

1 Enhancement of central norepinephrinergic neurotransmission contributes to  
2 inhibition of seizure-induced respiratory arrest by targeting the beta-1 adrenergic  
3 receptor ( $\beta$ 1-AR) locating in the cardiomyocytes in the DBA/1 mouse SUDEP model

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## 11 **Abstract**

12 Sudden unexpected death of epilepsy (SUDEP) is the key cause of of death in  
13 patients with epilepsy. Due to the complicated pathogenesis of SUDEP, however, the  
14 exact mechanism of SUDEP remains elusive. Currently, although it is recognized that  
15 the seizure-induced respiratory arrest (S-IRA) may be a main cause for SUDEP, other  
16 factors resulting in SUDEP can not be excluded e.g arrhythmias. Our previous  
17 findings indicated that the incidence of seizure-induced respiratory arrest S-IRA and  
18 SUDEP evoked by acoustic stimulation or pentetrazol (PTZ) injection was  
19 significantly reduced by atomoxetine, a norepinephrine reuptake inhibitor (NRI),  
20 suggesting that noradrenergic neurotransmission modulates S-IRA and SUDEP. Given  
21 that norepinephrine acts on the central and peripheral target to modulate respiratory  
22 and circulation function by targeting adrenergic receptor  $\alpha$  and beta ( $\alpha$ -AR and  $\beta$ -AR)  
23 and the arrhythmias can be contributed to SUDEP. Meanwhile, to further test whether  
24 cardiac factors are implicated in S-IRA and SUDEP, we choose esmolol hydrochloride,  
25 a selective antagonist of beta-1 adrenergic receptor ( $\beta$ 1-AR) to test it in our models.  
26 Our findings demonstrated that the lower incidence of S-IRA and SUDEP evoked by  
27 acoustic stimulation or PTZ in DBA/1 mice by administration with atomoxetine was  
28 significantly reversed by intraperitoneal (IP) of esmolol hydrochloride. Importantly,  
29 the data of electrocardiogram (ECG) showed that the cardiac arrhythmia evoked by  
30 acoustic stimulation including the ventricular tachycardia, ventricular premature beat

1 and atrioventricular block and administration of atomoxetine significantly reduced  
2 these arrhythmias and the incidence of S-IRA and SUDEP in our models. Thus, the  
3 dysfunction of respiratory and circulation may be implicated in the pathogenesis of  
4 S-IRA and SUDEP hand in hand and enhancing central norepinephrinergic  
5 neurotransmission contributes to inhibition of seizure-induced respiratory arrest by  
6 targeting  $\beta$ 1-AR locating in the cardiomyocytes. Our findings will show a new light  
7 on decoding the pathogenesis of SUDEP.

8 **Keywords:** sudden unexpected death in epilepsy (SUDEP); seizure-induced  
9 respiratory arrest S-IRA); esmolol hydrochloride (Esmolol); Electrocardiogram  
10 (ECG); locus coeruleus (LC); cardiac arrhythmia; pentetrazol (PTZ)

## 11 1. INTRODUCTION

12 It had been recognized that sudden unexpected death in epilepsy (SUDEP) is the  
13 key cause leading to the mortality of epilepsy patients, especially those who had  
14 undergone antiepileptic drug resistance <sup>[1-4]</sup>. Although some important advancements  
15 concerning the pathogenesis of SUDEP had been obtained, to our certain knowledge,  
16 decoding SUDEP seems to be a huge challenge in views of the complicated  
17 mechanism of SUDEP. Indeed, seizure-induced respiratory arrest (S-IRA) is an  
18 important cause resulting in the death of epilepsy including our previous findings <sup>[2-6]</sup>.  
19 However, more and more evidence hinted that the mechanisms of SUDEP are likely  
20 to be heterogeneous <sup>[5]</sup>. Some data showed that the dysfunction in circulation may be a  
21 contributor to SUDEP. In fact, both of the dysfunction of respiratory and circulation  
22 was in implicated in S-IRA and SUDEP. Previous findings showed that the cardiac  
23 and respiratory function were simultaneously suppressed to induce the S-IRA and  
24 SUDEP evoked by acoustic stimulation in DBA/1 mice, suggesting that the  
25 autonomic nerve including the sympathetic and parasympathetic nerve were involved  
26 in the course of S-IRA and SUDEP.

27 Our previous data unveiled that the incidence of S-IRA and SUDEP evoked by  
28 acoustic or PTZ can be significantly reduced by IP with atomoxetine, a  
29 norepinephrine reuptake inhibitor (NRI) <sup>[6]</sup>. Considering that the central and  
30 peripheral norepinephrine transmission was associated closely with the activity of the

1 sympathetic nerve. It was well known that norepinephrine transmission modulates the  
2 respiratory and circulation function by targeting the adrenergic  $\alpha 1$ ,  $\alpha 2$ ,  $\beta 1$ , and  $\beta 2$   
3 receptors. The findings from our and other laboratories showed  $\alpha 1$  and  $\alpha 2$  receptors  
4 were implicated in the S-IRA and SUDEP mediated by atomoxetine in the DBA/1  
5 mice models [7-8]. However, whether both of  $\beta 1$  and  $\beta 2$  receptors, especially  $\beta 1$   
6 receptor located in the cardiomyocytes that play a key role in modulating the cardiac  
7 function seems to essentially be explored based on our and other findings. Therefore,  
8 we choose esmolol hydrochloride, a selective antagonist of beta-1 adrenergic receptor  
9 ( $\beta 1$ -AR) to test whether the  $\beta 1$ -AR was implicated in the atomoxetine mediated  
10 S-IRA and SUDEP. Our findings showed that the lower incidence of S-IRA and  
11 SUDEP evoked by acoustic stimulation mediated by atomoxetine was significantly  
12 reversed by Esmolol. Thus, our data further demonstrated that the cardiac factors  
13 implicated in the reduction of the incidence of S-IRA and SUDEP by atomoxetine  
14 can't be excluded and further contribute to explaining the possibility of heterogeneous  
15 factors leading to SUDEP.

