Isoflurane anesthesia decreases excitability of inhibitory neurons in the basolateral amygdala leading to anxiety-like behavior in aged mice

MENGYUAN LI[1], RUIJIAO ZHANG[1], SHIYIN WU[1], LIQIN CHENG[1], HUAN FU[1] and LIANGCHAO QU[1,2]

[1]Medical Center of Anesthesiology and Pain, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi 330000, P.R. China; [2]Department of Anesthesia and Surgery, People's Hospital of Ganjiang New District, Nanchang, Jiangxi 341099, P.R. China

Received March 13, 2024; Accepted July 18, 2024

DOI: 10.3892/etm.2024.12688

Abstract. Anxiety after surgery can be a major factor leading to postoperative cognitive dysfunction, particularly in elderly patients. The role of inhibitory neurons in the basolateral amygdala (BLA) in anxiety-like behaviors in aged mice following isoflurane anesthesia remains unclear. Therefore, the present study aimed to investigate the role of inhibitory neurons in isoflurane-treated mice. A total of 30 C57BL/6 mice (age, 13 months) were allocated into the control and isoflurane anesthesia groups (15 mice/group) and were then subjected to several neurological assessments. Behavioral testing using an elevated plus maze test showed that aged mice in the isoflurane anesthesia group displayed significant anxiety-like behavior, since they spent more time in the closed arm, exhibited more wall climbing behavior and covered more distance. In addition, whole-cell patch-clamp recording revealed that the excitability of the BLA excitatory neurons was notably increased following mice anesthesia with isoflurane, while that of inhibitory neurons was markedly reduced. Following mice treatment with diazepam, the excitability of the BLA inhibitory neurons was notably increased compared with that of the excitatory neurons, which was significantly attenuated. Overall, the results of the current study indicated that anxiety-like behavior could occur in aged mice after isoflurane anesthesia, which could be caused by a reduced excitability of the inhibitory neurons in the BLA area. This process could enhance excitatory neuronal activity in aged mice, thus ultimately promoting the onset of anxiety-like behaviors.

Correspondence to: Dr Liangchao Qu, Medical Center of Anesthesiology and Pain, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, 17 Yong Wai Zheng Road, Donghu, Nanchang, Jiangxi 330000, P.R. China
E-mail: ndyfy03356@ncu.edu.cn

Key words: basolateral amygdala, inhibitory neurons, age, isoflurane, anxiety

Introduction

Elderly patients commonly experience postoperative cognitive dysfunction (POCD) following anesthesia and surgery. POCD is commonly characterized by anxiety, confusion, personality changes and impaired memory (1-3). Anxiety is a relatively common manifestation in patients postoperatively, even in the absence of other complications (4). Therefore, reducing post-operative anxiety has become a primary goal for preventing POCD.

Isoflurane is widely used as a maintenance drug for general anesthesia, due to its good anesthetic effect, easy adjustment of anesthesia depth, mild circulatory effects, low toxicity and rapid induction and recovery (5). However, inhalation anesthesia can exhibit toxic effects on several types of cells, including nerve cells. It has been reported that isoflurane has significant toxicity (6-8). Currently, the research on the effect of isoflurane on anxiety-like behavior in elderly patients undergoing anesthesia and its underlying mechanism is limited.

The amygdala is a key structure that processes anxiety-related information (9). It is composed of multiple parts, among which the basolateral amygdala (BLA) and central amygdala are particularly significant for the treatment of anxiety disorders (10). A previous study demonstrated that BLA is associated with pathological anxiety, while the excitability of a subpopulation of excitatory neurons in the BLA continue to increase during anxiety (11). Another study also showed that inhibitory neurons in the BLA are involved in the synaptic plasticity, which can regulate fear learning in the amygdala (12).

Therefore, in the current study an isoflurane anesthesia model was established in elderly mice to evaluate the neuronal status of the BLA and analyze its role in this process, thus uncovering the possible mechanism underlying the effect of isoflurane on inducing postoperative anxiety in the elderly.

Materials and methods

Ethics. All experiments were approved by the Laboratory Animal Committee of The First Affiliated Hospital of Nanchang University (approval no. CDYFY-IACUC-202205QR015) and

conformed to the National Research Council's Guide for the Care and Use of Laboratory Animals (13). This study complied with the Animal Research: Reporting of *In Vivo* Experiments guidelines (14). The number of, as well as the procedures introducing pain to the animals, were minimized according to the aforementioned regulations.

Experimental grouping and treatment. A total of 30 13-month-old C57BL/6 male mice (weight, 30-38 g) were included in the present study. Mice were given free access to food and water and were randomly allocated into the control and experimental group (n=15 mice/group).The mice were housed in groups of six animals per cage under a constant light-dark cycle (lights on from 08:00-20:00) and fed standard laboratory food and tap water in an air-conditioned room (23±1˚C with ~60% humidity). An anxiety model was established after 1.5% isoflurane anesthesia, as previously described, mice in the experimental group received 1.5% isoflurane in pure oxygen for 2 h and then breathed fresh air for 4 h (15,16). Mice in the control group only received fresh air for 6 h. After recovering, mice were allowed to eat and drink freely. Behavioral tests were performed on the following day. After the end of the behavioral study, mice were injected with 100 mg/kg sodium pentobarbital into the abdomen, mice were euthanized under deep anesthesia to remove the brain tissue for slicing, and then brain slices were isolated for electrophysiological recordings.

Behavioral tests. The elevated plus maze test is used to evaluate anxiety-like behavior in rodents (17,18). It consists of four arms, two open and two closed, arranged in a cross shape with a central area elevated off the ground. In the present study, the anxiety-like behavior of mice was assessed by comparing the time spent and distance traveled by the mice in the open and closed arms. Briefly, each mouse was placed in the central area of the maze, facing the open arm. The position of each mouse was consistent throughout the experiment. Subsequently, the number of entries of each mouse into the open and closed arms and the time spent in each arm were recorded by a camera for 5 min. The experiment was conducted in a quiet environment, while the researcher remained 1 m away from the maze. After recording was complete, the mouse was returned to its cage. The maze was cleaned with 5% acetic acid solution or 75% alcohol to eliminate any residual animal odor. Furthermore, mice were also subjected to open field test. This test is commonly used to investigate anxiety or depression in animals (18,19) by evaluating several behaviors of experimental animals in an open environment, such as the fear of the animals in a new environment. Therefore, animals mainly move in the peripheral area and less in the central one. However, due to their exploratory nature, animals are motivated to move in the central area, thus resulting in the development of anxiety symptoms. In the present study, the open field was set to 50x50 cm with a brightness of 700 lux. The mouse was placed in the experimental area to adapt for 10 min and then its behavior was recorded for 20 min. Periphery was defined as the area within 5 cm of the edge of the field, while the total distance traveled and the time spent in the center or periphery, measured in sec, were recorded. The distance traveled by the mouse to the central area was divided by the total distance covered to obtain the center distance/total distance ratio, which could be used as an anxiety index.

Preparation of mice brain slices. After the end of the behavioral study, mice were injected with 100 mg/kg sodium pentobarbital into the abdomen for deep anesthesia. Following anesthesia, the brain was quickly removed and placed in ice-cold sucrose-containing artificial cerebrospinal fluid [ACSF; containing 100 mM choline-Cl, 13 mM NaCl, 3 mM KCl, 1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11 mM D-glucose, 1 mM $CaCl_2$ and 5 mM $MgCl_2$ (pH 7.4 after bubbling with 95% O_2 and 5% CO_2]. Subsequently, 300 μm-thick horizontal slices were prepared using a vibratome. The aforementioned slices were then incubated in standard ACSF at 32˚C for 30 min, followed by resting at room temperature for 30 min.

