

# Repeated exposure to transient obstructive sleep apnea-related conditions causes an atrial fibrillation substrate in a chronic rat model



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**BACKGROUND** High night-to-night variability in obstructive sleep apnea (OSA) is associated with atrial fibrillation (AF). Obstructive apneas are characterized by intermittent deoxygenation-reoxygenation and intrathoracic pressure swings during ineffective inspiration against occluded upper airways.

**OBJECTIVE** We elucidated the effect of repeated exposure to transient OSA conditions simulated by intermittent negative upper airway pressure (INAP) on the development of an AF substrate.

**METHODS** INAP (48 events/4 h; apnea-hypopnea index 12 events/h) was applied in sedated spontaneously breathing rats (2% isoflurane) to simulate mild-to-moderate OSA. Rats without INAP served as a control group (CTR). In an acute test series (ATS), rats were either killed immediately ( $n = 9$  per group) or after 24 hours of recovery (ATS-REC:  $n = 5$  per group). To simulate high night-to-night variability in OSA, INAP applications ( $n = 10$ ; 24 events/4 h; apnea-hypopnea index 6/h) were repeated every second day for 3 weeks in a chronic test series (CTS).

**RESULTS** INAP increased atrial oxidative stress acutely, represented in decreases of reduced to oxidized glutathione ratio (ATS:

INAP:  $0.33 \pm 0.05$  vs CTR:  $1 \pm 0.26$ ;  $P = .016$ ), which was reversible after 24 hours (ATS-REC: INAP vs CTR;  $P = .274$ ). Although atrial oxidative stress did not accumulate in the CTS, atrial histological analysis revealed increased cardiomyocyte diameters, reduced connexin 43 expression, and increased interstitial fibrosis formation (CTS: INAP  $7.0\% \pm 0.5\%$  vs CTR  $5.1\% \pm 0.3\%$ ;  $P = .013$ ), which were associated with longer inducible AF episodes (CTS: INAP:  $11.65 \pm 4.43$  seconds vs CTR:  $0.7 \pm 0.33$  seconds;  $P = .033$ ).

**CONCLUSION** Acute simulation of OSA was associated with reversible atrial oxidative stress. Cumulative exposure to these transient OSA-related conditions resulted in AF substrates and was associated with increased AF susceptibility. Mild-to-moderate OSA with high night-to-night variability may deserve intensive management to prevent atrial substrate development.

**KEYWORDS** Atrial fibrillation; Night-to-night variability; Obstructive sleep apnea; Rats; Substrate

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## Introduction

Obstructive sleep apnea (OSA) is prevalent in up to 70% of all patients with atrial fibrillation (AF) and affects the efficacy of pharmacological and catheter-based strategies for rhythm control.<sup>1–3</sup> In nonrandomized observational clinical trials, treatment of severe OSA improved AF catheter ablation outcomes.<sup>1,2</sup> Most clinical and preclinical research mainly focused on the role of severe OSA. However, whether nonsevere OSA leads to an AF substrate remains unclear.

OSA episodes are characterized by high-frequency desaturation-reoxygenation, and intrathoracic pressure swings occur during ineffective inspiration against the occluded upper airways, which increases transmural pressure gradients and may expose the thin-walled atria to increased stretch.<sup>4,5</sup> Additionally, despite the described high prevalence of OSA in patients with AF, longitudinal assessment of sleep apnea severity in patients with AF and cardiovascular implantable electronic devices in the VARIOSIA-AF study (Variability of Sleep Apnea Severity and Risk of Atrial Fibrillation) indicated that most patients with AF do not demonstrate severe sleep apnea every day, but rather mild or moderate sleep apnea and considerable intraindividual night-to-night variability.<sup>6–8</sup> This creates a scenario of transient exposure to OSA-related conditions, characterized by intermittent hypoxia and intrathoracic pressure swings. Such effect of transient exposure to OSA-related stressors have not been considered in recent chronic animal models, which mainly exposed the animals to severe OSA every day.<sup>9</sup>

We hypothesized that transient exposure to obstructive respiratory events characterized by intrathoracic pressure changes leads to atrial damage and that repeated exposure to these transient OSA-associated conditions results in a structural substrate for AF.

In this study, we developed a novel rat model for OSA in order to study the transient and acutely reversible effects of high-frequency desaturation-reoxygenation in combination with negative intrathoracic pressure changes induced by intermittent negative upper airway pressure (INAP) simulating obstructive respiratory events. Additionally, the development of an atrial arrhythmogenic substrate as a cumulative consequence of repeated exposure to transient and acutely reversible conditions induced by INAP (simulating long-term mild-to-moderate OSA with high night-to-night variability) was characterized.

## Methods

For detailed methods, please see [Online Supplemental Methods](#).

### Animal model

All animal studies were performed in accordance with the German law for the protection of animals. The investigation conforms to the *Guide for the Care and Use of laboratory Animals* published by the US National Institutes of Health (eighth edition; revised 2011). The study was approved by

the regional Animal Welfare Inspectorate (Saarländisches Landesamt für Verbraucherschutz: #18/2014).

Forty-seven rats (male Sprague Dawley, 260–300 g body weight [BW]) were purchased from Charles River (Sulzfeld, Germany) and housed 2 per cage under standard conditions (room temperature 24°C; relative humidity 55%; 12-hour dark/light cycle). Rats had free access to a standard diet (#1320, sodium content 0.2%, Altromin, Lage, Germany) and tap drinking water ad libitum.

### Anesthesia and killing

During experiments, rats were anesthetized with 2% isoflurane and 98% oxygen administered via a nebulizer. For killing, rats were sedated with nebulized isoflurane (2% plus 98% oxygen) and a single intraperitoneal injection of ketamine hydrochloride (80 mg/kg BW) and xylazine hydrochloride (6 mg/kg BW). The thorax was opened bilaterally along the midaxillary line, and the diaphragm was removed. For immediate killing and tissue sampling, the heart was harvested and dissected in left and right atria and ventricles.

