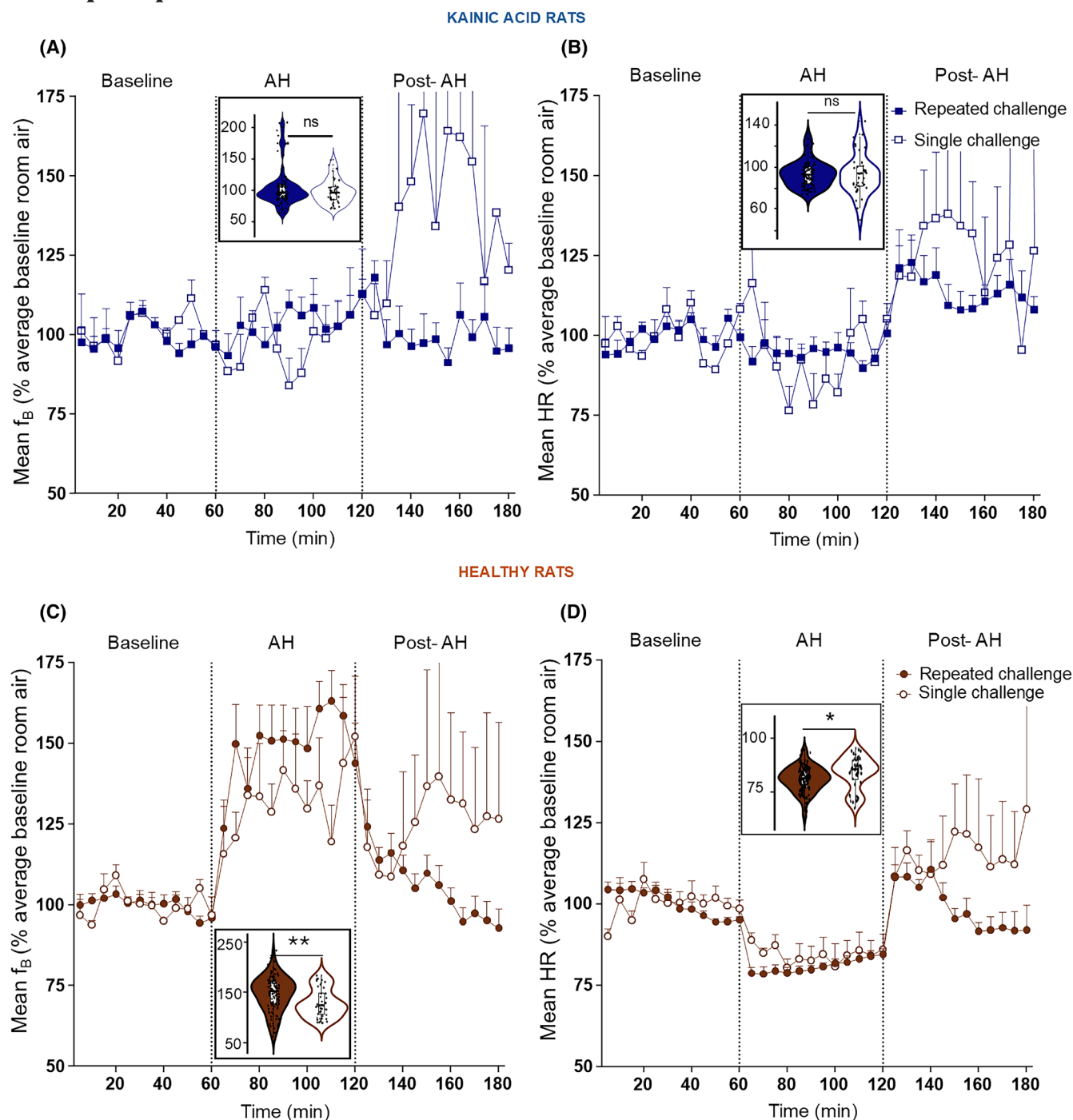


FIGURE 3 The effect of repetitive challenge on hypercapnic cardioventilatory response in kainic acid (KA) and healthy rats, showing time course of (A, C) breathing rate (f_B) and (B, D) heart rate (HR) as a percentage of the average baseline during 1 h room air (RA), 1 h acute hypercapnia (AH; 10% CO_2), and 1 h post-AH RA. Violin plots illustrate the mean of all individual data points (% average baseline), each representing the average of a 5-min interval (12 points per rat) during the AH period within repeated and single challenge groups. Violin plots illustrate the mean of all individual data points (% average baseline), each representing the average of a 5-min interval during the AH period (12 points per rat and 10 rats in total in KA-repeated challenge group make 120 points, three rats in KA-repeated challenge group make 36 points, 14 rats in healthy-repeated challenge group make 168 points per month, and five rats in healthy-repeated challenge group make 60 points). Panels A and B show the comparison between KA-repeated challenge group (filled squares) and KA-single challenge group (empty squares; unpaired t -test followed by Bonferroni correction). Panels C and D show the comparison between aged-matched healthy-repeated challenge group (filled circles) and healthy-single challenge group (empty circles). Error bars represent SEM. * $p < .05$, ** $p < .01$, significantly different by unpaired t -test followed by Bonferroni correction. ns, not significant.

Supplementary Table 3 for raw data for the AH challenge). In this cohort, 5-month-old animals corresponded to the 2-month post-KA timepoint in the KA group. A significant difference in mean f_B during AH was observed between 5- and 6-month-old healthy rats ($p < .0001$; **Figure 2A**); however, f_B remained elevated relative to baseline. No other age-dependent differences were observed. HR responses showed no significant changes across the 5-month recording period (**Figure 2B**), and all age groups returned to baseline during the post-AH recovery period.