In vitro whole-cell patch-clamp recording. The patch-clamp set-up was performed using the Olympus BX50WI microscope (Olympus Corporation) equipped with x60 water immersion lens (LUMPlanFL, NA 1.0). Brain slices were transferred into a recording chamber maintained at 32˚C and were continuously perfused with standard ACSF at a rate of 2-4 ml/min. Whole-cell patch-clamp recordings were obtained from the visually identified neurons in the lateral/basolateral amygdala complex. The internal solution composed of 130 mM K-gluconate, 5 mM KCl, 10 mM phosphocreatine, 10 mM HEPES, 0.5 mM EGTA, 2 mM Na2-ATP, 0.3 mM Na-GTP and 2 mM MgSO4 (pH 7.20-7.30, 290 mosmol/l). Membrane potential at resting state was recorded within the first 20 sec after membrane rupture, while input resistance was measured at resting membrane potential with current pulses (+10 pA; 500 ms). Additionally, the action potentials were recorded with a series of 1-sec depolarizing current pulses at the resting membrane potential. There are two main types of neurons in the BLA, namely the excitatory principal neurons and the local circuit inhibitory interneurons. Based on the action potential waveform (short depolarizing process, high membrane potential peak, fast membrane potential decay and hyperpolarizing afterpotential), the cells were classified as excitatory neurons. The most common features of inhibitory neuron action potentials are low peak amplitude, prolonged depolarization, absence of repolarization process and absence of after-hyperpolarization. All recordings were obtained using the Multiclamp 700B amplifier (Molecular Devices, LLC) and the PowerLab system (ADInstruments Ltd.) with a low-pass filter frequency of 4 kHz. The signals were digitized at 40 kHz for computer analysis using WinWCP software (V5.2.6; gift by Dr. John Dempster, University of Strathclyde). All experiments were carried out at 32˚C.

Statistical analysis. The results are expressed as the mean ± SD. The tests for mice were repeated 15 times for each group. All data were tested for normality by the Kolmogorov-Smirnov test. The animal behavior, resting membrane potential, input resistance, AP threshold, AP amplitude and AP half amplitude results between the two groups were compared by unpaired Student's t-test. One- and two-way ANOVA followed by Bonferroni's multiple comparison post hoc test were performed to compare the number of action potentials evoked by different current steps. All statistical analyses were carried out with Prism 7 (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

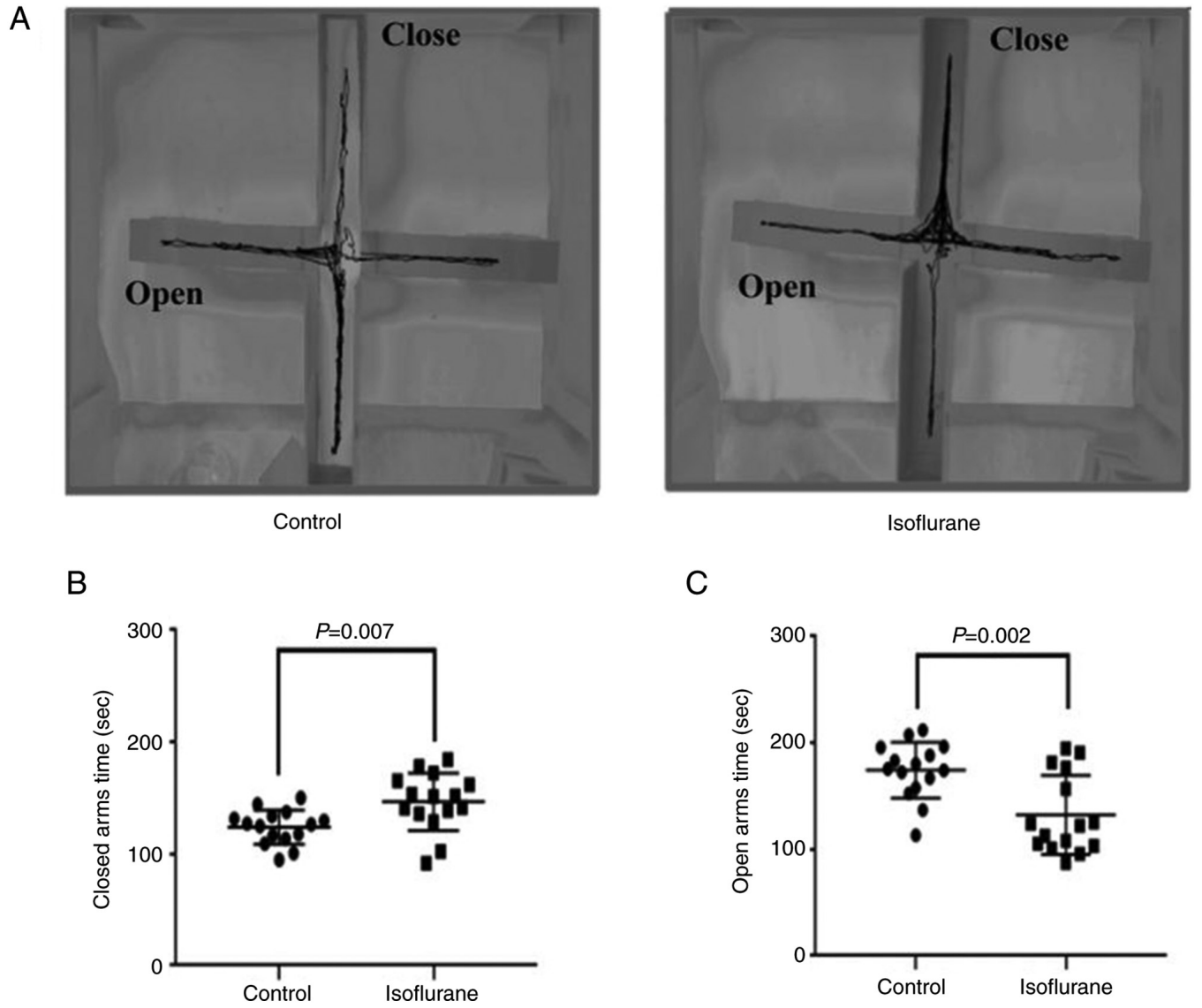

Figure 1. In the elevated plus maze test, aged mice spend more time in the closed arms following isoflurane anesthesia. (A) The movement trajectories of the two groups of mice in the elevated plus maze test are shown. The time spend in the (B) closed and (C) open arms by the two groups of mice was recorded (n=15).

Results

Elevated plus maze test. The movement trajectory results showed that mice in the control group had a particular movement trajectory in both the open and closed arms, while those in the isoflurane anesthesia group mainly moved in the closed arms (Fig. 1A). In addition, compared with the control group, mice in the isoflurane anesthesia group stayed a significantly longer and shorter time in the closed (P<0.05; Control vs. Isoflurane, 123.8±15.4 vs. 156.6±25.8 sec; Fig. 1B) and open (P<0.05; Control vs Isoflurane, 164.3±26.3 vs. 132.5±37.5 sec; Fig. 1C) arms, respectively.