## 16 **2. MATERIALS AND METHODS**

### 17 **2.1 Animals**

18 All experimental procedures were the agreement with the National Institutes of Health  
19 Guidelines for the Care and Use of Laboratory Animals and approved by the Animal  
20 Advisory Committee of Zhejiang University. DBA/1 mice were housed and bred in  
21 the Animal Center of Zhejiang University School of Medicine and provided with  
22 rodent food and water ad libitum. DBA/1 mice of either sex were used in this  
23 experiment, as previous studies have shown, gender is not a variable that affects the  
24 S-IRA of DBA/1 mice. Aiming at acoustic stimulation murine model, DBA/1 mice  
25 were “primed” starting from postnatal days 26-28 to establish the consistent  
26 susceptibility to audiogenic seizures and S-IRA and the PTZ-evoked S-IRA model  
27 was established as previously described [6-7].

### 28 **2.2 Seizure induction and resuscitation**

29 S-IRA was established by acoustic stimulation as we previously described [6-7]. For  
30 the acoustic stimulation model, each mouse was placed in a cylindrical plexiglass

1 chamber in a sound-isolated room, and audiogenic seizures (AGSZs) were evoked by  
2 using an electric bell (96 dB SPL, Zhejiang People's Electronics, China). Acoustic  
3 stimulation was given for a maximum duration of 60 s or until the onset of tonic  
4 seizures and S-IRA in most mice in each group. Mice with S-IRA have resuscitated  
5 within 5 s post the final respiratory gasp by a rodent respirator (YuYan Instruments,  
6 Shanghai, China). S-IRA was also established in all non-primed DBA/1 mice by IP  
7 administration of a single dose of PTZ (Cat #P6500; Sigma-Aldrich, St. Louis, MO)  
8 at a dose of 75 mg/kg.

### 9 **2.3 Effects of IP administration of Esmolol on the atomoxetine-mediated** 10 **suppression of S-IRA evoked by acoustic stimulation**

11 The susceptibility of DBA/1 mice to S-IRA was confirmed 24 hours before  
12 atomoxetine or vehicle administration. To IP administration, atomoxetine and esmolol  
13 were dissolved in saline. Different dosages of esmolol were applied to a vehicle  
14 control group and treatment groups, in which DBA/1 mice would be acoustically  
15 stimulated later. Atomoxetine (15 mg/kg, Ca # Y0001586; Sigma-Aldrich) or vehicle  
16 was intraperitoneal (IP) injection in DBA/1 mice 120min prior to acoustic stimulation,  
17 115min after infusion of atomoxetine (or vehicle), IP injection of esmolol (25mg/kg,  
18 50mg/kg, H19991058; Qilu Pharmaceutical Co., Ltd.) or vehicle 5min before acoustic  
19 stimulation (n=5-7/per group). The incidence of S-IRA, latency to AGSZs, duration of  
20 wild running, clonic seizures, tonic-clonic seizures, and seizure scores were  
21 videotaped for offline analysis. DBA/1 mice from the different pre-treatment groups,  
22 the monitoring of ECG was performed before acoustic stimulation and post-S-IRA.  
23 To exclude the effects of esmolol on the incidence of the mortality of DBA/1 mice, the  
24 group, subjected to confirmation of S-IRA before 24 hours to experiment, was  
25 pre-treated with the esmolol (50mg/kg, i.p) without administration of atomoxetine and  
26 acoustic stimulation in the same manner to observe the incidence of the mortality of  
27 DBA/1 mice (n= 6-7/per group).

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## 1 **2.4 Effects of IP administration of Esmolol on the atomoxetine-mediated** 2 **suppression of S-IRA evoked by PTZ**

3 The experiments were performed as follows: 1) For the control group, saline (i.p)  
4 was administered 120 min prior to and 10 min post PTZ (75 mg/kg, i.p), respectively  
5 (n=8); 2) For the Esmolol group, saline (i.p) was administered 120 min and prior to  
6 and Esmolol (50 mg/kg, i.p ) was administered 10 min post PTZ (75 mg/kg, i.p),  
7 respectively (n=7); 3) For the atomoxetine group, atomoxetine (15 mg/kg, i.p ) was  
8 administered 120 min and prior to and saline (i.p) was administered 10 min post PTZ  
9 (75 mg/kg, i.p), respectively (n=5); 4) For the atomoxetine with Esmolol group,  
10 atomoxetine (15 mg/kg, i.p ) was administered 120 min prior to and Esmolol (50  
11 mg/kg, i.p) was administered 10 min post PTZ (75 mg/kg, i.p), respectively (n=5).  
12 The S-IRA latency to AGSz, duration of wild running and clonic seizures, duration  
13 tonic-clonic seizures, and seizure scores were determined by offline analysis of video  
14 recordings (n= 6-7/per group).

## 15 **2.6 Data analysis**

16 Statistical analyses were performed using SPSS 23(SPSS Software Inc., USA).  
17 The incidence of S-IRA was compared in different groups using Wilcoxon Signed  
18 Rank test, as these data are nonparametric. The data on seizure scores and the latency  
19 to AGSZs, the duration of wild running, clonic seizures, and tonic-clonic seizures  
20 were evaluated using the one-way ANOVA tests, which were presented as the mean  $\pm$   
21 SEM. Statistical significance was inferred if  $p < 0.05$ .