3.3 | Comparison between repeated and single challenges in KA and healthy rats

The effect of repeated AH exposure was evaluated in both KA-injected and healthy rats. In KA rats, f_B and HR responses to AH did not differ between rats receiving repeated monthly challenges (1 per month for 6 months) and age-matched rats receiving a single challenge at month 6; responses were impaired in both groups (**Figure 3A,B**). In healthy rats, the AH-induced f_B and HR responses relative to baseline were preserved in both groups but were reduced in rats receiving a single challenge compared to those exposed repeatedly (f_B : $p < .01$, HR: $p < .05$; **Figure 3C,D**). During post-AH recovery, neither KA nor healthy rats in the single-challenge group returned to baseline f_B or HR levels.

3.4 | Influence of seizure severity on cardioventilatory response during acute hypercapnia

To monitor epileptogenesis, EEG recordings were analyzed in KA rats for 1 week per month over 6 months. Seizures emerged from 2-month post-KA injection (**Supplementary Table 5**). By the end of 6 months, of the 10 rats, three exhibited stage 1–2 seizures, three showed stage 3–5 seizures, two experienced both stage 1–2 and stage 3–5 seizures, and two displayed no seizures during the recording periods, despite detectable interictal activity.

Cardioventilatory responses were analyzed in relation to seizure burden expressed as mean seizures per day per month. No correlation was found between seizure frequency and f_B or HR responses during AH (**Supplementary Table 6**). Seizure severity was then assessed by grouping rats into stage 0–2 or stage 3–5 seizures. In both groups, AH-induced f_B responses were abolished from 4 months after KA onward, with comparable temporal profiles (**Figure 4A,B**). In contrast, HR responses diverged by

severity. In rats with stage 0–2 seizures, the HR response to CO_2 was preserved throughout 6 months after KA injection and even significantly amplified at 4 months (**Figure 4C**). In contrast, rats with stage 3–5 seizures displayed a significantly blunted HR response by 6 months post-KA (**Figure 4D**).