Open field test. The results of movement trajectory revealed that mice in the control group displayed a certain movement trajectory in both the central and peripheral areas, while mice in the isoflurane anesthesia group mainly moved in the peripheral area (Fig. 2A). Furthermore, mice in the isoflurane anesthesia group traveled a significantly longer total distance (P<0.05; Control vs. Isoflurane, 3040.3±338.2 vs. 4069.6±419.3 cm; Fig. 2B), spent a significantly shorter time in the central area (P<0.05; Control vs. Isoflurane, 507.3±48.2 vs. 318.5±59.3 sec; Fig. 2C) and markedly longer time in the peripheral area (P<0.05; Control vs. Isoflurane, 687.2±59.3 vs. 871.6±89.3 sec; Fig. 2D), compared with the control group.

Electrophysiological changes of the BLA excitatory neurons in aged mice after isoflurane anesthesia. The activity of excitatory neurons were recorded by whole-cell patch-clamp in both the control (Fig. 3A) and isoflurane (Fig. 3B) groups. To detect the basic electrophysiological properties of the BLA principal neurons, the activity of neurons (n=24) located in the BLA were recorded using a whole-cell patch-clamp. The results demonstrated that, compared with the control group, the resting membrane potential of the excitatory neurons in the isoflurane anesthesia group was enhanced (P<0.05; Control vs. Isoflurane, -66.7±6.5 vs. -62.3±5.2; Fig. 3C). However, no significant difference was recorded in input resistance between the isoflurane and control groups (P>0.05; Control vs. Isoflurane, 177.9±42.4

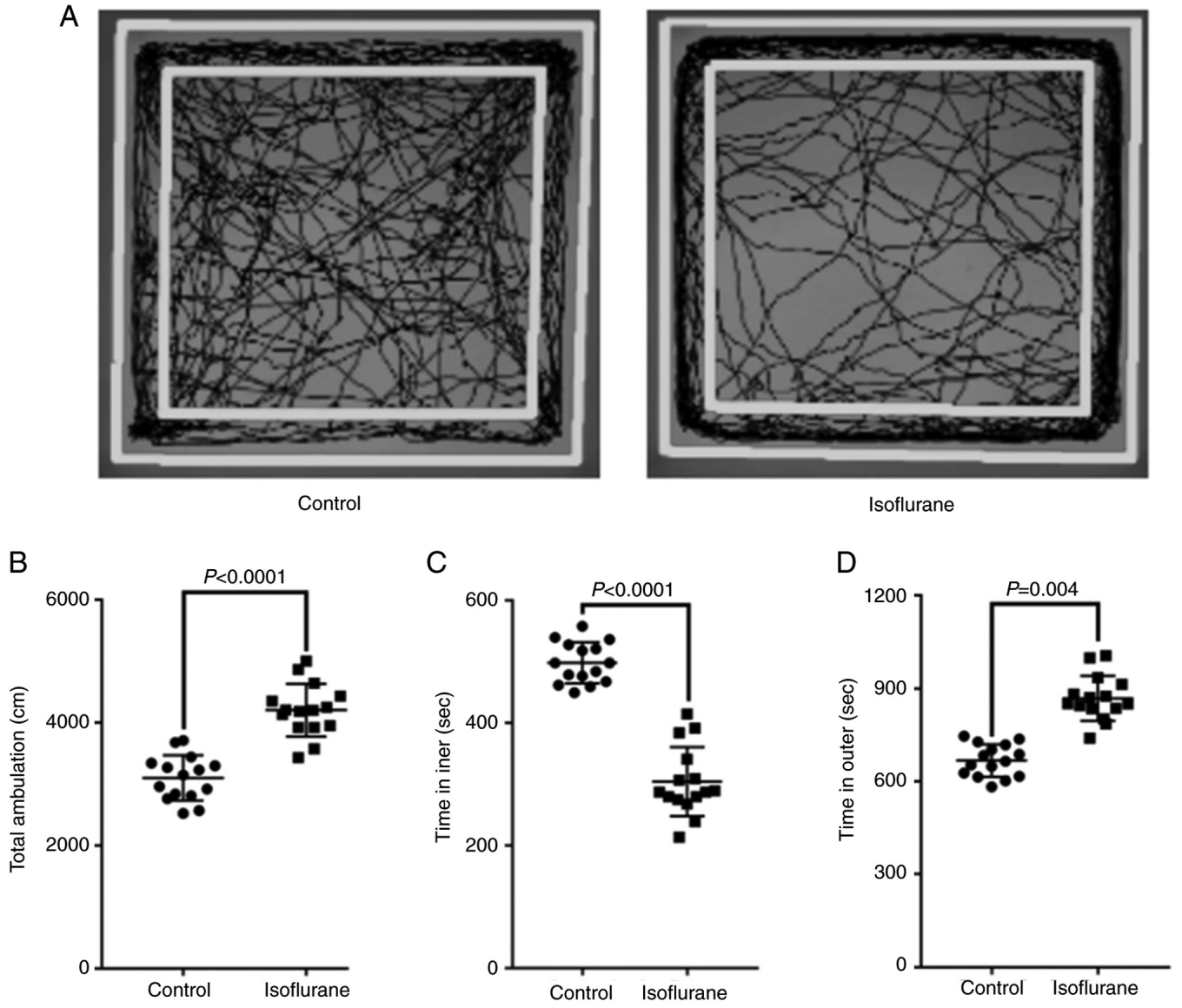

Figure 2. In the open field test, aged mice spend more time in the outer area following isoflurane anesthesia. (A) The movement trajectories of the two groups of mice in the open field test are shown. (B) The total distance traveled by the two groups of mice is presented. The time spend in the (C) central and (D) peripheral area by the two groups of mice was recorded (n=15).

vs. 182.3±40.3; Fig. 3D). As the input current was increased, the number of action potentials generated by the excitatory neurons of mice in the isoflurane anesthesia group was notably elevated compared with the control group (P<0.05; Fig. 3E). Additionally, the action potential threshold was higher in the control group compared with the isoflurane anesthesia group (P<0.05; Control vs. Isoflurane, -39.4±5.3 vs. -36.6±7.9; Fig. 3F). However, there was no difference in the amplitude of the action potential or the half-amplitude of the action potential between the two groups (P>0.05; Fig. 3G and H).

Electrophysiological changes of BLA inhibitory neurons in aged mice following isoflurane anesthesia. The activity of inhibitory neurons were recorded by whole-cell patch-clamp in both the control (Fig. 4A) and isoflurane (Fig. 4B) groups. To investigate the basic electrophysiological properties of the BLA inhibitory neurons, the activity of 20 neurons in the BLA was recorded using the whole-cell patch-clamp technique. The results showed that compared with the control group, the resting potential of the isoflurane anesthesia group was significantly lower (P<0.05; Control vs. Isoflurane, -61.2±5.2 vs. -67.3±6.5; Fig. 4C). Consistently, the input resistance was also markedly reduced (P<0.05; Control vs. Isoflurane, 172.5±19.7 vs. 157.1±23.7; Fig. 4D). As the input current was elevated, the number of evoked action potentials also gradually increased. However, notably fewer action potentials were recorded in the isoflurane anesthesia group compared with the control group (P<0.05; Fig. 4E). Additionally, the threshold of action potential was also significantly enhanced (P<0.05; Control vs. Isoflurane, -43.8±6.1 vs. -36.9±5.4; Fig. 4F). However, no difference between the two groups was obtained in terms of the amplitude of action potential and action potential half-duration (P>0.05; Fig. 4G and H).