### Application of hypoxia combined with INAP

INAP was applied via customized animal masks (#E5-5100, Breezer, Medico-Lab, Winsen, Germany) by a negative pressure device, which consists of a 50-L negative pressure container and a vacuum pump controlled by a manometer (Manometer Type 831, WIKA, Alexander Wiegand SE & Co. KG, Germany). The animal mask was connected via a flexible tube to the vacuum container, and a solenoid valve was opened and the tube and the vacuum container created a closed system (Figure 1A). The *Mueller maneuver*, which is defined as forced inspiration against airway obstruction, is used in the clinical setting to simulate conditions occurring during obstructive respiratory events, in particular negative thoracic pressure.<sup>10</sup> As a modification of this maneuver, we noninvasively applied a defined negative pressure of –40 hPa. During INAP, respiration efforts (inhalation and expiration) and pressure leakage of any kind were detectable in real-time recordings via a pressure sensor (Isotec/Sigma-Aldrich Chemie GmbH, Munich, Germany) with a data acquisition and analysis software (Notocord-hem Evolution, Notocord, Croissy, France) (Figure 1B). Vital parameters were visualized by a multichannel monitor (Vismo PVM-2701k, Nihon Kohden, Tokyo, Japan), and an SpO<sub>2</sub> probe was attached to the lower limb of the rat to record oxygen saturation (Figure 1C).

### Acute and chronic trials

In the acute test series (ATS), INAP was applied for 1 minute followed by a 4-minute resting period. INAP (INAP-ATS, n = 8) sequence was repeated 48 events/4 h (apnea-hypopnea index [AHI] 12 events/h). In pilot experiments, a 4-minute resting period between 2 consecutive INAP applications ensured an optimal recovery of vital parameters. Rats were killed immediately after the last INAP application (isoflurane anesthesia 2% plus 98% oxygen) and an intraperitoneal

injection of ketamine hydrochloride (80 mg/kg BW) and xylazine hydrochloride (6 mg/kg BW). As a control group (CTR-ATS,  $n = 9$ ), rats were anesthetized during the time period of the experiments but no INAP was applied. Additional rats underwent the same ATS protocol but were killed after 24 hours of the resting period (CTR-ATS-REC:  $n = 5$ ; INAP-ATS-REC:  $n = 5$ ) (Figure 1D). After killing, blood and cardiac tissue for biochemical analysis were preserved. Wet lung tissue was weighed and dried for 48 hours in a 60°C incubator and afterward weighed again for evaluation of fluid accumulation due to lung edema.

In the chronic test series (CTS), INAP was applied for 1 minute followed by a 9-minute resting period. The INAP (INAP-CTS,  $n = 10$ ) sequence was repeated 24 events/4 h (AHI 6 events/h) every second day throughout 3 weeks to simulate high night-to-night variability. As a control group (CTR-CTS:  $n = 10$ ), rats were anesthetized during the time period of the experiments but INAP was not applied. Twenty-four hours after the last INAP application, rats were killed (Figure 1D).

In CTS rats, blood pressure (by telemetry), left ventricular (LV) hemodynamics (by invasive pressure measurements), and AF inducibility (by transesophageal atrial stimulation) were determined. Cardiac tissue was harvested for histologi-

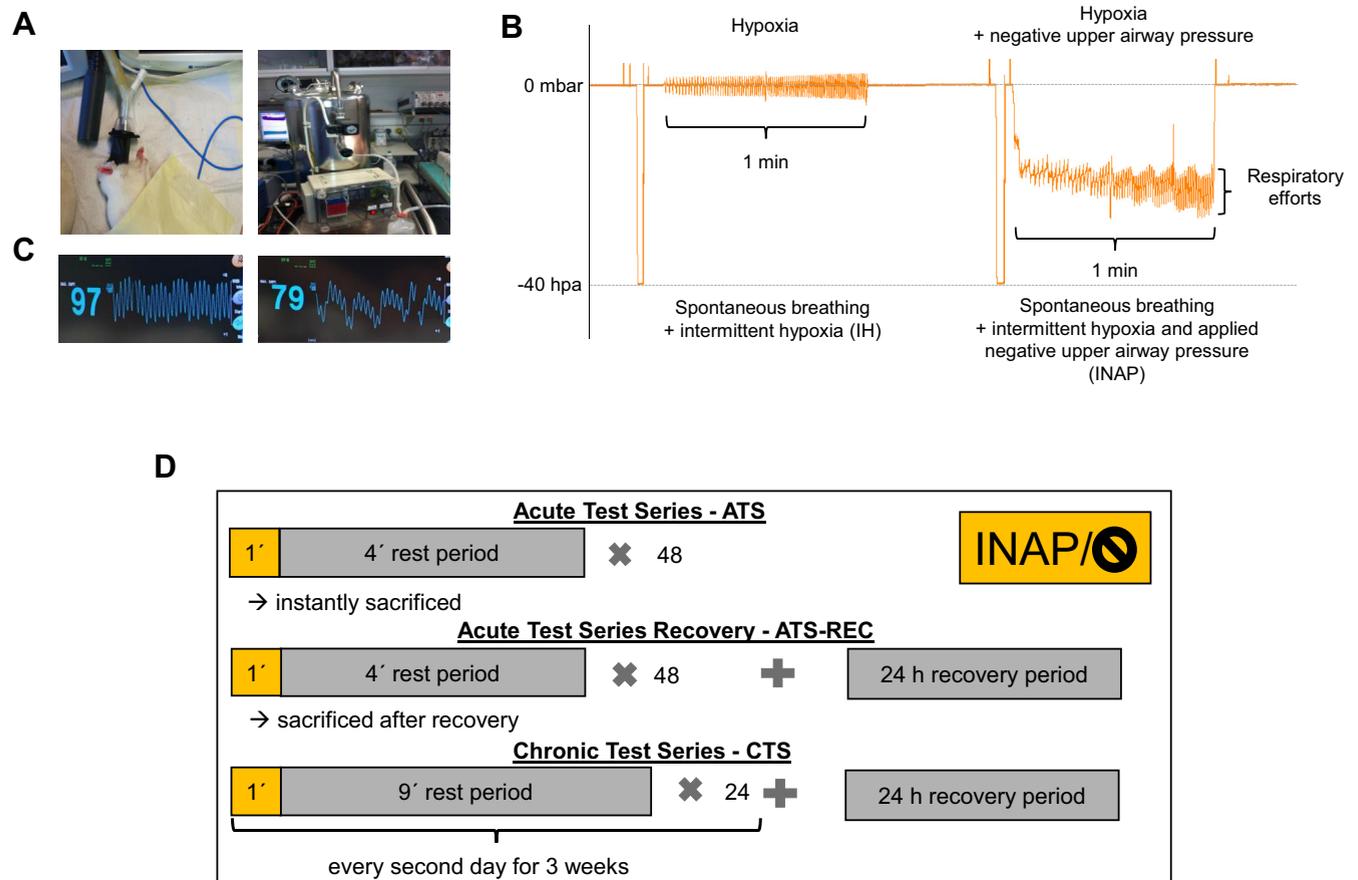
cal, immunohistochemical, biochemical, and microarray analyses, which were performed to characterize the development of an AF substrate.