3.5 | Comparison of the cardioventilatory response to CO_2 between day and night in 6-month-old epileptic KA and healthy rats

Day–night differences in cardioventilatory responses to CO_2 were assessed at 6 months post-KA in KA rats and age-matched saline-injected controls. In KA rats, nighttime recordings showed a transient increase in f_B during the first 30 min of AH, and HR tended to be decreased throughout the AH period. However, mean responses during AH did not differ from daytime recordings in KA rats (**Figure 5A,B**). In healthy rats, no day–night differences were observed in hypercapnic f_B or HR responses (**Figure 5C,D**).

During post-AH recovery, f_B and HR returned to baseline in healthy rats, regardless of the time of day (**Figure 5C,D**). In contrast, in KA rats, nighttime f_B remained elevated and HR failed to normalize both during the day and at night (**Figure 5B**).

4 | DISCUSSION

Here, we investigated the longitudinal progression of hypercapnic cardioventilatory responses during epileptogenesis and assessed the day/night pattern to identify mechanisms potentially linked to SUDEP risk. Before KA injection, rats exhibited a normal hypercapnic response, characterized by increased f_B and decreased HR, with both parameters inversely correlated. By 4 months, the f_B response was abolished and remained absent thereafter, whereas the HR response was initially preserved but was lost by 6 months, indicating delayed cardiac impairment. The inverse f_B and HR relationship was disrupted during this period and reemerged at 6 months. Recovery after hypercapnia was also disrupted in KA rats, unlike controls. When stratified by seizure severity, reduced hypercapnic HR response was observed only in stage 3–5 seizures, whereas the f_B response was ablated across all seizure stages. Overall, seizure frequency was not correlated with the level of cardioventilatory dysfunction. Day/night variation and repeated CO_2 exposure had minimal impact, highlighting the specificity of KA-induced autonomic decline.