Effect of diazepam on the BLA inhibitory neurons in aged mice following isoflurane anesthesia. To further investigate the effects of isoflurane anesthesia on BLA neurons, the

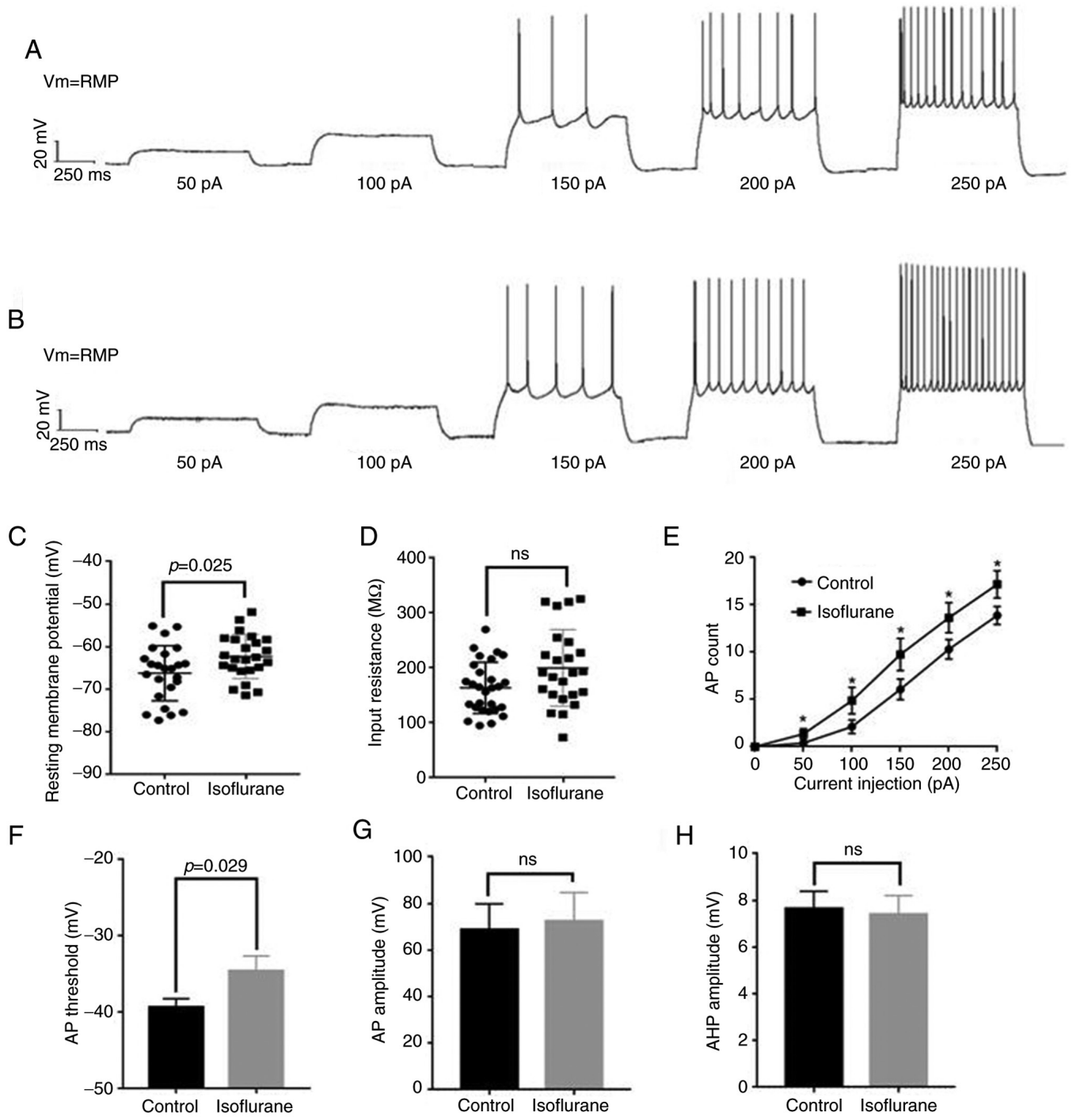

Figure 3. Anesthesia of aged mice with isoflurane enhances the excitability of basolateral amygdala neurons. (A) Action potentials of excitatory neurons in the control group injected with different currents at resting potential. (B) Action potentials of excitatory neurons in mice in the isoflurane anesthesia group injected with different currents at resting potential. (C) Resting potentials of excitatory neurons in brain slices isolated from mice in the control and isoflurane groups. (D) The input resistance of both groups of excitatory neurons is presented. (E) The number of action potentials evoked by different input currents of excitatory neurons in brain slices from mice in the control and isoflurane groups is shown. (F) The threshold of action potential of excitatory neurons in brain slices of control and isoflurane-treated mice. The amplitude of (G) action potential and (H) action potential half-duration of excitatory neurons in brain slices derived from mice in the control and isoflurane groups was also recorded (n=24). *P<0.05, control group vs. isoflurane group. AP, action potential; AHP, action potential half-duration.

ACSF perfusion solution was supplemented with diazepam (500 ng/ml) (18), to enhance the excitability of inhibitory neurons (20). Subsequently, the action potentials of 19 inhibitory neurons at resting potential was recorded using the whole-cell patch-clamp technique (Fig. 5A and B). Therefore, compared with the control group, there was no significant difference in the resting potential (Fig. 5D) or input resistance (Fig. 5E) of the BLA inhibitory neurons in the isoflurane anesthesia group (P>0.05). As the input current increased, the number of evoked action potentials also gradually enhanced. However, significantly fewer action potentials were recorded following treatment with diazepam (P<0.05; Fig. 5C), while