### AF inducibility and duration

A surface electrocardiogram (ECG) was recorded using Notocord-hem Evolution. A lead with the optimal P-wave amplitude was selected to allow an optimal visualization of atrial activation. Susceptibility to AF was tested by using transesophageal atrial burst stimulation. In sedated rats, a 4-F catheter was placed in the esophagus in the height of the left atrium. An initial stimulation (cycle length 200 ms; amplitude twice the diastolic threshold; stimulus width 8.6 ms) was performed to ensure atrial capture in the surface ECG. Repetitive 3-second bursts of stimuli (cycle length 10 ms; amplitude twice the diastolic threshold; stimulus width 8.6 ms) were applied. When the surface ECG showed indistinguishable P waves and irregularly alternating RR intervals for at least 1 second, AF was diagnosed. Each subject was burst paced 20 times independent of successful AF induction.

### Statistical analysis

Data are expressed as mean  $\pm$  SEM. An unpaired Student  $t$  test (2-tailed) was used for statistical analysis comparing 2



**Figure 1** Experimental setup and protocol. **A:** Male Sprague Dawley rat breathing spontaneously under isoflurane sedation (2%, 98% O<sub>2</sub>) (left); negative pressure device (right). **B:** Representative recording of the upper airway pressure during 1-minute intermittent hypoxia (left) or intermittent negative upper airway pressure (INAP; right). **C:** Oxygen saturation monitoring using an oximetry probe attached to the lower limb of the rat. **D:** Protocol of the acute test series (ATS) and acute recovery series (ATS-REC) and chronic test series (CTS).

independent groups, a paired analysis was used for intrasubject analysis.  $P$  values  $< .05$  were regarded as statistically significant. Statistical analysis was performed using GraphPad Prism version 8.0.1 (GraphPad Software, La Jolla, CA).

## Results

### ATS: Transient short-term effects of INAP

INAP significantly increased respiratory rate compared with sole isoflurane deprivation (Figure 2A) and led to increases in respiratory efforts (Figure 2B). In the ATS-REC groups, mean oxygen desaturation did not differ from that in the ATS groups, demonstrating valid reproducibility during the ATS protocols (Figure 2C).

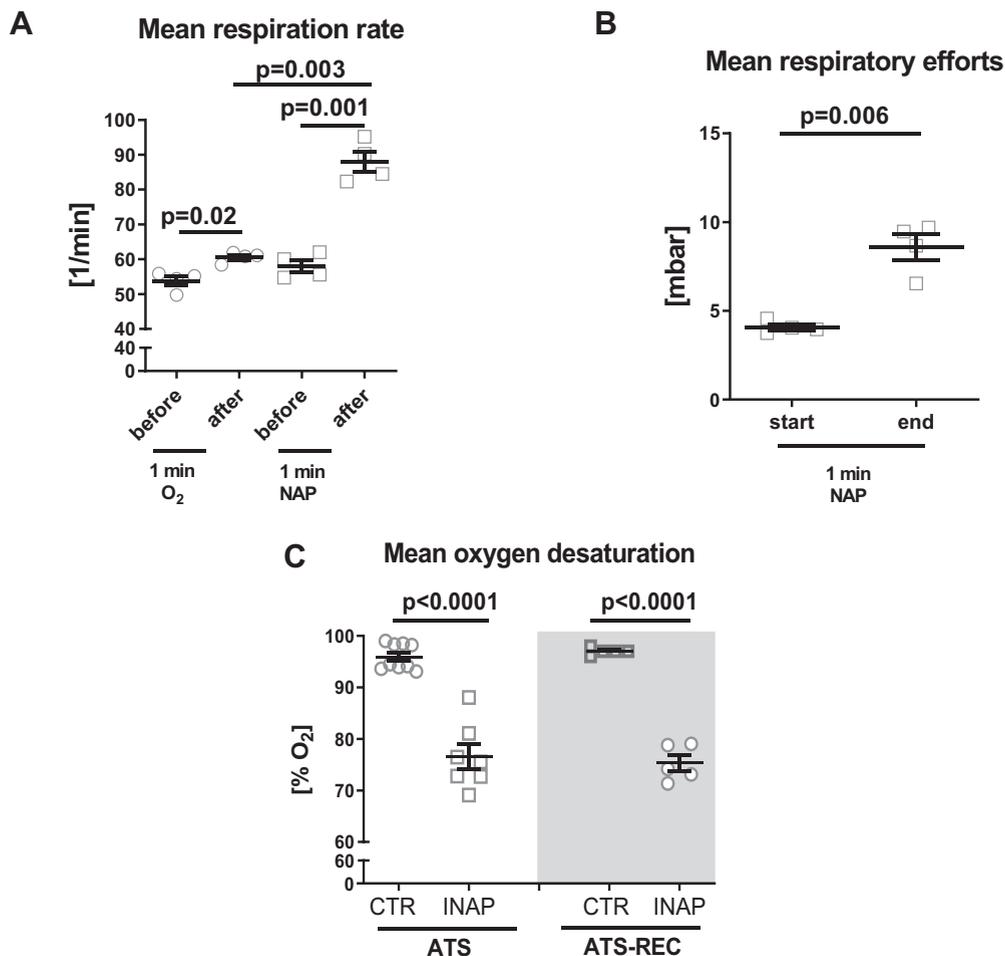
### Left ventricular expression of brain natriuretic peptide