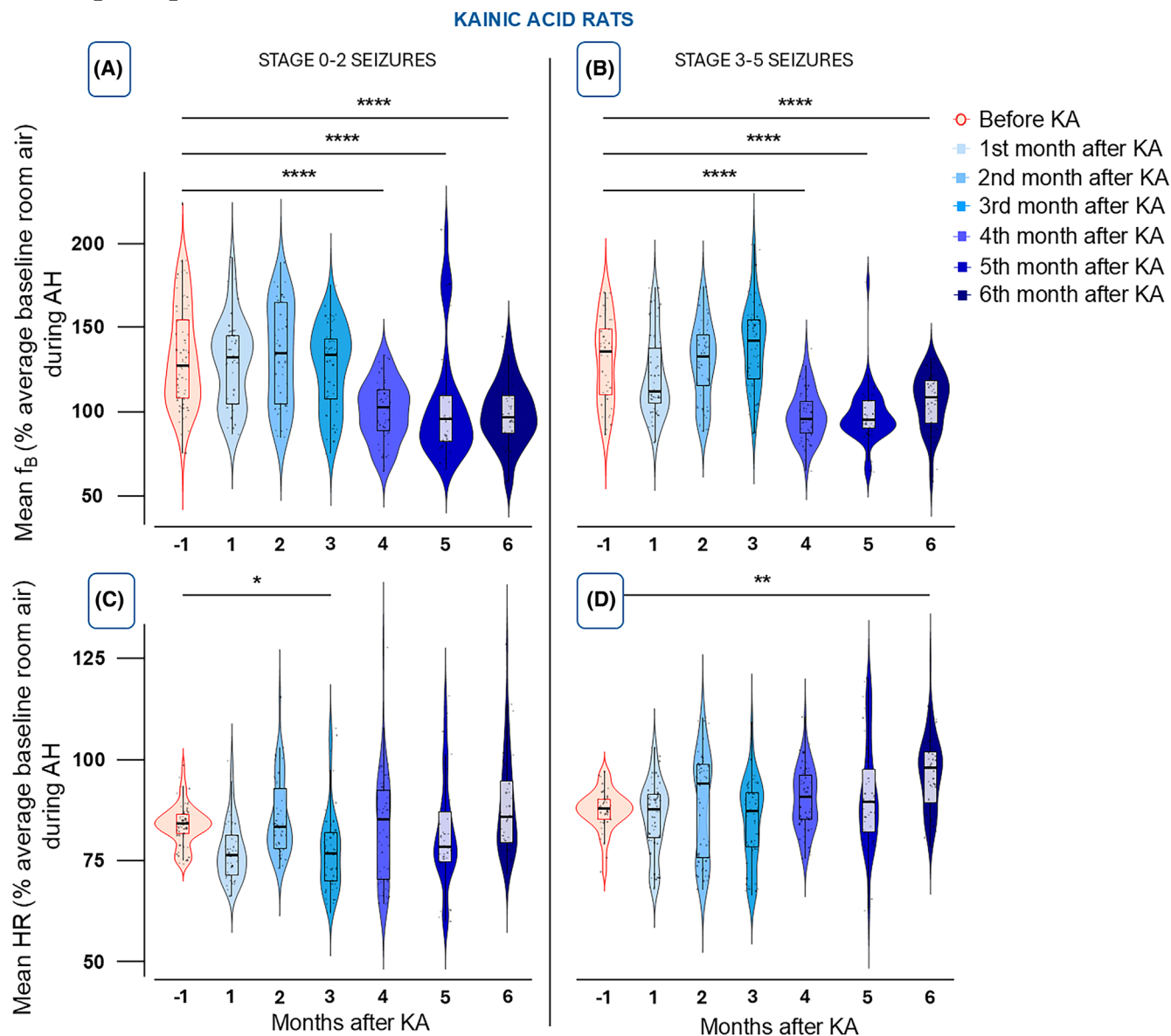
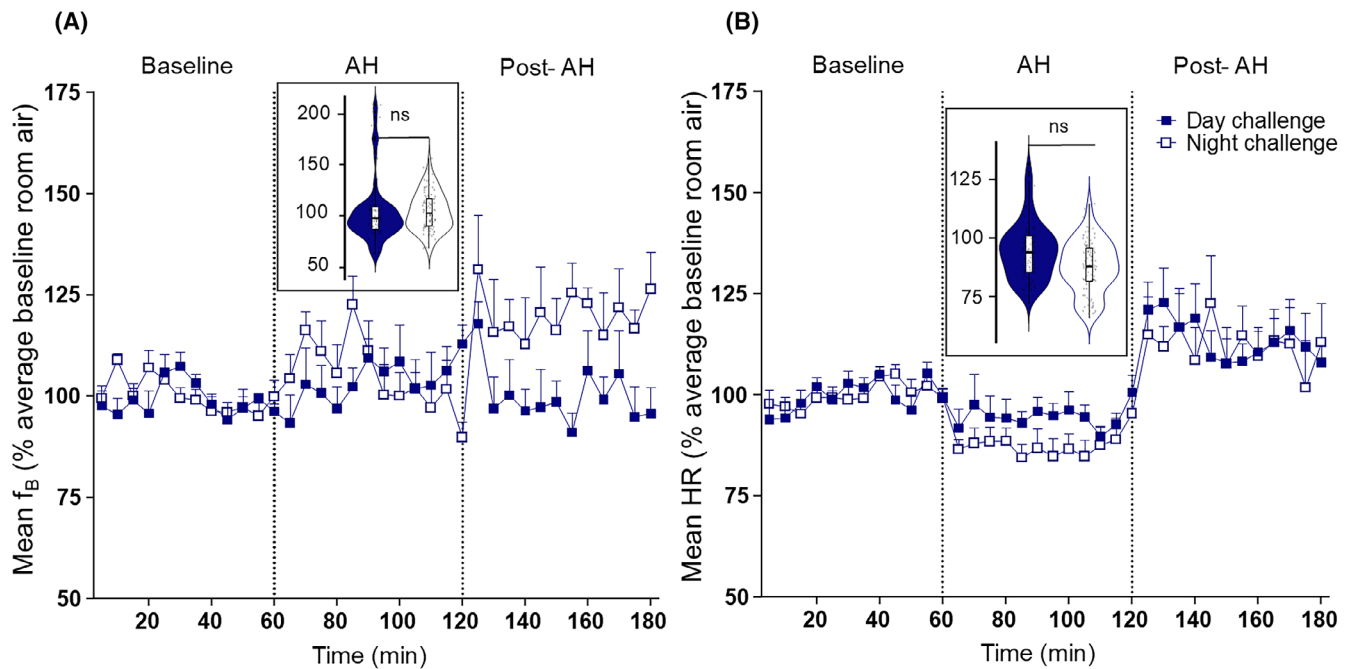