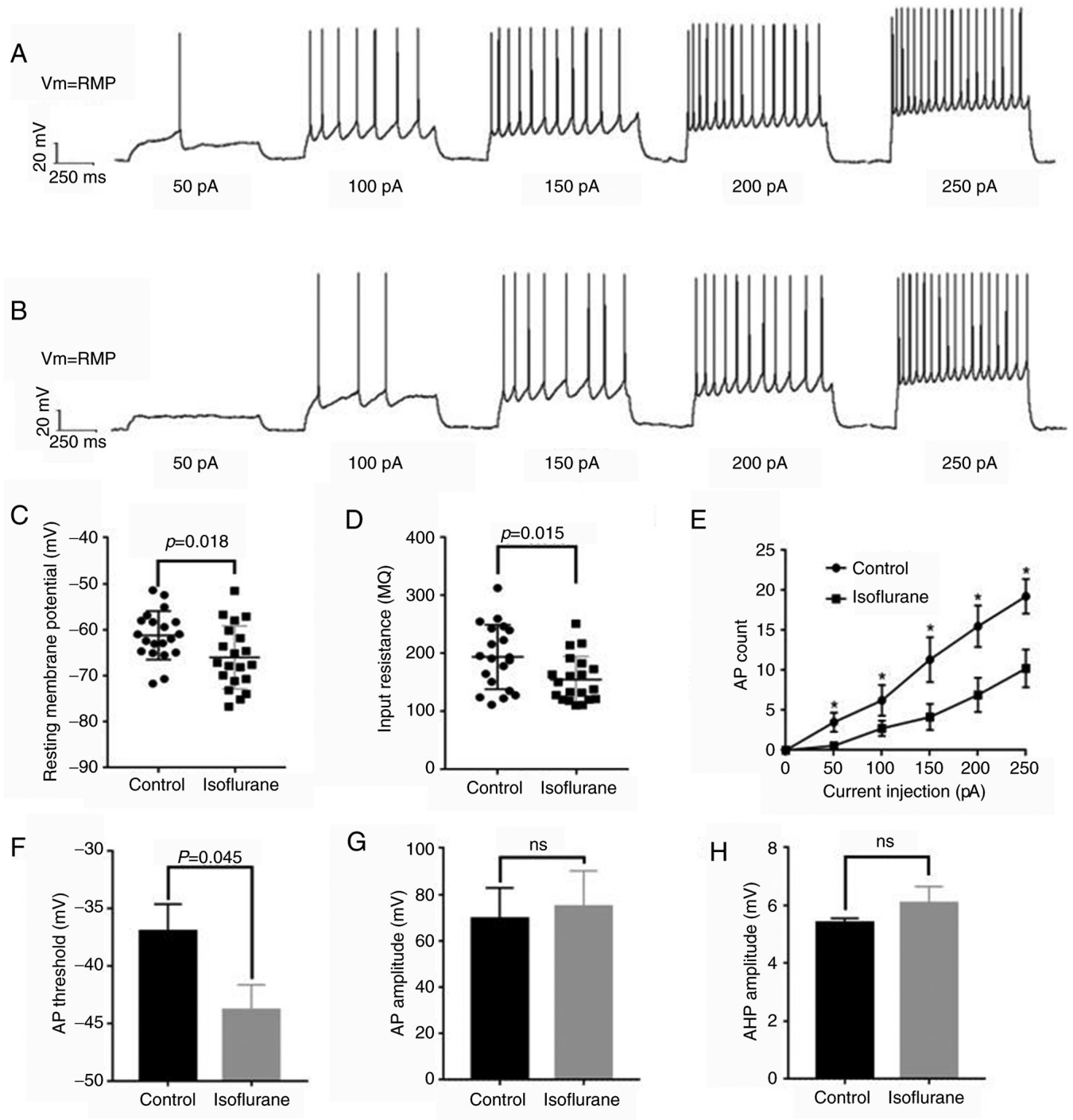

Figure 4. Isoflurane anesthesia inhibits the electrophysiological activity of basolateral amygdala inhibitory neurons in aged mice. The action potentials of inhibitory neurons in mice in the (A) control and (B) isoflurane anesthesia groups injected with different currents at resting potential are shown. (C) Resting potentials of inhibitory neurons in brain slices derived from mice in the control and isoflurane groups. (D) Input resistance of inhibitory neurons in brain slices from control and isoflurane-treated mice. (E) The number of action potentials evoked by different input currents of inhibitory neurons in brain slices isolated from mice in the control and isoflurane groups is shown. (F) Threshold of action potential of inhibitory neurons in brain slices from control and isoflurane-treated mice. The amplitude of (G) action potential and (H) action potential half-duration of inhibitory neurons in brain slices derived from mice in the control and isoflurane groups was recorded (n=20). *P<0.05 control group vs. isoflurane group. AP, action potential; AHP, action potential half-duration.

the threshold of action potential was significantly reduced (P<0.05; Iso vs. Iso + Diaz, -36.7±5.4 vs. -41.2±7.2; Fig. 5F).

Effect of diazepam on the BLA excitatory neurons in aged mice following isoflurane anesthesia. The action potentials of 18 excitatory neurons after injecting different currents at resting potential were recorded using the whole-cell patch-clamp technique (Fig. 6A and B). Therefore, compared with the isoflurane anesthesia group, the resting potential of excitatory neurons in the diazepam group was significantly lower (P<0.05; Iso vs. Iso + Diaz, 172.5±19.8 vs. 157.1±23.7; Fig. 6D). In addition, the input resistance was also notably reduced in the isoflurane anesthesia group compared with the diazepam group (P<0.05; Iso vs. Iso + Diaz, -61.2±5.2 vs. -67.3±6.5;

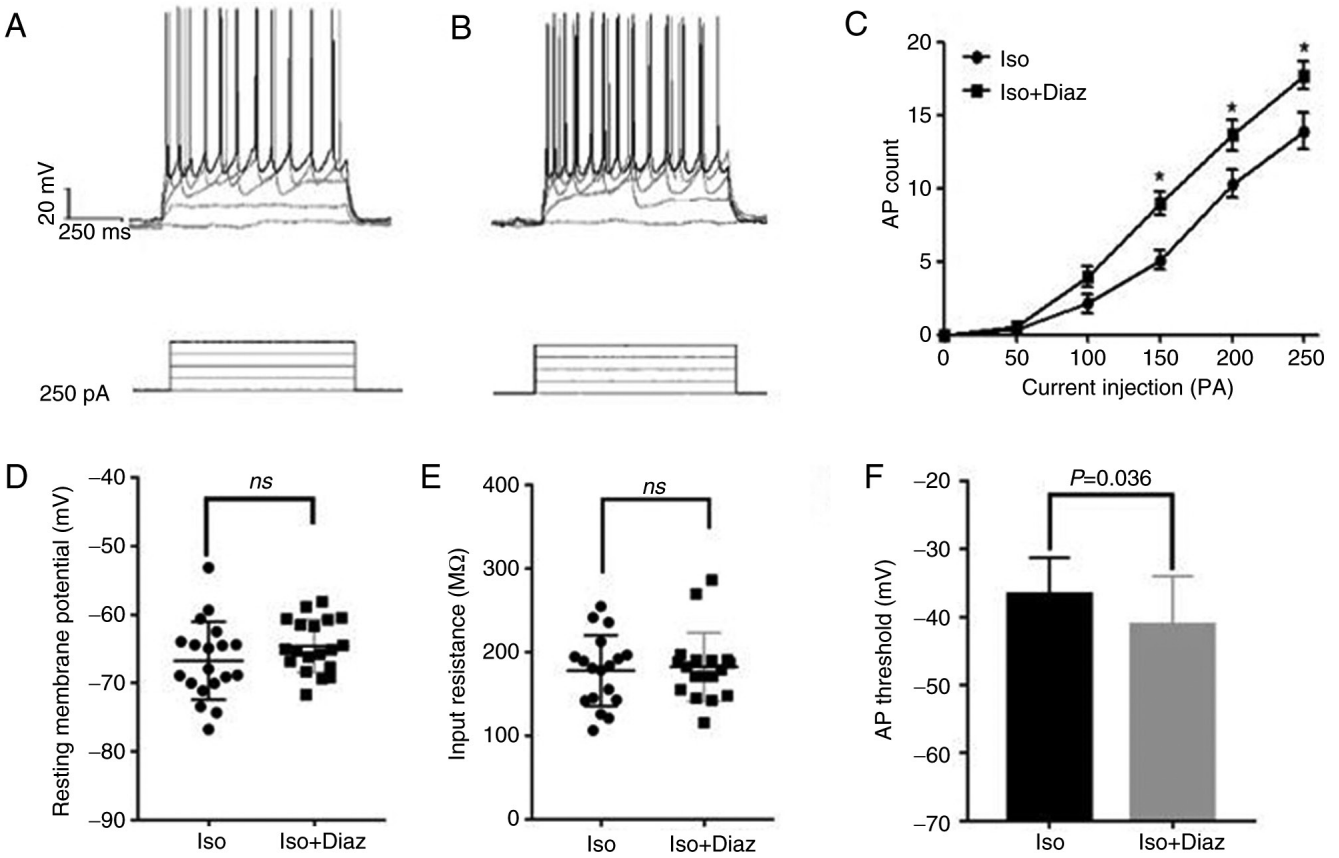

Figure 5. Diazepam increases the excitability of inhibitory neurons in the basolateral amygdala of aged mice after isoflurane anesthesia. The action potentials of inhibitory neurons in mice in the (A) isoflurane anesthesia and (B) diazepam groups injected with different currents at resting membrane potential. (C) The number of action potentials of inhibitory neurons in the brain slices isolated from isoflurane-treated mice with or without the addition of diazepam under different input currents. (D) The resting membrane potential of inhibitory neurons in the brains of isoflurane-treated mice with or without the addition of diazepam is shown. (E) Input resistance of inhibitory neurons in the brains of isoflurane-treated mice co-treated or not with diazepam. (F) Action potential threshold of inhibitory neurons in the brains of isoflurane-treated mice with or without the addition of diazepam (n=19). *P<0.05 Iso group vs. Iso + Diaz group. AP, action potential; Iso, isoflurane; Diaz, diazepam.