## 22 **3. Results**

### 23 **3.1 Administration of Esmolol significantly reversed the atomoxetine-mediated** 24 **suppression of S-IRA evoked by acoustic stimulation**

25 To investigate the effects of Esmolol on the incidence of S-IRA and SUDEP  
26 medicated by atomoxetine based on our previous findings. The following experiments  
27 were performed. The incidence of S-IRA evoked by acoustic stimulation in primed  
28 DBA/1 mice was obviously reduced by atomoxetine(15 mg/kg,i.p )compared with the  
29 vehicle group(n=6, n=7,  $p < 0.001$ ). There was no significant difference between the  
30 vehicle group and the group pre-treated with esmolol ( 50 mg/kg,i.p ) and with vehicle

1 (n=7,n=6,  $p > 0.05$ ). However, the incidence of -S-IRA in the group pre-treated with  
2 atomoxetine(15 mg/kg,i.p) and esmolol(50 mg/kg,i.p) was significantly increased than  
3 the group pre-treated with atomoxetine(15 mg/kg,i.p) and the vehicle(n=6, n=7,  $p <$   
4  $0.05$ ). The incidence of -S-IRA in the group pre-treated with atomoxetine(15  
5 mg/kg,i.p) and the vehicle was significantly decreased than in the group pre-treated  
6 with the vehicle and with Esmolol(50 mg/kg,i.p) (n=6, n=6,  $p < 0.001$ ). The  
7 difference of latencies to AGSZs, durations of wild running, clonic seizures,  
8 tonic-clonic seizures, and seizure scores in the treatment group showed administration  
9 of esmolol significantly reversed the atomoxetine-mediated suppression of S-IRA  
10 evoked by acoustic stimulation without sensitivity to seizures. These data showed the  
11 atomoxetine-mediated suppression of S-IRA evoked by acoustic stimulation can be  
12 significantly reversed by esmolol, which means that administration of atomoxetine  
13 reduced the incidence of S-IRA by targeting the  $\beta$ 1-AR locating in the  
14 cardiomyocytes in our models. (FIG 1)

### 15 **3.2 Administration of Esmolol specifically reversed the atomoxetine-mediated** 16 **suppression of S-IRA by targeting the $\beta$ 1-AR locating in the cardiomyocytes**

17 To exclude whether the dose of Esmolol ( 50 mg/kg,i.p ) produces the morality of  
18 DBA/1 mice to affect the specificity to reverse the reversed atomoxetine-mediated  
19 suppression of S-IRA in our models. The following experiments were performed in  
20 different groups. There was no significant difference in the morality of DBA/1 mice  
21 between the group pre-treated with the vehicle and the group pre-treated with Esmolol  
22 ( 50 mg/kg,i.p ) ( $P>0.05$ ), suggesting that the atomoxetine-mediated suppression of  
23 S-IRA can be specifically reversed by esmolol by targeting the  $\beta$ 1-AR locating in the  
24 cardiomyocytes. (FIG 2)

### 25 **3.3 Obvious cardiac arrhythmia immediately occurred with tonic seizures and** 26 **S-IRA evoked by acoustic stimulation**

27 To observe the changing of ECG in the different treatment groups to analyze the role  
28 of atomoxetine and/or esmolol in cardiac electrophysiology. We observed that the  
29 ventricular tachycardia, ventricular premature beat, and atrioventricular block  
30 frequently occurred immediately following tonic seizures and S-IRA evoked by

1 acoustic stimulation in DBA/1 mice in a group pre-treated with the vehicle and with  
2 the vehicle. However, Compared with the vehicle group, the arrhythmia was  
3 significantly decreased in the group pre-treated with atomoxetine ( 15 mg/kg,i.p ) and  
4 vehicle. We also observed the arrhythmia frequently occurred in the group pre-treated  
5 with esmolol and the vehicle and the group pre-treated with atomoxetine (15  
6 mg/kg,i.p) and esmolol, suggesting that Esmolol can specifically reverse the  
7 atomoxetine-mediated suppression of S-IRA by targeting the  $\beta$ 1-AR locating in the  
8 cardiomyocytes without resulting in extra arrhythmia. (FIG 3-4)

### 9 **3.4 Administration of Esmolol significantly reversed the atomoxetine-mediated** 10 **suppression of S-IRA evoked by PTZ**

11 To further investigate whether administration of Esmolol significantly reversed the  
12 atomoxetine-mediated suppression of S-IRA depends on S-IRA and SUDEP models  
13 or not. The PTZ-induced S-IRA was accepted to test in the present study. Compared  
14 with the vehicle group, the lower incidence of S-IRA by PTZ was reversed by  
15 atomoxetine ( $p<0.01$ ). No obvious difference in the group of vehicle and in the group  
16 of Esmolol ( $p>0.05$ ), meaning that administration of Esmolol (50 mg/kg,i.p)  
17 produced no effects on the incidence of mortality caused by PTZ itself. However,  
18 Compared with the group treated with atomoxetine and vehicle, the incidence of  
19 S-IRA and SUDEP significantly increased in the group treated with atomoxetine and  
20 Esmolol ( $p<0.01$ ), suggesting that administration of Esmolol can specifically reverse  
21 the atomoxetine-mediated suppression of S-IRA evoked by PTZ. Thus, it can be  
22 speculated from our findings that enhancing the norepinephrinergic neurotransmission  
23 contributes to inhibition of S-IRA via targeting the ( $\beta$ 1-AR) localized in the  
24 cardiomyocytes in the DBA/1 mouse SUDEP model.