FIGURE 4 The effect of seizure severity on hypercapnic cardioventilatory response in kainic acid (KA) rats. Violin plots show the mean (A, B) breathing rate (f_B) and (C, D) heart rate (HR) during acute hypercapnia (AH) of the rats before KA injection and each month after KA injection. Violin plots illustrate the mean of all individual data points (% average baseline), each representing the average of a 5-min interval during the AH period (12 points per rat and five rats in total with stage 0–2 seizures make 60 points per months, and five rats with stage 3–5 seizures make 60 points per months). Rats with stage 0–2 seizures are represented by panels A and C, whereas rats with stage 3–5 seizures are shown in panels B and D. * $p < .05$, ** $p < .01$, significantly different from before KA by linear mixed model with Sidak correction for multiple testing. ns, not significant.

CO₂ homeostasis is regulated by central respiratory chemoreception. CO₂ sensitive neurons within brainstem nuclei detect elevations in P_{CO₂}, and relay signals to the ventral respiratory column, to activate breathing through the phrenic nucleus.²⁷ There is growing evidence that the HCVR is impaired in epilepsy⁸; however, its longitudinal trajectory across epileptogenesis remains largely unexplored. Only a few animal studies have addressed this question, including investigations in chronic seizure models including the pilocarpine

model at 15 and 30 days postinduction²⁸ and the KA mouse model at 3, 5, and 7 weeks postinjection.²⁹ These studies typically collect data at two or three timepoints after seizure induction, thus leaving the overall trajectory of HCVR throughout epileptogenesis unexplored. Our study addresses this critical gap by systematically assessing HCVR before seizure onset and monthly for 6 months, providing the first comprehensive characterization of its evolution during chronic epilepsy. We found that the f_B response to CO₂ was abolished by 4 months