Fig. 6E). As the input current increased, the number of evoked action potentials was also gradually enhanced. However, the number of action potentials evoked by excitatory neurons in the diazepam group was markedly reduced compared with the isoflurane anesthesia group (P<0.05; Fig. 6C). Finally, the threshold of action potential was significantly higher in mice in the isoflurane anesthesia group compared with the diazepam group (P<0.05; Iso vs. Iso + Diaz, -42.1±5.4 vs. -38.9±3.2; Fig. 6F).

Discussion

POCD is a type of cognitive impairment that occurs after surgery and is characterized by decreased memory, lack of concentration and impaired executive function (21,22). It has been suggested that anxiety can be a major factor associated with the onset of POCD after surgery, particularly in elderly patients (3). Anxiety is a physiological mechanism that is crucial for survival. However, anxiety circuit dysregulation caused by chronic stress, traumatic brain injury or drugs, can result in pathological anxiety (23).

In the present study, an aged mouse model of isoflurane anesthesia was established and elevated plus maze and open field tests were performed to assess anxiety behavior. The results indicated that aged mice displayed anxiety-like behavior after receiving isoflurane anesthesia. More particularly, the results demonstrated that mice spent more time in the closed arms, showed wall-hugging behavior and traveled longer distances, thus indicating fear and avoidance behavior towards new environments and objects. Additionally, a previous study revealed that volatile anesthetics can cause neurodevelopmental toxicity in rodents and primates and lead to more exaggerated anxiety-like behavior in response to future stress (24).

The processing of anxiety-related information involves a widespread network of brain areas, with the amygdala being a key structure in this network (25). Among the multiple branches of the amygdala, the BLA and central amygdala (CeA) serve a significant role in anxiety processing (26,27). The BLA is a cortical structure predominantly composed of excitatory principal projection neurons and local inhibitory interneurons, which not only modulate the output of the CeA, but also play multiple roles in shaping information flow through the amygdala circuits (28). It has been reported that the overactivity of the BLA is associated with pathological anxiety. Previous studies also showed that a subset of inhibitory interneurons in the BLA continued to increase their firing rate during anxiety-like behavior (29,30). Inhibitory interneurons in the

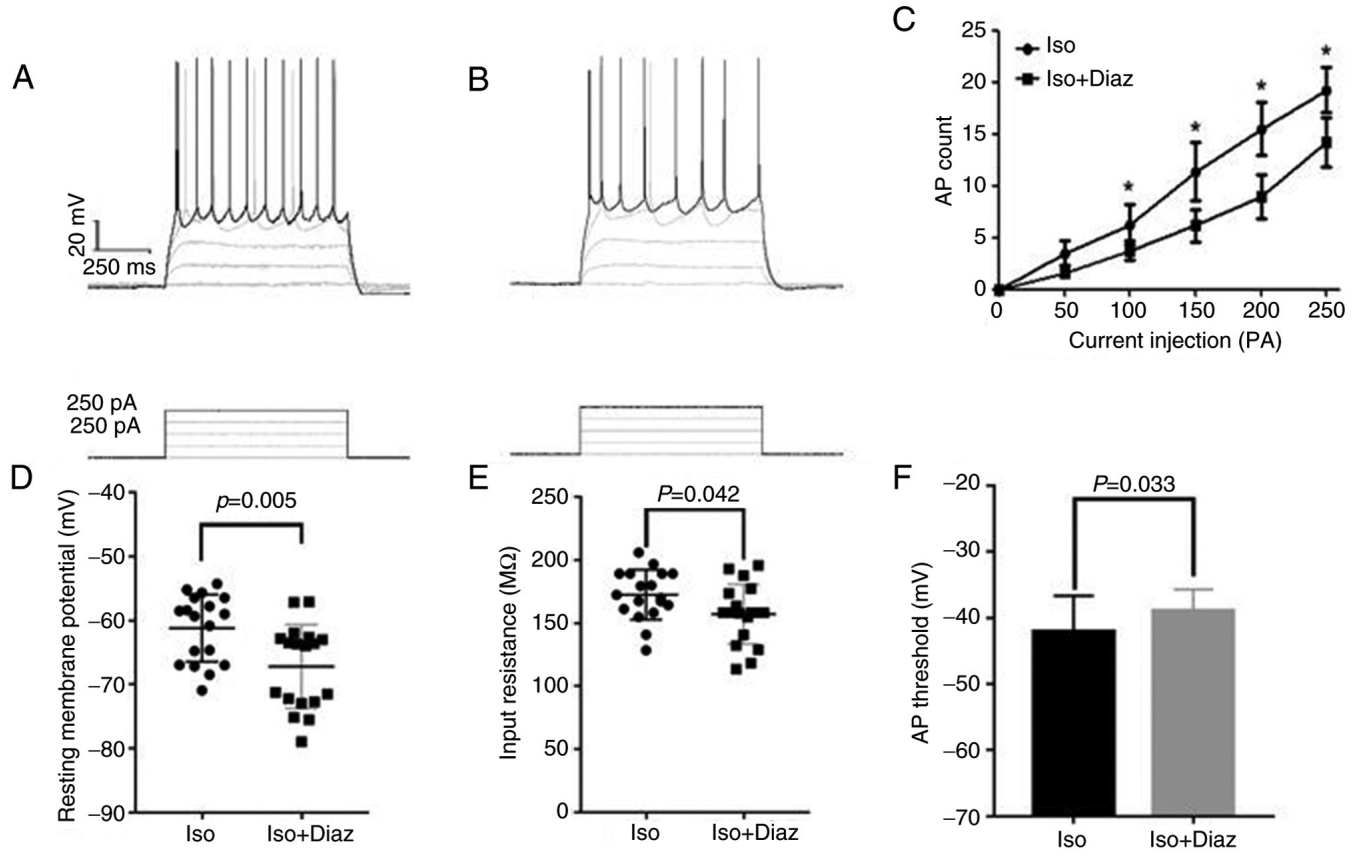

Figure 6. Diazepam inhibits the excitability of excitatory neurons in the basolateral amygdala of aged mice anesthetized by isoflurane. The action potentials of excitatory neurons in aged mice in the (A) isoflurane anesthesia and (B) diazepam groups injected with different currents at resting potential are presented. (C) The number of action potentials evoked by different input currents and (D) the resting potentials of excitatory neurons in the brains of isoflurane-treated mice with or without the addition of diazepam are shown. (E) Input resistance of excitatory neurons in the brains of mice in the isoflurane group co-treated or not with diazepam. (F) Threshold of action potential of excitatory neurons in the brains of isoflurane-treated mice with or without the addition of diazepam (n=18). *P<0.05 Iso group vs. Iso + Diaz group. AP, action potential; Iso, isoflurane; Diaz, diazepam.