## 25 **4. DISCUSSION**

26 There are about 70 million people in the world who suffer from epilepsy, and 2.4  
27 million have been diagnosed with epilepsy every year, nearly 90% occurred in  
28 developing countries<sup>[1]</sup>. The latest epidemiological studies of the American Epilepsy  
29 Society show that the incidence of SUDEP in western developed countries is about  
30 1.11 per 1000 people per year<sup>[2]</sup>, while the results of the multi-center epidemiological



1 survey in China show that the incidence of SUDEP in China is about 2.3 per 1000  
2 people per year<sup>[3]</sup>, and the number of domestic epilepsy patients dying from SUDEP is  
3 more than 20,000 per year. More importantly, SUDEP is the main factor that leads to  
4 the death in young patients with epilepsy with an incidence two to three times that of  
5 other diseases that can lead to sudden death in young adults, and children's SUDEP  
6 incidence is also close to adults<sup>[4]</sup>. Japan's latest epidemiological survey shows that  
7 the incidence of SUDEP in pregnant women is significantly higher than the incidence  
8 of non-pregnant women<sup>[5]</sup>. However, due to the complexity of its pathogenesis and the  
9 difficulty to obtain the real-time monitoring data of SUDEP in patients with epilepsy,  
10 the understanding of the SUDEP mechanism is still limited, and it is difficult to  
11 develop a prevention strategy. Many previous studies have shown that the  
12 pathophysiology of SUDEP is complex, including the dysfunction of heart, autonomic  
13 nerves, respiratory, brain structural obstacles, and polygenesis.

14 In the current study, we firstly reported that the suppressant effects of S-IRA and  
15 SUDEP in DBA/1 mice by atomoxetine can be significantly reversed by  
16 administration of esmolol, a selective  $\beta_1$  receptor blocker. Our previous findings  
17 showed that administration of atomoxetine inhibited S-IRA and SUDEP in DBA/1  
18 mice independently of seizure induction methods, and its inhibition of S-IRA induced  
19 by acoustic stimulation was dose-dependent<sup>[6]</sup>. Given the current clinical reports that  
20 atomoxetine can produce the effects of peripheral circulation. For example, after oral  
21 administration of atomoxetine, ADHD (Attention-Deficit/ Hyperactivity Disorder)  
22 patients showed symptoms such as increased heart rate and other symptoms<sup>[7]</sup>,  
23 considering that atomoxetine may play a role in SUDEP passing through the central -  
24 peripheral - cardiac sympathetic mechanisms by affecting  $\beta_1$  receptors as dominate  
25 receptors in the heart. In the present study, we used the  $\beta_1$  receptor blocker esmolol to  
26 block the  $\beta_1$  receptor which locates preferentially in the cardiomyocytes of the heart  
27 to test whether the lower incidence of S-IRA by atomoxetine can be reversed by  
28 Esmolol. It turned out that the suppressant effects of S-IRA by atomoxetine were  
29 markedly reversed by esmolol, suggesting that administration of atomoxetine  
30 significantly reduced the incidence of S-IRA by enhancing the concentration of



1 norepinephrine of the cleft between the sympathetic synapse and cardiomyocytes of  
2 the heart to combine with  $\beta_1$  receptors of cardiomyocytes. Of course, atomoxetine  
3 medicating the reduction of S-IRA by enhancing the concentration of norepinephrine  
4 to target the adrenergic receptor  $\alpha_1$  and  $\alpha_2$  in the brain was the main pathway as well.  
5 To be exact, atomoxetine medicates the reduction of S-IRA by interacting between  $\alpha_1$ ,  
6  $\alpha_2$ , and  $\beta_1$  receptors located in central and peripheral sympathetic nerves. In fact,  
7 although S-IRA was the key cause leading to SUDEP, other factors are involved in the  
8 course can't be excluded as well. A previous study showed that S-IRA evoked by  
9 acoustic stimulation in DBA/1mice was characterized by the simultaneous  
10 suppression of respiration and circulation though the respiratory arrest preceded the  
11 cardiac arrest. What's more, the recent study showed that the tonic phase apnea  
12 evoked by acoustic stimulation is not sufficient for seizure-induced death in a  
13 W/+<sup>Emx1-Cre</sup> mouse. Therefore, the factors resulting in the S-IRA and SUDEP  
14 including respiration and circulation may be heterogeneous.

15 The previous research has shown that the main risk factors of SUDEP are  
16 generalized tonic-clonus and nocturnal seizures. The neuronal network of generalized  
17 tonic-clonic seizures may lead to hypoventilation, apnea, and cardiovascular failure  
18 by inhibiting brainstem respiration or autonomic nerve control centers, leading to  
19 death [10, 11]. Since the most sudden death cases occur at night or in sleep, with no  
20 witnesses, leaving many questions unanswered. In recent years, more and more  
21 researches have shown that apnea and heart failure are important potential  
22 mechanisms in SUDEP. Studies in animal models and SUDEP patients have shown  
23 that seizure-induced respiratory arrest (S-IRA) is one of the main induced factors that  
24 lead to death in many cases<sup>[12-14]</sup>. However, a respiratory arrest is not the only lethal  
25 cause, tonic-clonic seizures caused sudden, simultaneous respiratory and cardiac  
26 depression in mice, and the time of recovery of respiration and heart rate during  
27 resuscitation is also closely matched<sup>[15]</sup>. Therefore, the heart also plays an important  
28 role in the mechanism of SUDEP.

29 Seizure-associated arrhythmias are common and have been considered as the  
30 potential pathogenesis of SUDEP, such as tachycardia, bradycardia, and cardiac arrest