KAINIC ACID RATS



HEALTHY RATS

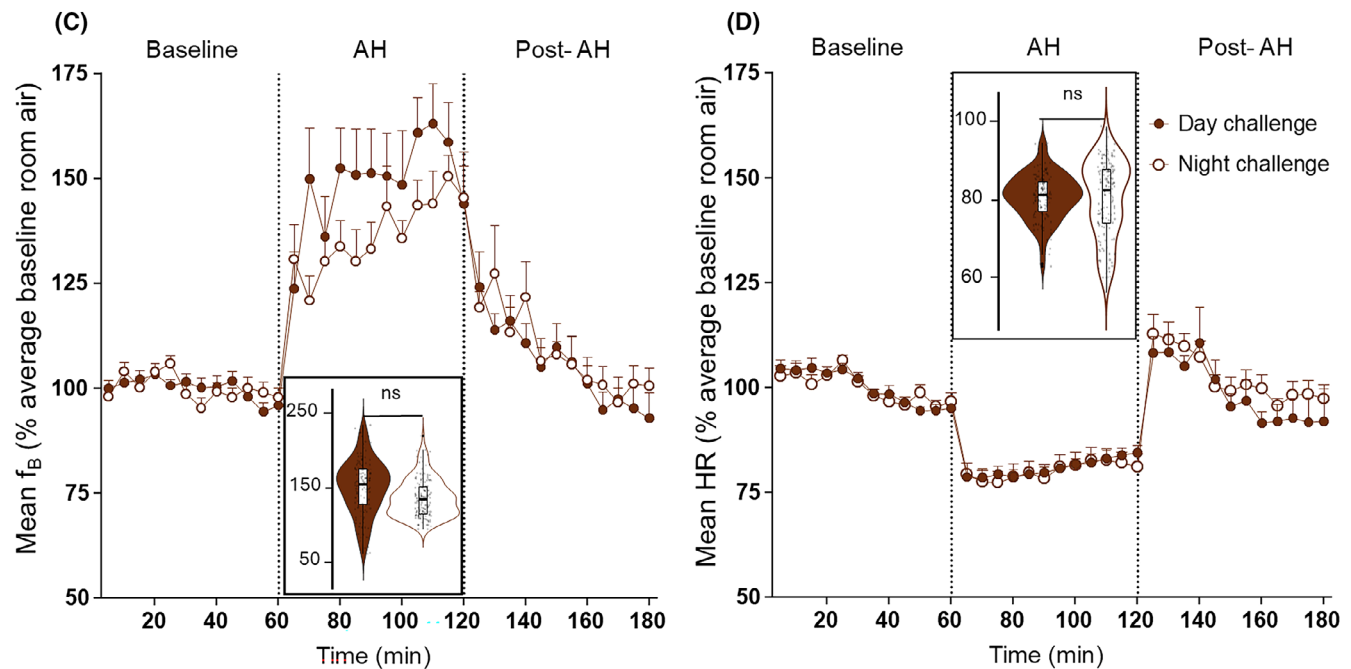


FIGURE 5 The effect of day or night on hypercapnic cardioventilatory response in kainic acid (KA) and healthy rats, showing time course of (A, C) breathing rate (f_B) and (B, D) heart rate (HR) as a percentage of the average baseline during 1 h room air (RA), 1 h acute hypercapnia (AH; 10% CO_2), and 1 h post-AH RA. Violin plots illustrate the mean of all individual data points (% average baseline), each representing the average of a 5-min interval during the AH period (12 points per rat and 10 rats in total in KA rats group make 120 points per condition, and 14 rats in healthy rats group make 168 points per condition). Panels A and B show the comparison during acute hypercapnia between day (filled squares) and night challenges (empty squares) in KA rats 6 months post-KA. Panels C and D show the comparison between day (filled circles) and night (empty circles) in aged-matched healthy rats. Error bars represent SEM. Paired t -test was followed by Bonferroni correction. ns, not significant.

after KA injection, which is consistent with the epileptogenic timeline of this model, in which seizure frequency gradually increases over time and plateaus at approximately 30 weeks (~3.5 months) after seizure induction.²⁶ Notably, one report described a patient with pharmacoresistant epilepsy who exhibited a complete loss of the HCVR and died from SUDEP 11 months later.³⁰ Together, these observations suggest that longitudinal assessment of HCVR during epileptogenesis may have potential as a biomarker of SUDEP risk, particularly if consistent trajectories are identified in humans.

In rodents, hypercapnia typically induces an increase in mean arterial pressure accompanied by a reduction in HR,^{8,9} mediated by parasympathetic baroreflex activation.⁹ In contrast, healthy humans exhibit a tachycardic response to CO₂.¹⁰ The current findings are consistent with established rodent physiology and extend prior reports by demonstrating, for the first time, the longitudinal evolution of HR responses to CO₂ across epileptogenesis. Notably, the HR response was abolished only at 6 months after KA injection, approximately 2 months after the loss of the f_B response. This temporal dissociation parallels observations from the MORTEMUS study, in which respiratory failure precedes cardiovascular collapse in SUDEP patients.⁵ Although the current study did not directly assess SUDEP or mortality, focusing instead on the longitudinal hypercapnic cardioventilatory responses during chronic epilepsy, the data suggest that impaired ventilatory responsiveness to CO₂ may represent an early vulnerability that is compounded by subsequent loss of cardiac responsiveness.