Four hours of repetitively applied INAP resulted in an enhanced expression of brain natriuretic peptide (BNP) messenger RNA (mRNA), a marker for increased cardiac volume and pressure overload, in the LV of INAP-ATS compared with CTR-ATS ( $1.72 \pm 0.16$  vs  $1.10 \pm 0.14$  relative gene expression/glyceral-

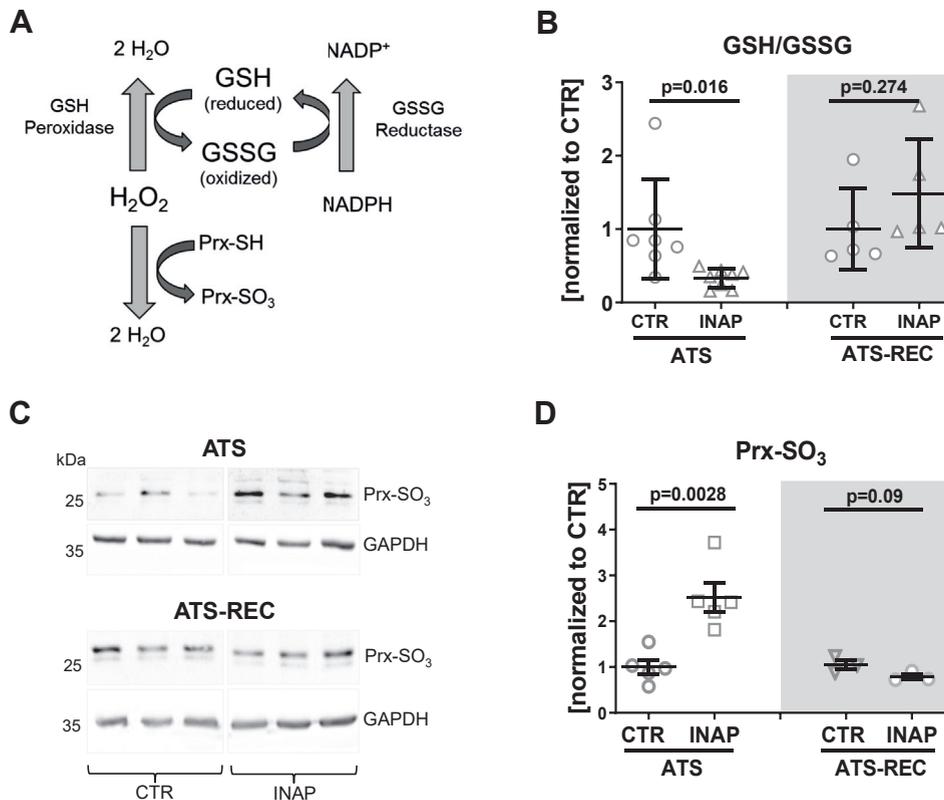
dehyde 3-phosphate dehydrogenase, respectively;  $P = .009$ ). In INAP-ATS-REC, LV BNP mRNA levels were normalized after 24 hours of recovery (CTR-ATS-REC:  $1.21 \pm 0.23$ ; INAP-ATS-REC:  $1.16 \pm 0.11$  relative gene expression/glyceraldehyde 3-phosphate dehydrogenase;  $P = .913$ ).

### Atrial oxidative stress

In order to determine the level of oxidative stress in the left atrium, the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) was determined. The atrial GSH/GSSG ratio was significantly reduced in INAP-ATS, indicating a lower cellular antioxidative capacity (Figures 3A and 3B) after INAP. Additionally, Western blot analysis demonstrated that peroxiredoxins were hyperoxidized after acute INAP (Figures 3C and 3D). These effects on left atrial antioxidative capacity and peroxiredoxin oxidation were transient and reversible after 24 hours of recovery in INAP-ATS-REC (Figures 3B and 3D). Of note, the phosphorylation status of connexin 43 was not affected in INAP-ATS (Online Supplemental Figure 1).



**Figure 2** Acute test series (ATS)—respiratory responses. **A:** Respiration rate at 1 minute before and 1 minute immediately after applied negative upper airway pressure (NAP) maneuver ( $n = 4$ ). Application of 1-minute oxygen (O<sub>2</sub>) served as controls for sole awakening reaction due to isoflurane deprivation ( $n = 4$ ). **B:** Respiratory efforts measured via the pressure difference of end expiration and end inhalation at the beginning and at the end of 1-minute intermittent negative upper airway pressure (INAP) maneuvers ( $n = 4$ ). **C:** Mean oxygen desaturation during the 4-hour-long experiment (ATS: CTR:  $n = 9$ ; INAP:  $n = 7$  and ATS recovery [ATS-REC]: CTR:  $n = 5$ ; INAP:  $n = 5$ ). Data are expressed as mean  $\pm$  SEM. For statistical analysis, a paired  $t$  test was used for panels A and B and an unpaired  $t$  test for panel C.



**Figure 3** Acute test series (ATS)—atrial oxidative stress. **A:** Scheme for the detoxification of oxidative stressor hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Within the cell, H<sub>2</sub>O<sub>2</sub> is enzymatically neutralized to water (H<sub>2</sub>O) by glutathione peroxidase by using reduced glutathione (GSH) as a substrate. As a product, oxidized glutathione (GSSG) is generated. GSSG is reduced by GSSG reductase and consumption of nicotinamide adenine dinucleotide phosphate (NADPH). Additionally, H<sub>2</sub>O<sub>2</sub> may be detoxified by reduced peroxiredoxins (Prx-SH), resulting in the hyperoxidized form Prx-SO<sub>3</sub>. **B:** Ratio of left atrial GSH and GSSG content (normalized to respective control groups [CTR or CTR-REC]; ATS: CTR: n = 7; intermittent negative upper airway pressure [INAP]: n = 8 and ATS recovery [ATS-REC]: CTR: n = 5; intermittent hypoxia: n = 5; INAP: n = 5). **C:** Representative Western blot of hyperoxidized peroxiredoxin (Prx-SO<sub>3</sub>) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a loading CTRs. **D:** Quantification of Prx-SO<sub>3</sub> Western blot analysis. ATS, and ATS-REC (normalized to respective CTR groups [CTR or CTR-REC]; ATS: CTR: n = 5; INAP: n = 5 and ATS-REC: CTR: n = 3; INAP: n = 3). Data are expressed as mean ± SEM. For statistical analysis, an unpaired *t* test was used.