1 after the seizures, resulting in cardiac repolarization, changes in electrolyte levels,  
2 blood pH, and catecholamine release in patients with epilepsy, which latter three  
3 factors may promote arrhythmias and lead to death by affecting cardiac excitability<sup>[16]</sup>.  
4 Tachycardia and several factors that increase susceptibility to tachyarrhythmias,  
5 known as pathological cardiac repolarization, are considered as risk factors for sudden  
6 cardiac death (SCD) in healthy individuals and patients with epilepsy which, in this  
7 particular setting, are important factors in triggering SUDEP events<sup>[17, 18]</sup>. However,  
8 the exact mechanism of SUDEP and cardiac arrest induced by seizures remains  
9 unclear. Seizures may interfere with the normal functioning of the heart by causing  
10 autonomic disturbances <sup>[19]</sup>, leading to fatal arrhythmias. Studies have found that  
11 cardiac electrical instability and autonomic dystonia are caused by cumulative cardiac  
12 damage during repeated attacks<sup>[20]</sup>. Studies also have shown that catecholamine  
13 (norepinephrine and adrenaline) released from seizures is related to a wide range of  
14 calcium (Ca<sup>2+</sup>)-mediated physiological changes, which can lead to damage to the  
15 heart structure. Our previous studies found that repeated S-IRA in DBA / 1 mice will  
16 result in necrotic damage in the ventricle which is caused by the local Ca<sup>2+</sup> +  
17 homeostasis disturbances, and the incidence and severity of injury depend on the total  
18 number of S-IRA<sup>[21]</sup>. Relevant clinical data have found that paroxysmal arrhythmias  
19 (including arrest, atrioventricular block, and rare atrial and ventricular fibrillation)  
20 usually occur after convulsive episodes and are often associated with (near)SUDEP<sup>[22]</sup>.  
21 Studies have shown that chronic epilepsy can lead to hypoxemia and the increase of  
22 catecholamine, causing damage of the heart and vascular, resulting in cardiac  
23 electrical, and mechanical dysfunction<sup>[23,24]</sup>. Some fatal incentives may be the acidosis  
24 caused by persistent hypoxemia and hypercapnia, leading to bradycardia or cardiac  
25 arrest, while in others it may be the seizures due to malignant arrhythmias<sup>[25]</sup>. This  
26 suggests that SUDEP is inextricably linked to arrhythmia.

27 In the present study, the ECG of DBA/ 1 mice with administration of esmolol (50  
28 mg/kg, i.p) that can significantly reverse the lower incidence of S-IRA evoked by  
29 acoustic stimulation by administration of atomoxetine showed the mixture of sinus  
30 bradycardia, atrioventricular block, ventricular premature beat, and ventricular

1 tachycardia in order. In the meantime, the ECG of DBA/1 mice suffered from the  
2 S-IRA evoked by acoustic stimulation characterized by the mixture of sinus  
3 bradycardia, atrioventricular block, ventricular premature beat, and ventricular  
4 tachycardia as well in our model. However, no obvious arrhythmia appeared in the  
5 DBA/1 mice without suffering from S-IRA other than the sinus bradycardia and no  
6 apparent mortality occurred in the group of DBA/1 mice with pre-treatment with the  
7 dose of esmolol 50 mg/kg (i.p). Furthermore, the arrhythmias can be significantly  
8 reduced by atomoxetine in our models. Thus, the lower incidence of S-IRA by  
9 acoustic stimulation or PTZ can be significantly as well as especially reversed by  
10 esmolol by blocking the  $\beta$ 1-AR localized in cardiomyocytes to combine the  
11 norepinephrine released by cardiac sympathetic nerve synaptic terminal in our models.  
12 Additionally, it is possible that the locus coeruleus (LC), as the largest nuclei to  
13 synthesize and release norepinephrine in the brain, may be implicated in the course of  
14 administration of esmolol to reverse the lower incidence of S-IRA and SUDEP and  
15 plays a key role in modulating S-IRA and SUDEP by regulating the respiratory and  
16 circulation function in our models. In future experiments, we will further explore it.

## 17 **5. Conclusions**

18 The current finding in our models suggested that enhancing central  
19 norepinephrinergic neurotransmission contributes to inhibition of seizure-induced  
20 respiratory arrest by targeting  $\beta$ 1-AR locating in the cardiomyocytes and the pathway  
21 of norepinephrinergic neurotransmission- $\beta$ 1-AR between brain and heart may be a  
22 potential and specific target to prevent SUDEP.

## 23 **6. Funding**

24 The work was supported by the National Natural Science Foundation of China  
25 (Grant.NO: 81771403 and 81974205); by the Natural Science Foundation of Zhejiang  
26 Province (LZ20H090001); by the Program of New Century 131 outstanding young  
27 talent plan top-level of Hang Zhou to HHZ