To further explore this relationship, we assessed the correlation between mean f_B and HR during hypercapnia. Consistent with our previous work,⁸ pre-KA, rats exhibited a positive association, whereby greater increases in f_B were accompanied by larger reductions in HR. After KA, this correlation was lost when the f_B response was abolished, whereas HR responses were still preserved, indicating that cardiac modulation during hypercapnia had become dissociated from ventilatory drive. The persistence of HR responses at this stage may reflect contributions from alternative autonomic cardiac reflexes³¹ or intrinsic cardiac mechanisms.³² Interestingly, the mean f_B –HR correlation reemerged at 6 months postinjection, coinciding with the loss of both ventilatory and cardiac responses. This reappearance may reflect a parallel failure of cardioventilatory control mechanisms rather than restoration of physiological coupling. Notably, in our previous study, the correlation remained absent at the corresponding timepoint,⁸ suggesting that methodological differences—such as rat strain (Wistar vs. Sprague Dawley) or the route of KA administration (intraperitoneal vs. intrahippocampal)—may influence the temporal evolution of cardioventilatory

dysfunction. Intraperitoneal KA administration may lead to a slower or more progressive impairment of ventilatory and cardiac responses. Future studies should dissect the underlying mechanisms and extend analyses to the postictal period, a critical window of vulnerability for SUDEP.

To assess the potential influence of aging on cardioventilatory responses to CO₂, we applied the same longitudinal protocol in healthy control rats and observed preserved physiological responses throughout the study period. This is consistent with evidence from healthy humans indicating that age does not affect ventilatory responses to CO₂.³³ Taken together, these findings indicate that the loss of f_B and HR response to CO₂ in KA rats is attributable to epilepsy-related mechanisms rather than age-associated changes.

Although both animal and human studies have shown that elevated CO₂ can suppress or terminate seizures,³⁴ its impact on physiological CO₂ responses in epileptic animals remains unclear. To delineate potential effects of repeated CO₂ exposure, we included additional cohorts exposed to a single hypercapnia challenge at 6 months following KA or saline injection. Similar to animals subjected to repeated challenges, KA rats exhibited a markedly impaired cardioventilatory response to CO₂, whereas healthy rats maintained normal responses. Although some baseline variability and slightly attenuated hypercapnic f_B responses were observed in singly challenged animals compared with repeatedly challenged cohorts, the overall response pattern was preserved. This difference likely reflects reduced stress and habituation in repeatedly challenged rats due to familiarity with the recording environment and PPG collar. Consistent with previous work showing that repeated CO₂ exposure does not alter cardioventilatory responses,³⁵ these findings indicate that KA-induced impairment of CO₂ responsiveness is independent of challenge frequency.

We also examined seizure severity to determine its potential influence on central respiratory chemoreception. No clear association was observed between seizure burden and cardioventilatory responses to CO₂, although interpretation is limited by small subgroup sample size ($n=5$ per stage). Previous research showed a correlation between stage 3–5 seizures and HR during AH in KA rats, suggesting a progressive deterioration of cardiovascular autonomic control in parallel with increased seizure severity.⁸ In humans, SUDEP risk increases with seizure frequency of generalized tonic–clonic seizures or multiple seizure types,^{12,36} likely reflecting cumulative impairment of cardioventilatory autonomic regulation. Overall, given that seizure frequency is a major risk factor for SUDEP, longitudinal monitoring of HCVR in patients with frequent seizures, particularly generalized tonic–clonic seizures, may have potential utility in risk stratification and mortality reduction.

Because SUDEP occurs predominantly at night,^{15,17} we assessed hypercapnic cardioventilatory responses during the night. Unlike humans, rats are nocturnal³⁷ and exhibit higher metabolic and ventilatory responses to CO₂ at night.^{20,38} In the present study, although a trend toward reduced f_B responses was observed at night in healthy rats, no significant day–night differences in f_B or HR were detected in either healthy or KA rats. Accumulating evidence indicates that sleep state exerts a stronger influence on respiratory control than circadian phase.^{39–41} During non-rapid eye movement (NREM) sleep, cortical input to brainstem chemoreceptors is reduced, increasing vulnerability, as breathing depends predominantly on intrinsic brainstem mechanisms.⁴¹ Because SUDEP risk is also elevated during NREM sleep in humans,⁴² dysfunction within central respiratory chemoreceptor networks is likely to be particularly relevant. Together, these findings suggest that sleep-related mechanisms, rather than time of day alone, may be more relevant for understanding cardioventilatory vulnerability in epilepsy and should be a focus of future studies, with EEG recordings assessing the sleep state.