### CTS: Effects of repeated exposure to transient INAP-induced stress

CTS rats were repeatedly exposed to acutely reversible conditions induced by 4 hours of INAP every second day for 3 weeks to allow intermittent recovery from transient short-term effects of OSA maneuvers. Mean oxygen desaturation associated with INAP applications was comparable in INAP-CTS and INAP-ATS throughout the CTS protocol (Figures 4A and 2C). Heart rate, mean arterial blood pressure, and arterial pulse pressure measured by telemetry measured during 3 weeks of the INAP protocol did not show any differences between the CTR and INAP groups (Figures 4C–4E). Blood pressure measured using the tail-cuff method during the CTS protocol confirmed the finding of telemetry-based measurements (Online Supplemental Table 1).

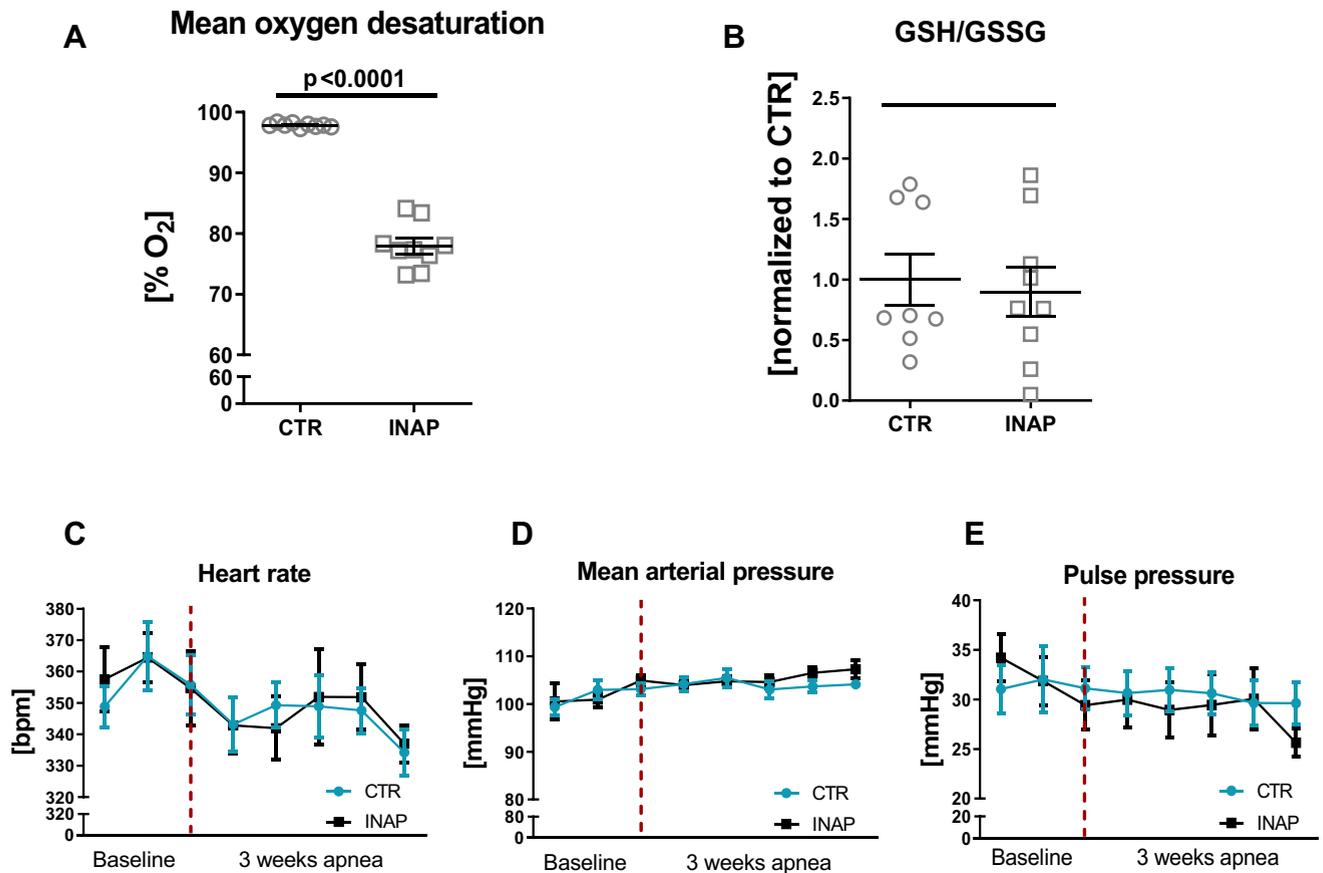
#### LV hemodynamics and remodeling

Chronic application of INAP had no effect on heart weight/tibia length ratio, nor on LV cardiomyocyte diameter,

GSH/GSSG ratio, or BNP mRNA expression as compared with CTR application (Online Supplemental Table 1). The ratio of wet to dry lung tissue did not indicate the development of lung edema throughout the protocol (CTR: 4.43 ± 0.26 vs INAP: 4.39 ± 0.32; *P* = .93). However, INAP-CTS rats demonstrated increased LV fibrosis formation compared with CTR-CTS rats (Online Supplemental Figure 2). Invasive LV hemodynamic measurements revealed no significant changes in diastolic or systolic parameters (Online Supplemental Table 2).