## 28 **7. DISCLOSURE**

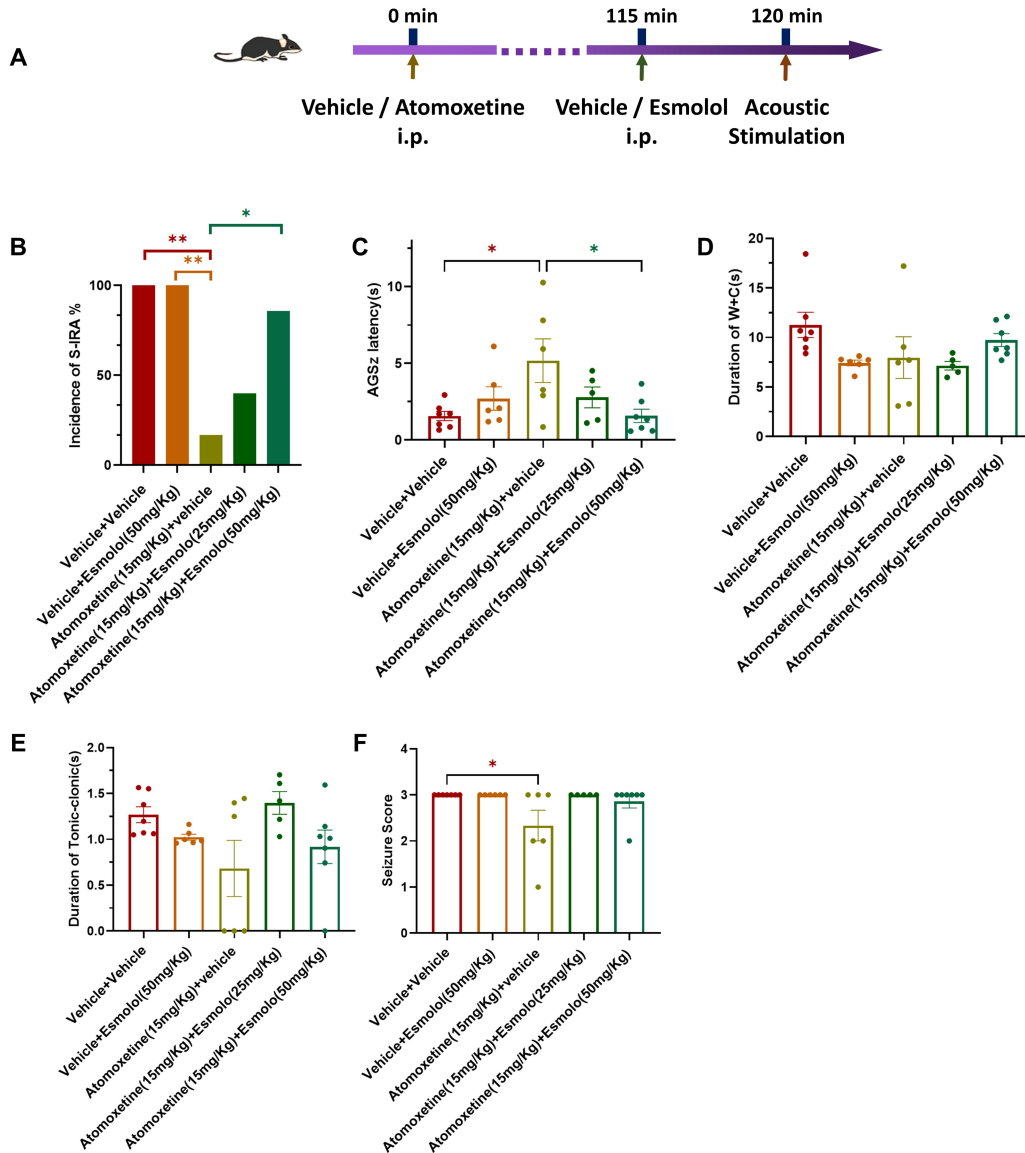
29 All authors declare no competing interests. We had confirmed that we have read the  
30 Journal's position on issues involved in ethical publication and affirm that this study

1 was in accordance with those guidelines.

2

### 3 8. Figure legends

**Figure 1**



4

5

6 Figure 1 Administration of Esmolol significantly reversed the atomoxetine-mediated  
7 suppression of S-IRA evoked by acoustic stimulation

8 **A.** The protocol to explore the influence of administration of esmolol on  
9 atomoxetine-mediated reduction of S-IRA evoked by acoustic stimulation. **B.**

10 Compared to the group treated with vehicle (n = 7) or vehicle and esmolol (n = 6),

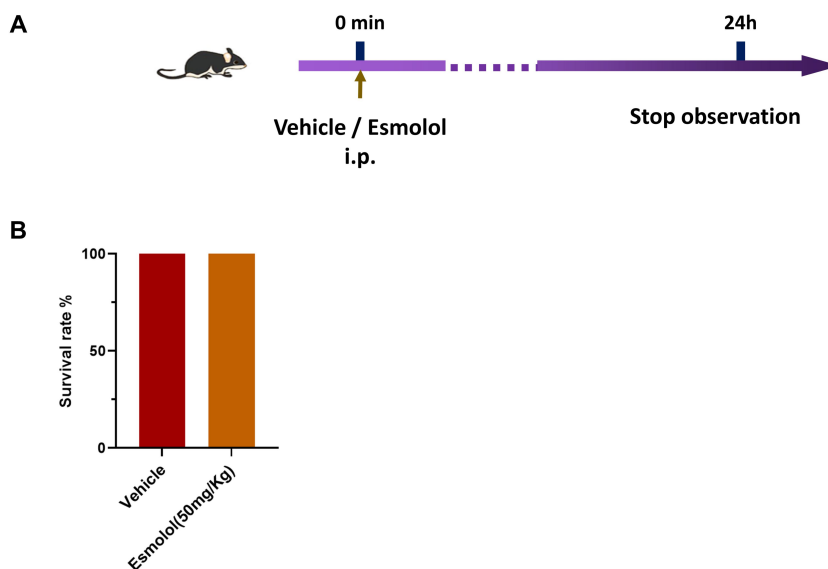
11 S-IRA evoked by acoustic stimulation was markedly lower in groups treated with i.p.

12

1 atomoxetine (n = 6, \*\*p < 0.01) at 15mg/Kg. However, the protective effect of  
2 atomoxetine in primed DBA/1 mice was significantly reversed by esmolol doses of  
3 50mg/kg (n = 7, \*p < 0.05). **C.** Furthermore, compared with the group treated with  
4 atomoxetine and vehicle (n = 6), the latencies to AGSZs in the control group (n = 7)  
5 or the group treated with atomoxetine and esmolol (n = 7) was significantly increased  
6 (p < 0.05). **D-E.** There were no intergroup differences in durations of wild running  
7 plus clonic seizures (W+C), tonic-clonic seizures (p > 0.05). **F.** Compared to the  
8 control group (n = 7), the seizure score was lower in the group treated with  
9 atomoxetine and vehicle (n = 6, \*p < 0.05).