BLA can regulate the output of excitatory principal projection neurons to limit the magnitude of anxiety behaviors.

In the present study, whole-cell patch clamp electrophysiology showed that the excitability of the BLA excitatory neurons in aged mice was significantly increased after isoflurane anesthesia, as evidenced by the significantly higher resting membrane potential and input resistance, lower action potential threshold and the markedly increased number of action potentials fired. By contrast, the excitability of inhibitory neurons was markedly decreased, as evidenced by the notably lower resting membrane potential and input resistance, the higher action potential threshold and the significant decrease in the number of action potentials fired compared with the control group.

It has been reported that isoflurane and other anesthetics can affect postsynaptic γ-aminobutyric acid sub-type A (GABAA) receptors and increase their inhibitory function via allosteric modulation (31,32). Therefore, when isoflurane is present, the GABAA receptor-mediated charge transfer is increased, primarily due to the prolongation of the inhibitory current decay. The aforementioned effect has been observed in evoked inhibitory postsynaptic potentials in the BLA (33). Therefore, a previous study demonstrated that repeated exposure to isoflurane promotes a long-term increase in spontaneous GABAA receptor-mediated synaptic transmission (34). Inhibitory interneurons in the amygdala regulate the output of excitatory principal projection neurons to prevent overt behavioral responses to anxiety-provoking stimuli. Therefore, it was hypothesized that they could serve a critical role in defining the valence of incoming sensory stimuli (20). In the present study, to further investigate the role of inhibitory neurons in the increased excitability of the BLA excitatory neurons following isoflurane anesthesia, the perfusate of the brain slices was supplemented with diazepam. Diazepam is the most commonly used psychotropic medication for the treatment of anxiety disorders (35). It enhances the excitability of central inhibitory neurons primarily by enhancing the inhibitory effects of GABA at the GABA A receptor (36). Diazepam binds to specific sites on the GABA A receptor, thus inducing the inhibitory effects of GABA (37). In turn, the aforementioned process facilitates the opening of chloride ion channels, allowing more chloride ions to enter the neurons, thus strengthening the inhibitory effects of GABA (36). The aforementioned enhanced inhibitory activity can reduce neuronal excitability, thus resulting in sedative, anxiolytic and anticonvulsant effects (38). In the present study, co-treatment of isoflurane anesthesia-treated aged mice with diazepam significantly increased the excitability of the BLA inhibitory neurons, while that of excitatory neurons was notably decreased. This finding suggested that the reduced

excitability of inhibitory neurons in aged mice following isoflurane anesthesia could lead to attenuated inhibition of excitatory neurons, thus resulting in the increased excitability and electrical activity of excitatory neurons, ultimately leading to anxiety-like behaviors.

Interneurons in the BLA can form local circuits, thus promoting feedforward and feedback inhibition to projection neurons and other interneurons (28). These interneurons can be classified into different subgroups based on the expression of calcium binding proteins and neuropeptides, such as parvalbumin, somatostatin, cholecystokinin, calbindin and calretinin. The aforementioned interneurons can differ in soma size and dendritic tree shape, while they can target distinct compartments of their postsynaptic targets within the BLA (28). Therefore, emerging evidence has suggested that inhibition of interneurons in the BLA plays a crucial role in regulating anxiety.

In conclusion, aged mice displayed anxiety-like behavior after receiving isoflurane anesthesia, possibly due to the decreased excitability of the inhibitory neurons in the BLA area. This process resulted in an enhanced excitability and electrical activity of excitatory neurons, eventually leading to anxiety-like behavior. However, the mechanism involved was not clarified, and further animal experiments are required to elucidate the effects of isoflurane anesthesia on anxiety-like behavior. Anesthesia-induced consciousness disturbances are usually short-term, with anxiety-like behaviors in aged mice following isoflurane anesthesia being most prominent 2 to 3 days post-anesthesia, gradually resolving even without drug intervention. Therefore, the present study only explored behavioral changes in aged mice after isoflurane anesthesia and electrophysiological alterations in the BLA region, thereby providing a potential direction for future research on anxiety-like behavior changes in elderly patients following anesthesia.

Acknowledgements

Not applicable.

Funding

All funds used for experiments were supported by the Foundation of Science and Technology of Jiangxi Provincial Health and Health Commission (grant no. 202130211).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LQ conceived and designed this study. ML, RZ, SW, LC and HF performed the experiments. ML, RZ, SW, LC, and HF contributed reagents, materials or analysis tools. LQ and ML confirm the authenticity of all the raw data. ML, RZ, SW, LC, HF and LQ wrote the paper. Critical revision of the manuscript was given by all authors. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments were approved by the Laboratory Animal Committee of the First Affiliated Hospital of Nanchang University (approval no. CDYFY-IACUC-202205QR015) and conformed to the National Research Council's Guide for the Care and Use of Laboratory Animals. This study was reported in accordance with ARRIVE guidelines.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Beloeil H, Garot M, Lebuffe G, Gerbaud A, Laviolle B, Dubout E, Oger S, Nadaud J, Becret A, *et al*: Balanced opioid-free anesthesia with dexmedetomidine versus balanced anesthesia with remifentanil for major or intermediate noncardiac surgery. Anesthesiology 134: 541-551, 2021
2. Wang CM, Chen WC, Zhang Y, Lin S and He HF: Update on the mechanism and treatment of sevoflurane-induced postoperative cognitive dysfunction. Front Aging Neurosci 13: 702231, 2021.
3. Lin X, Chen Y, Zhang P, Chen G, Zhou Y and Yu X: The potential mechanism of postoperative cognitive dysfunction in older people. Exp Gerontol 130: 110791, 2020.
4. Bruce SL, Ching THW and Williams MT: Pedophilia-themed obsessive-compulsive disorder: Assessment, differential diagnosis, and treatment with exposure and response prevention. Arch Sex Behav 47: 389-402, 2018.
5. Kozu F, Shirahama-Noda K, Araki Y, Kira S, Niwa H and Noda T: Isoflurane induces Art2-Rsp5-dependent endocytosis of Bap2 in yeast. FEBS Open Bio 11: 3090-3100, 2021.
6. Hao X, Ou M, Zhang D, Zhao W, Yang Y, Liu J, Yang H, Zhu T, Li Y and Zhou C: The effects of general anesthetics on synaptic transmission. Curr Neuropharmacol 18: 936-965, 2020.
7. Yamamoto T, Iwamoto T, Kimura S and Nakao S: Persistent isoflurane-induced hypotension causes hippocampal neuronal damage in a rat model of chronic cerebral hypoperfusion. J Anesth 32: 182-188, 2018.
8. Chírico MTT, Guedes MR, Vieira LG, Reis TO, Dos Santos AM, Souza ABF, Ribeiro IML, Noronha SISR, Nogueira KO, Oliveira LAM, *et al*: Lasting effects of ketamine and isoflurane administration on anxiety- and panic-like behavioral responses in Wistar rats. Life Sci 276: 119423, 2021.
9. Feinstein JS, Gould D and Khalsa SS: Amygdala-driven apnea and the chemoreceptive origin of anxiety. Biol Psychol 170: 108305, 2022.
10. Morel C, Montgomery SE, Li L, Durand-de Cuttoli R, Teichman EM, Juarez B, Tzavaras N, Ku SM, Flanigan ME, Cai M, *et al*: Midbrain projection to the basolateral amygdala encodes anxiety-like but not depression-like behaviors. Nat Commun 13: 1532, 2022.
11. Puggioni P, Pelko M, Rossum MV and Duguid I: Behavioral state differentially regulates input sensitivity and firing rates of motor cortex pyramidal neurons. Bmc Neurosci 14 (Suppl 1): P114, 2013.
12. Perumal MB and Sah P: Inhibitory circuits in the basolateral amygdala in aversive learning and memory. Front Neural Circuits 15: 633235, 2021.
13. National Research Council (US): Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals, 8th edition. National Academies Press, Washington, DC, 2011.
14. Kilkenny C, Browne W, Cuthill IC, Emerson M and Altman DG; NC3Rs Reporting Guidelines Working Group: Animal research: reporting in vivo experiments: The ARRIVE guidelines. J Gene Med 12: 561-563, 2010.