#### Atrial remodeling

In INAP-CTS, atrial antioxidative capacity (GSH/GSSG ratio) measured 24 hours after the last applied INAP maneuver did not differ between groups (Figure 4B), suggesting a transient exposure to reversible INAP-related oxidative stress throughout the 3 weeks of the CTS protocol. To gain further insight into gene programs activated in left atrial tissue by chronic INAP, we performed a gene microarray using



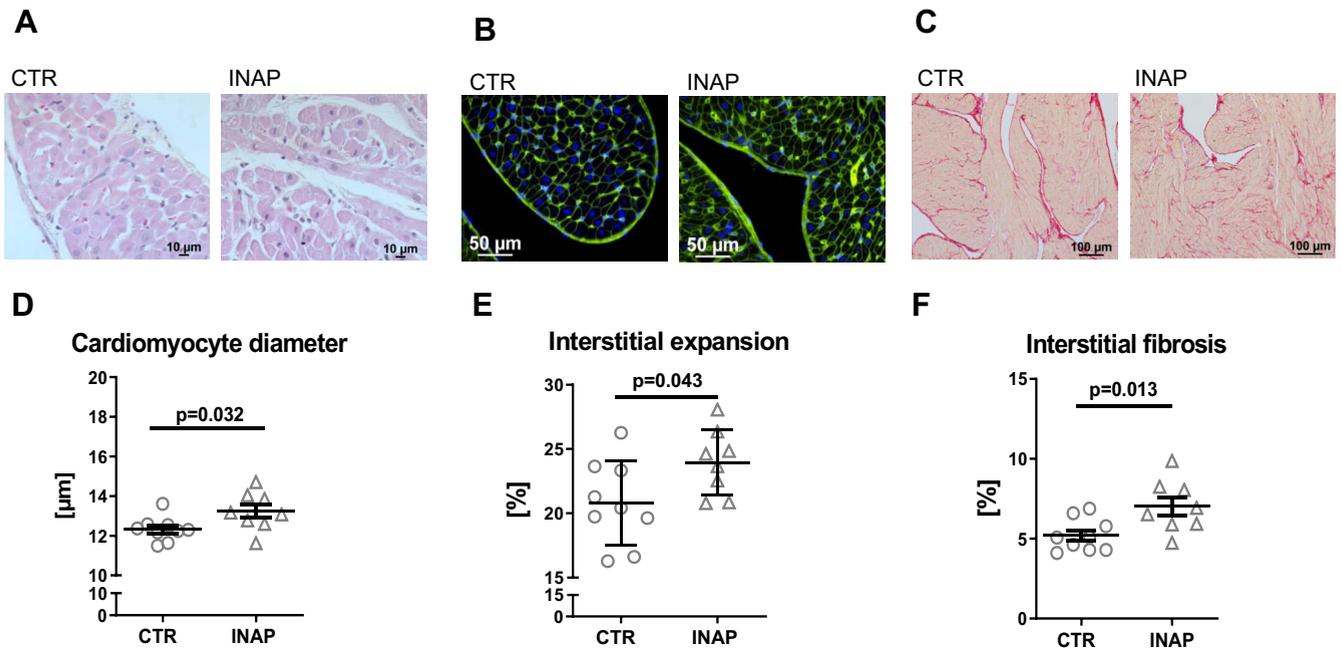
**Figure 4** Chronic test series (CTS)—oxygen desaturation and telemetry hemodynamics. **A:** Mean oxygen desaturation during 3 weeks of the CTS experiments (control group [CTR]: n = 9; intermittent negative upper airway pressure [INAP]: n = 9). **B:** Ratio of left atrial reduced glutathione (GSH) and oxidized glutathione (GSSG) content (normalized to CTR; CTR: n = 8; INAP: n = 9). **C–E:** Catheter-based telemetry recording of heart rate (panel C), mean arterial pressure (panel D), and pulse pressure in CTR (n = 5) and INAP (n = 7) rats (panel E) throughout the protocol. Data are expressed as mean  $\pm$  SEM.

biotinylated antisense complementary DNA derived from the left atria of INAP-CTS and CTR-CTS rats. One thousand thirty-one genes were significantly dysregulated in the left atria of INAP-CTS 24 hours after the final INAP maneuver. A KEGG pathway analysis revealed a differential expression of genes associated with the development of atrial remodeling and arrhythmia, such as the adenosine monophosphate-activated protein kinase signaling pathway,<sup>11</sup> the insulin signaling pathway,<sup>12</sup> and the peroxisome proliferator-activated receptor signaling pathway<sup>13</sup> in the left atrium of INAP-CTS (Online Supplemental Figures 3–5). Histological analysis of the left atria presented slightly enhanced myocyte diameters, mild but significant expansion of the left atrial interstitium, and increased collagen formation in INAP-CTS (Figure 5). Immunofluorescence staining of atrial connexin 43 demonstrated a striking decrease in connexin 43 protein expression after chronic INAP (Figure 6 and Online Supplemental Figure 6). Of note, left atrial remodeling was neither associated with increased expression of inflammatory marker genes such as tumor necrosis factor, interleukin 1b (IL1b), IL6, or IL10 nor with macrophage or neutrophil infiltration of atrial tissue (Online Supplemental Figure 7). Right

atrial histological staining revealed changes in neither cardiomyocyte diameters nor interstitial fibrosis (Online Supplemental Figure 8). P-wave durations (INAP-CTS:  $21.93 \pm 1.59$  ms vs CTR-CTS:  $19.24 \pm 0.5$  ms;  $P = .15$ ) and PR intervals (INAP-CTS:  $52.39 \pm 1.74$  ms vs CTR-CTS:  $50.36 \pm 1.88$  ms;  $P = .44$ ) were not significantly altered in INAP-CTS compared with CTR-CTS (Online Supplemental Table 1). However, AF inducibility and durations were significantly increased in INAP-CTS (Figure 7). Telemetry recordings did not reveal any spontaneous AF periods throughout the CTS.

## Discussion

To investigate the effect of mild-to-moderate OSA with high night-to-night variability on AF substrates, we developed and described a novel rat model for OSA-related atrial remodeling, which represents a unique approach (1) to investigate effects of transient OSA-related conditions (namely, intermittent negative airway pressure) and (2) to study the development of an arrhythmogenic substrate as a cumulative result of repeated exposure to transient and acutely reversible biological responses to OSA.



**Figure 5** Chronic test series (CTS)—left atrial (LA) structural remodeling processes. Representative histological staining of atrial (A) cardiomyocyte hypertrophy (hematoxylin and eosin staining), (B) interstitial expansion (wheat germ agglutinin staining), and (C) interstitial fibrosis (Picro Sirius Red staining) in the CTS. Quantification of LA (D) cardiomyocyte diameters, (E) interstitial expansion, and (F) interstitial fibrosis in the CTS (control group [CTR]:  $n = 9$ ; intermittent negative upper airway pressure [INAP]:  $n = 8$ ). Data are expressed as mean  $\pm$  SEM. For statistical analysis, an unpaired  $t$  test was used.