10

## Figure 2



11

12 Figure 2 The dose of Esmolol ( 50 mg/kg,i.p. ) does not produce the incidence of  
13 morality of DBA/1 mice.

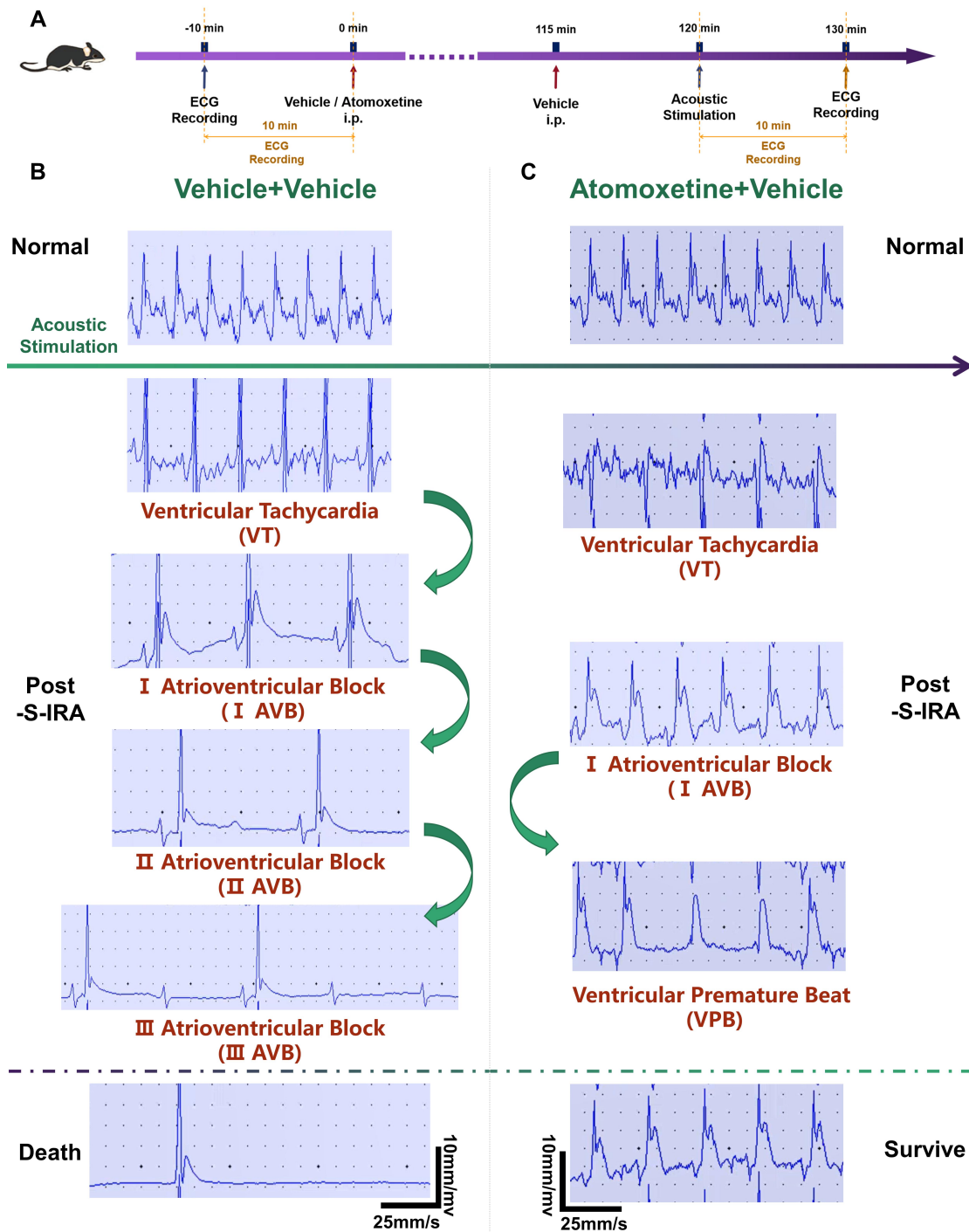
14 **A.** The protocol to explore the dose of esmolol (50 mg/kg,i.p.) whether produce the  
15 morality of DBA/1 mice. **B.** There was no significant difference in the morality of  
16 DBA/1 mice between the group pre-treated with the vehicle and the group pre-treated  
17 with esmolol(50 mg/kg, i.p, n=7 ) (p > 0.05)

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**Figure 3**



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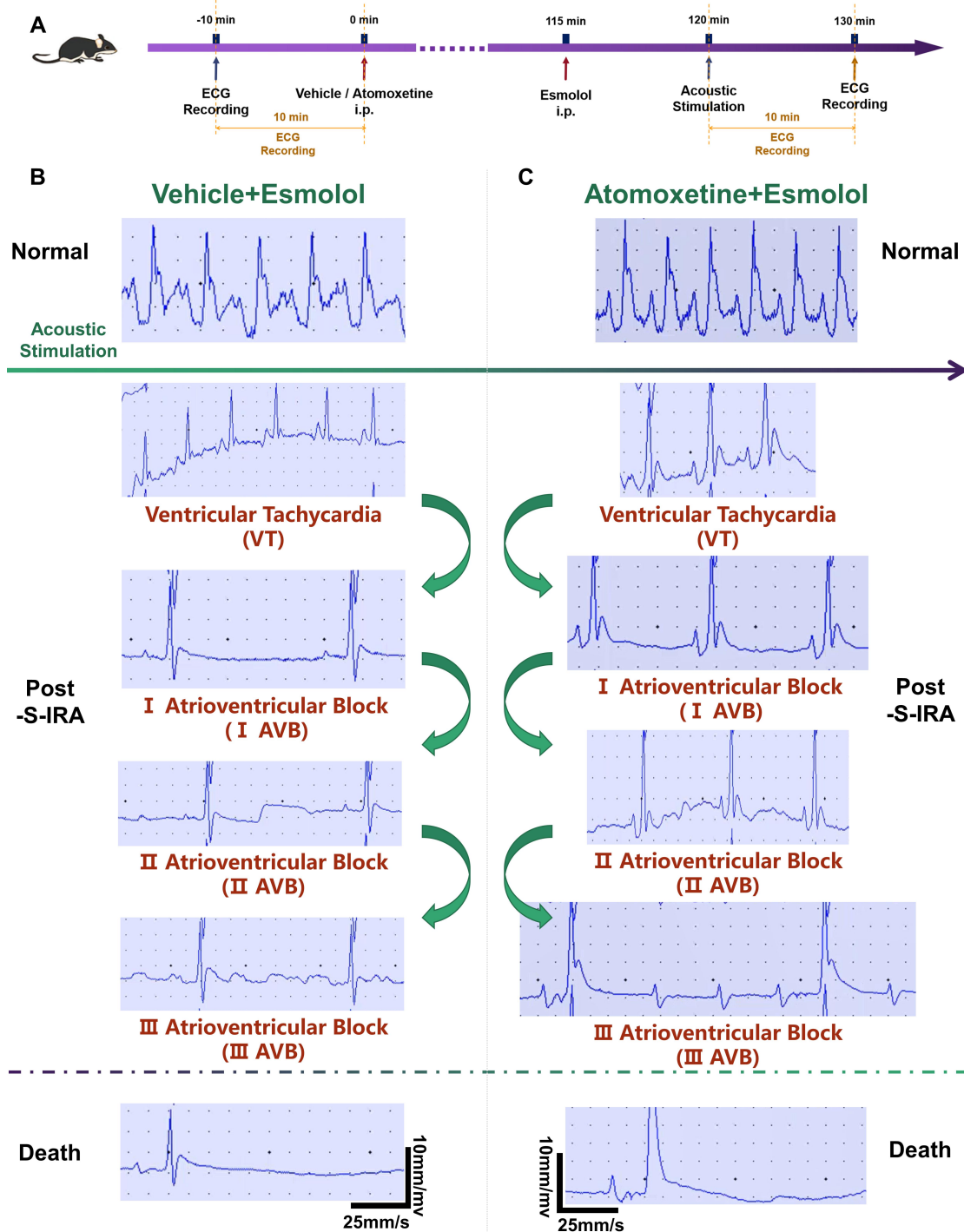
5 Figure 3 The effects imposed by tonic seizures on the changing of ECG in both