Several limitations should be noted. First, the KA model is an induced model of epilepsy and does not directly replicate SUDEP; however, it recapitulates key SUDEP-relevant features, including progressive epileptogenesis, secondary generalized tonic–clonic seizures,^{26,43,44} impaired central respiratory chemoreception, and autonomic dysregulation. Notably, mortality associated with spontaneous seizures in this model remains relatively low, which supports its utility for longitudinal studies of hypercapnic cardiorespiratory responses without the confounding effects of high seizure-related mortality.⁸ Second, although the intrahippocampal KA model is well established for inducing epilepsy, we did not perform histological evaluation to confirm uniform pathology across animals. Future studies should integrate histopathological analysis of hippocampal and brainstem regions to correlate structural damage with functional cardiorespiratory changes. Third, f_B was derived exclusively from PPG as previously employed in other studies^{45–47}; however, future investigations should incorporate additional respiratory measures such as tidal volume by doing whole body plethysmography and arterial blood gas analyses. Fourth, the absence of continuous seizure monitoring limited precise quantification of seizure frequency, as the intermittent recording period (1 week/month) may not fully represent the seizure burden following KA administration. Additionally, our modest sample size ($n=10$ KA rats) precluded analysis of individual Racine scores, necessitating grouping the epileptic animals to maintain statistical power. Finally, hypercapnic challenges were conducted at monthly intervals at different starting times; higher temporal resolution with precise timing, particularly around 4 months post-KA, may better

delineate the critical transition period during which ventilatory responses are lost.

5 | CONCLUSIONS

In summary, during epileptogenesis, KA rats exhibit a marked loss of both f_B and HR responses to CO₂ by the 4-month timepoint postinduction, independent of aging, hypercapnic challenge repetition, and time of day. Clinically, SUDEP risks assessment currently relies on the presence and frequency of generalized tonic–clonic seizures and nocturnal seizures.⁴⁸ Given that SUDEP is often preceded by severe hypercapnia,^{5,49} incorporating tests of f_B and HR responses to hypercapnic challenges could provide valuable mechanistic insights and potential value for identifying patients at elevated risk.

AUTHOR CONTRIBUTIONS

Auriane Apaire, Ayse S. Dereli, and Riem El Tahry conceptualized and designed the work. Auriane Apaire, Elise Collard, and Abigail Niyibizi performed the surgeries and recordings. Auriane Apaire and Abigail Niyibizi acquired the data. Enrique Germany Morrison and Javier Chavez Cerda created the analysis tools and scripts. Javier Chavez Cerda designed the recording cages. Auriane Apaire analyzed the data. All authors contributed to the interpretation of data. Auriane Apaire drafted the manuscript. Auriane Apaire, Ayse S. Dereli, Riem El Tahry, Natasha N. Kumar, and Enrique Germany Morrison substantially revised the manuscript. All authors have revised and approved the submitted version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

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.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available at the institutional Dataverse

repository of Epilepsy and Neuromodulation lab (DOI: <https://doi.org/10.14428/DVN/XPWLEF>) accessible upon request from the authors.

ORCID

Auriane Apaire  <https://orcid.org/0009-0003-2062-104X>

Elise Collard  <https://orcid.org/0009-0008-1576-9142>

Abigail Niyibizi  <https://orcid.org/0009-0006-2864-9591>

Natasha N. Kumar  <https://orcid.org/0000-0003-4712-8792>

Enrique Germany Morrison  <https://orcid.org/0000-0002-3100-187X>

Javier Chavez Cerda  <https://orcid.org/0000-0003-1155-6701>

Ayşe S. Dereli  <https://orcid.org/0000-0002-5584-197X>

Riem El Tahry  <https://orcid.org/0000-0002-2440-0194>

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SUPPORTING INFORMATION

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

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