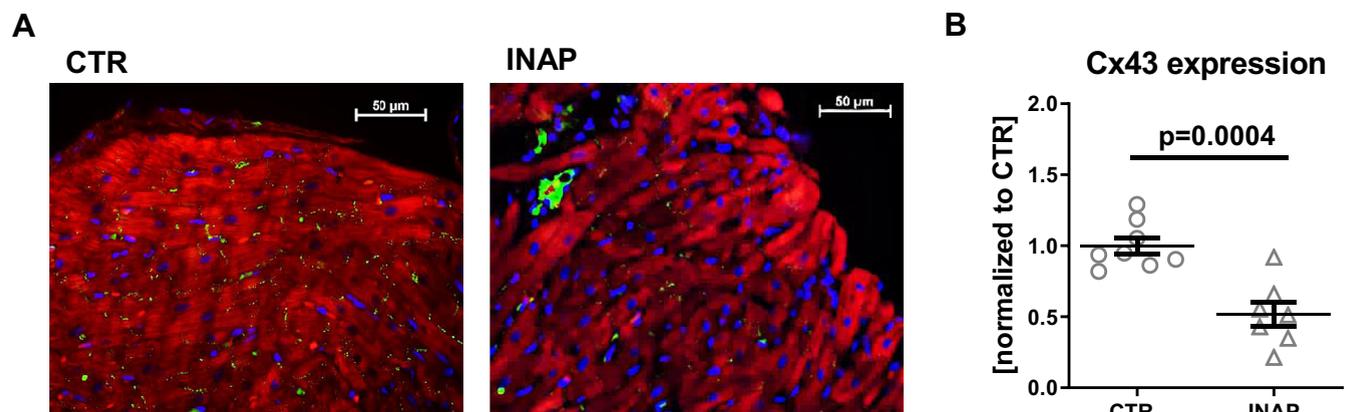
### Major findings

Four hours of simulated OSA transiently increased atrial oxidative stress, which completely reversed within 24 hours. Mild-to-moderate OSA with high night-to-night variability simulated by repeated exposure to these transient biological responses related to OSA for 3 weeks resulted in an arrhythmogenic substrate for AF.

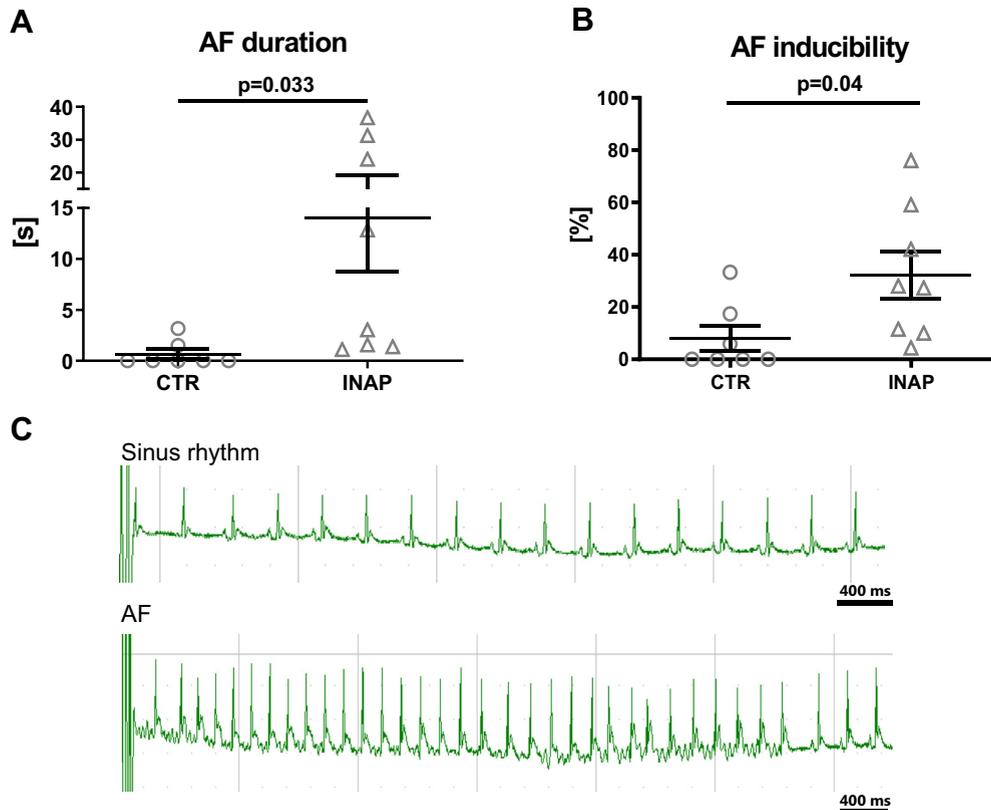
### Comparison with previous animal studies

Several mechanisms have been identified to contribute to AF in the setting of severe OSA. Acute apnea-associated atrial electrophysiological changes and increased occurrence of triggers due to intermittent hypoxemia followed by reoxygenation,

intrathoracic pressure changes during ineffective breathing attempts, and arousal-related sympathovagal activation create a complex and dynamic substrate for AF during sleep.<sup>4,14</sup> Additionally, long-term severe OSA has been shown to be associated with atrial enlargement, voltage reduction, and site-specific conduction abnormalities in humans<sup>15</sup> and with connexin dysregulation and increased AF inducibility in rodent models.<sup>9</sup> In recently described animal models for sleep apnea (SA) with daily orotracheal intubation during deep sedation,<sup>9</sup> implantable tracheal balloons,<sup>16</sup> or sleep chambers,<sup>17</sup> noninvasive induction of upper airway occlusions during light anesthesia has not been possible to simulate conditions of OSA. Herein, we introduce a rat model with INAP-inducing standardized and reproducible simulated collapses of the upper airways. Four hours of INAP



**Figure 6** Chronic test series (CTS)—left atrial connexin 33 remodeling. A: Representative immunofluorescence staining of atrial connexin 33 (Cx43). B: Quantification of left atrial Cx43 expression in the CTS (control group [CTR]:  $n = 8$ ; intermittent negative upper airway pressure [INAP]:  $n = 7$ ). Data are expressed as mean  $\pm$  SEM. For statistical analysis, an unpaired Student  $t$  test (2-tailed) was used.