6 groups treated with vehicle or atomoxetine

7 **A.** The protocol to explore the changes of electrocardiogram on

1 atomoxetine-mediated reduction of S-IRA evoked by acoustic stimulation. **B.** The  
 2 ventricular tachycardia and atrioventricular block occurred immediately following  
 3 tonic seizures and S-IRA evoked by acoustic stimulation in DBA/1 mice in the  
 4 control group. **C.** What's more, the group pre-treated with the atomoxetine (15mg/Kg)  
 5 was observed the ventricular tachycardia, atrioventricular block, and ventricular  
 6 premature beat after acoustic stimulation, and the DBA/1 mice were then recovered.

**Figure 4**





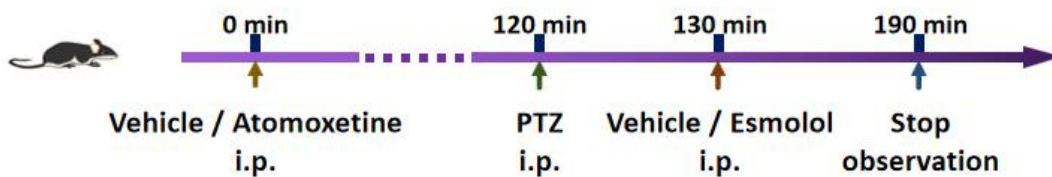
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2 **Figure 4 The effects imposed by tonic seizures on the changing of ECG in both**  
3 **groups treated with atomoxetine or esmolol**

4 A. The protocol to explore the changes of electrocardiogram on the administration of  
5 esmolol reversed the atomoxetine-mediated reduction of S-IRA evoked by acoustic  
6 stimulation. **B.** The ventricular tachycardia and atrioventricular block occurred  
7 immediately following tonic seizures and S-IRA evoked by acoustic stimulation in  
8 DBA/1 mice in the group treated with vehicle and Esmolol (50mg/Kg). **C.** The group  
9 pre-treated with the atomoxetine(15mg/Kg) and treated with esmolol (50mg/Kg) was  
10 observed the ventricular tachycardia and atrioventricular block after acoustic  
11 stimulation.

12 **Figure 5 Administration of Esmolo significantly reversed the**  
13 **atomoxetine-mediated suppression of S-IRA evoked by PTZ**

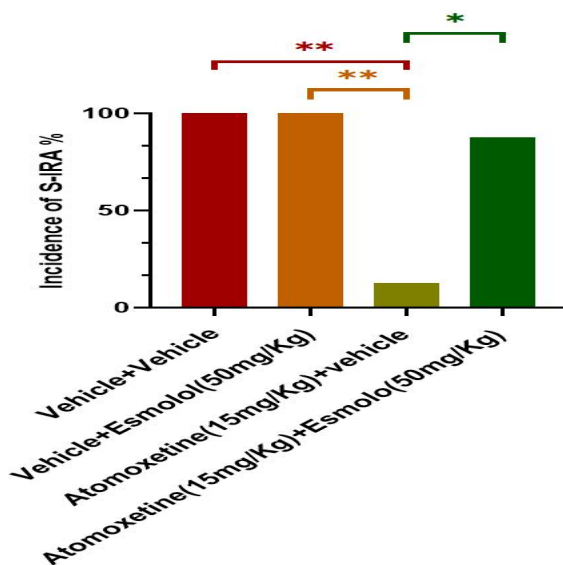
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1 Compared with the group treated with vehicle (n = 5) or vehicle and esmolol (n =5),  
2 S-IRA evoked by acoustic stimulation was markedly lower in groups treated with i.p.  
3 atomoxetine (n = 7, \*\*p < 0.01) . However, Compared with the group treated with i.p.  
4 atomoxetine and vehicle, the incidence of S-IRA significantly increased in the group  
5 treated with i.p. atomoxetine and esmolol (n = 7, \*\*p < 0.05)

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