15. Zuo CL, Wang CM, Liu J, Shen T, Zhou JP, Hao XR, Pan YZ, Liu HC, Lian QQ and Lin H: Isoflurane anesthesia in aged mice and effects of A1 adenosine receptors on cognitive impairment. CNS Neurosci Ther 24: 212-221, 2018.
16. Wang Z, Meng S, Cao L, Chen Y, Zuo Z and Peng S: Critical role of NLRP3-caspase-1 pathway in age-dependent isoflurane-induced microglial inflammatory response and cognitive impairment. J Neuroinflammation 15: 109, 2018.
17. Wang J, Zhu S, Lu W, Li A, Zhou Y, Chen Y, Chen M, Qian C, Hu X, Zhang Y and Huang C: Varenicline improved laparotomy-induced cognitive impairment by restoring mitophagy in aged mice. Eur J Pharmacol 916: 174524, 2022.
18. Rabat Y, Henkous N, Corio M, Nogues X and Beracochea D: Baclofen but not diazepam alleviates alcohol-seeking behavior and hypothalamic-pituitary-adrenal axis dysfunction in stressed withdrawn mice. Front Psychiatry 10: 238, 2019.
19. Rana T, Behl T, Sehgal A, Singh S, Sharma N, Abdeen A, Ibrahim SF, Mani V, Iqbal MS, Bhatia S, *et al*: Exploring the role of neuropeptides in depression and anxiety. Prog Neuropsychopharmacol Biol Psychiatry 114: 110478, 2022.
20. Jones SK, McCarthy DM, Vied C, Stanwood GD, Schatschneider C and Bhide PG: Transgenerational transmission of aspartame-induced anxiety and changes in glutamate-GABA signaling and gene expression in the amygdala. Proc Natl Acad Sci USA 119: e2213120119, 2022.
21. Wang S, Cardieri B, Mo Lin H, Liu X, Sano M and Deiner SG: Depression and anxiety symptoms are related to pain and frailty but not cognition or delirium in older surgical patients. Brain Behav 11: e02164, 2021.
22. Hem S, Albite R, Loresi M, Rasmussen J, Ajler P, Yampolsky C, Chabot JD, Gerszten PC and Goldschmidt E: Pathological changes of the hippocampus and cognitive dysfunction following frontal lobe surgery in a rat model. Acta Neurochir (Wien) 158: 2163-2171, 2016.
23. Craske MG and Stein MB: Anxiety. Lancet 388: 3048-3059, 2016.
24. Zhong J, Li C, Peng L, Pan Y, Yang Y, Guo Q and Zhong T: Repeated neonatal isoflurane exposure facilitated stress-related fear extinction impairment in male mice and was associated with ΔFosB accumulation in the basolateral amygdala and the hippocampal dentate gyrus. Behav Brain Res 446: 114416, 2023.
25. Liu WZ, Zhang WH, Zheng ZH, Zou JX, Liu XX, Huang SH, You WJ, He Y, Zhang JY, Wang XD and Pan BX: Identification of a prefrontal cortex-to-amygdala pathway for chronic stress-induced anxiety. Nat Commun 11: 2221, 2020.
26. Zheng ZH, Tu JL, Li XH, Hua Q, Liu WZ, Liu Y, Pan BX, Hu P and Zhang WH: Neuroinflammation induces anxiety- and depressive-like behavior by modulating neuronal plasticity in the basolateral amygdala. Brain Behav Immun 91: 505-518, 2021.
27. Tye KM, Prakash R, Kim SY, Fenno LE, Grosenick L, Zarabi H, Thompson KR, Gradinaru V, Ramakrishnan C and Deisseroth K: Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature 471: 358-362, 2011.
28. Babaev O, Piletti Chatain C and Krueger-Burg D: Inhibition in the amygdala anxiety circuitry. Exp Mol Med 50: 1-16, 2018.
29. Prager EM, Bergstrom HC, Wynn GH and Braga MFM: The basolateral amygdala γ-aminobutyric acidergic system in health and disease. J Neurosci Res 94: 548-567, 2016.
30. Lee SC, Amir A, Haufler D and Pare D: Differential recruitment of competing valence-related amygdala networks during anxiety. Neuron 96: 81-88.e5, 2017.
31. Hemmings HC Jr, Akabas MH, Goldstein PA, Trudell JR, Orser BA and Harrison NL: Emerging molecular mechanisms of general anesthetic action. Trends Pharmacol Sci 26: 503-510, 2005.
32. Krasowski MD and Harrison NL: General anaesthetic actions on ligand-gated ion channels. Cell Mol Life Sci 55: 1278-1303, 1999.
33. Ranft A, Kurz J, Deuringer M, Haseneder R, Dodt HU, Zieglgänsberger W, Kochs E, Eder M and Hapfelmeier G: Isoflurane modulates glutamatergic and GABAergic neurotransmission in the amygdala. Eur J Neurosci 20: 1276-1280, 2004.
34. Long Ii RP, Aroniadou-Anderjaska V, Prager EM, Pidoplichko VI, Figueiredo TH and Braga MF: Repeated isoflurane exposures impair long-term potentiation and increase basal GABAergic activity in the basolateral amygdala. Neural Plast 2016: 8524560, 2016.
35. Wesołowska A: Potential role of the 5-HT6 receptor in depression and anxiety: An overview of preclinical data. Pharmacol Rep 62: 564-577, 2010.
36. Tong X, Zhang Z, Zhu J, Li S, Qu S, Qin B, Guo Y and Chen R: A comparison of epileptogenic effect of status epilepticus treated with diazepam, midazolam, and pentobarbital in the mouse pilocarpine model of epilepsy. Front Neurol 13: 821917, 2022.
37. Kasaragod VB, Malinauskas T, Wahid AA, Lengyel J, Knoflach F, Hardwick SW, Jones CF, Chen WN, Lucas X, El Omari K, *et al*: The molecular basis of drug selectivity for α5 subunit-containing $GABA_A$ receptors. Nat Struct Mol Biol 30: 1936-1946, 2023.
38. Courtney CD, Sobieski C, Ramakrishnan C, Ingram RJ, Wojnowski NM, DeFazio RA, Deisseroth K and Christian-Hinman CA: Optoα1AR activation in astrocytes modulates basal hippocampal synaptic excitation and inhibition in a stimulation-specific manner. Hippocampus 33: 1277-1291, 2023.

Copyright © 2024 Li et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.