**Figure 7** Chronic test series (CTS)—atrial fibrillation (AF) inducibility. Inducible AF duration (A) and inducibility of AF (B) after transesophageal burst stimulation in the CTS (control group [CTR]; n = 7; intermittent negative upper airway pressure [INAP]; n = 8). C: Representative surface electrocardiographic recordings of sinus rhythm (*top*) and AF (*bottom*) after transesophageal burst stimulation. Data are expressed as mean  $\pm$  SEM. For statistical analysis, an unpaired *t* test was used.

transiently increased atrial oxidative stress, which was reversible within 24 hours. Pilot data in our rat model suggest that repetitive mechanical cardiac pressure alterations as in this model induced by INAP is crucial for a prominent decrease in antioxidative capacity in the atria, which could not be induced by intermittent hypoxia alone.

### Arrhythmogenic consequences of transient exposure to sleep apnea due to high night-to-night variability

Clinical and preclinical studies show that long-term severe OSA is associated with progressive atrial remodeling.<sup>9,14–16</sup> However, as most patients with AF show mild-to-moderate OSA with considerable intraindividual night-to-night variability in OSA severity,<sup>6</sup> we applied a relatively low number of INAP events per hours (6 events/h) and repeated INAP maneuvers just every second day to mimic mild-to-moderate OSA with high night-to-night variability in OSA severity in our rat model. Despite acute reversibility of INAP-induced atrial oxidative stress within 24 hours, 3 weeks of repeated exposure to transient oxidative stress every other day increased AF susceptibility. This AF susceptibility reflects the arrhythmogenic consequence of the underlying AF substrate independent from acute transient INAP-associated factors as it was tested 24 hours after the last INAP maneuver

when atrial oxidative stress was not increased anymore. Our results provide insights into the underlying mechanisms of increased AF inducibility including the development of a structural atrial remodeling process predominantly characterized by reduced connexin 43 expression.

Importantly, the development of an atrial arrhythmogenic substrate was independent of the development of hypertension or overt LV diastolic or systolic dysfunction. Intrathoracic pressure swings, as observed during INAP, result in cyclical atrial stretch, which mechanically stress atrial tissue.<sup>4,18</sup> Rodent models confirm that repeated stretch transiently activates stretch-sensitive and oxidative stress sensitive profibrotic signaling pathways and that stretch in combination with low doses of H<sub>2</sub>O<sub>2</sub> sensitize cells to early afterdepolarizations, which may further contribute to increased AF susceptibility.<sup>19</sup> Affymetrix analysis in our study identified a differential expression of multiple components of the adenosine monophosphate-regulatory protein kinase signaling pathway in rats with INAP, which has been shown to be involved in the initiation, maintenance, and progression of atrial arrhythmias.<sup>11</sup> INAP resulted in a differential mRNA regulation of members of the insulin signaling pathway, such as insulin-like growth factor 1 receptor and ETS transcription factor 1, both shown to play an important role in the pathogenesis of AF.<sup>12,20</sup>

## Study limitations

In this novel animal model for OSA, we applied stable and reproducible INAP maneuvers at  $-40$  hPa, which was also measured in patients with obstructive OSA.<sup>10</sup> The actual pressure in the thoracic cavity, however, was not measured, which might differ from the negative pressure applied during INAP maneuvers. In order to address the effect of experimental conditions, such as volatile sedation,<sup>21</sup> on the transient increase in oxidative stress, we introduced a CTR group in our study. Affymetrix analysis (Affymetrix, Santa Clara, USA), was performed in atria of rats killed 24 hours after the last INAP maneuver. Similar to the transient increase in atrial oxidative stress, gene expression levels may be partially reversible within this period, which may have contributed to an inhomogeneous gene expression pattern in the INAP group. Inflammatory cytokines and infiltration of macrophages were studied in this model, indicating increased systemic inflammation, which is associated with OSA.<sup>21</sup> However, precise inflammasome signaling was not studied in depth. Moreover, neither ion channel expression nor function was studied. In this model, we investigated the isolated effects of OSA on atrial remodeling. Because of the small size of rat atria, an analysis of the spatial distribution of atrial remodeling processes was not possible. Moreover, additional classical contributory factors related to common comorbidities in patients with AF and sleep apnea, including hypertension or obesity,<sup>22</sup> were not incorporated in the present rat model. Whether treatment of OSA, without targeting concomitant risk factors within an aggressive risk factor modification program, can prevent progression of AF or reverse structural remodeling processes in the atrium is unknown.<sup>22</sup> Clinical investigation is needed to validate the applicability of our findings in humans.

## Clinical perspectives

OSA is highly prevalent in patients with AF and affects the efficacy of pharmacological and catheter-based strategies for rhythm control.<sup>1,2,15,23</sup> Although clinical assessment of OSA-severity and OSA treatment initiation is primarily guided by the AHI, representing the number of apneas and hypopneas per hour of sleep, a more detailed characterization of the dynamicity of apneas in patients with AF may result in a better disease-based assessment of OSA in patients with AF. Moreover, our results have relevance to therapeutic approaches. Although treatment of OSA is mainly recommended in patients with AF and severe sleep-disordered breathing,<sup>24</sup> our findings suggest that even mild-to-moderate OSA with high night-to-night variability is sufficient to result in an arrhythmogenic substrate and may therefore warrant consequent management. Identification of mild-to-moderate OSA with high night-to-night variability may easily be missed by a single night sleep study spot assessment of sleep-disordered breathing. High night-to-night variability in OSA severity may require a long-term OSA screening approach to capture the daily OSA pattern and severity.<sup>6</sup>

## Conclusion

Simulated acute OSA by INAP transiently increased atrial oxidative stress. Although acute INAP-induced oxidative stress was completely reversible, cumulative exposure to transient INAP-related conditions every second day resulted in an arrhythmogenic AF substrate. Not just severe, but even mild-to-moderate OSA with high night-to-night variability may represent a treatment target to prevent the progression of AF substrates.

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## Appendix Supplementary data

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.hrthm.2020.10.011>.

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