Environmental enrichment improves the recognition memory in adult mice following social isolation via downregulation of Kv4.2 potassium channels

Qing Shang
Ningbo Hospital of Ningbo University: Ningbo City First Hospital
Yi-Bei Dong
Ningbo University School of Medicine: Ningbo University Medical School
Le Xu
Ningbo University School of Medicine: Ningbo University Medical School
Jian-Hong Yang
Ningbo Hospital of Ningbo University: Ningbo City First Hospital
Jia-Wen Li
Ningbo University School of Medicine: Ningbo University Medical School
Wei-Yi Yu
Ningbo University School of Medicine: Ningbo University Medical School
Jie Sun
Ningbo Hospital of Ningbo University: Ningbo City First Hospital
Xiang Gao
Ningbo Hospital of Ningbo University: Ningbo City First Hospital
Yi Huang
Ningbo Hospital of Ningbo University: Ningbo City First Hospital
Xiao-Qin Zhang (zhangxiaoqin1@nbu.edu.cn)
Ningbo University Medical School https://orcid.org/0000-0003-0254-7598

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Abstract

The recognition memory is a cognitive process that enables us to distinguish familiar objects and situations from new items, which is essential for mammalian survival and adaptation to a changing environment. Social isolation (SI) has been implicated as a detrimental factor for recognition memory. The medial prefrontal cortex (mPFC) has been shown to carry information concerning the relative familiarity of individual stimuli, and modulating neuronal function in this region may contribute to recognition memory. The present study aimed to investigate the neuronal mechanisms in the mPFC of environmental enrichment (EE) on recognition memory in adult mice following SI. Mice were assigned into three groups: Control, SI, and SI + EE group. Novel location recognition (NLR) and Novel object recognition (NOR) tests were performed to evaluate the recognition memory. The levels of Kv4 channels were assessed by qRT-PCR and Western blotting. The effects of SI and SI + EE on the intrinsic excitability of pyramidal neurons in the mPFC were measured using whole-cell recording. We found that SI led to a reduction in the intrinsic excitability of pyramidal neurons. Specifically, we have identified that the reduction in the firing activity of pyramidal neurons resulted from alterations in the function and expression of Kv4.2 channels. Furthermore, EE regulated Kv4.2 channels, normalized the activity of pyramidal neurons and restored the behavioral deficits following SI. Thus, the roles of Kv4.2 channels in intrinsic excitability of pyramidal neurons suggest that the Kv4.2 channels present a promising therapeutic target for recognition memory impairment.

Introduction

Recognition memory is defined as the ability of a person to identify as familiar a stimulus or a situation that has been encountered previously. This ability is an important part of declarative episodic memory and a vital cognitive function. Social isolation (SI) has been implicated as a detrimental factor for recognition memory in humans and animals. In particular, a higher level of SI during the COVID-19 outbreak or among the older individuals was associated with a greater decline in memory [1–3]. SI also impairs social recognition memory in adult mice [4, 5]. One of the most frequently described brain region for recognition memory is the medial prefrontal cortex (mPFC) [6]. Neurons in the mPFC have been shown to carry information concerning the relative familiarity of individual stimuli [7, 8] and lesions to this region impair recognition memory tasks [9, 10]. Notably, SI in rodents has been shown to reduce the synaptic and intrinsic excitability of pyramidal neurons and enhance the activity of inhibitory neuronal circuits in the mPFC [11, 12]. Furthermore, the expression and function of ion channels in neurons are important determinants of their excitability and responsiveness to synaptic plasticity [13]. However, it is unknown how SI affects the ion channel expression or function of mPFC neurons, and understanding the molecular mechanisms underlying ion channel function may uncover novel therapeutic targets for recognition memory impairment.

Environmental enrichment (EE) refers to modifications that act to enhance an individual or animals social or physical surroundings [14]. EE exposure produces wide-ranging effects in the brain at molecular, cellular, network, and behavioral levels [15]. Specifically, EE has been found to improve recognition

memory in animal models of normal aging and disease by stimulating synaptic plasticity via increased numbers of dendritic spines and enhanced synaptic function [16–18]. EE is also known to have an effect on neuronal activity through regulating the expression of ion channels and synaptic proteins of many brain areas including the PFC [19], hippocampus [20, 21] and NAc [22]. Thus, it is not surprising that EE may exert neuroplasticity and neuroprotective effects in adult mice following SI. However, the exact mechanisms underlying ion channels of EE on SI of adult mice remain unclear.

Kv4 channels are expressed in a variety of tissue, with particularly high levels in the brain and heart [23]. Especially, Kv4.2 channels may integrate a variety of intracellular signaling cascades into a coordinated output that dynamically modulates membrane excitability [23]. The changes in Kv4.2 channels are mostly responsible for the neuronal activity [24, 25], synaptic plasticity [26], neurotransmitters [24] and neurogenesis [27], which correlate with learning and memory.

In the present study, our findings demonstrate that SI is associated with a diminished intrinsic excitability of pyramidal neurons in the mPFC. Specifically, we have identified that the dampening of firing activity of pyramidal neurons is attributable to the abnormal function and expression of Kv4.2 channels. Additionally, we have established that EE exerts a modulatory effect on Kv4.2 channels, leading to the normalization of the firing activity of pyramidal neurons and the amelioration of the behavioral deficits that manifest after SI.

Materials and methods

Animals

All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and these were approved by the Animal Care and Use Committees of Ningbo University, China. Male C57BL/6J mice were purchased from Shanghai Sino-British SIPPR/BL Lab and were housed under standard conditions at 22°C and a 12 h light: dark cycle with free access to food and water. After weaning at postnatal day (PND) 21, mice from the same litter were randomly divided in equal numbers for isolated or group-rearing [28]. Each isolated mouse was housed in standard conditions (SE, cages with 30 × 18 × 12cm) or enriched environment (EE, cages with 50 × 35 × 20cm) until sampling [29]. EE often consists of social enhancement (group-housed) or cage enhancements, including toys, exercise wheels, and housing structures (e.g., PVC piping), here, we provided adult mice the cage enhancements following social isolation (Fig. 1A).

Novel location recognition (NLR) and Novel object recognition (NOR)

The NLR and NOR tests were conducted to assess recognition memory in mice, following established protocols[30]. For the NLR test, the experimental apparatus consisted of an open field box (25 cm L × 25 cm W × 25 cm H, with one wall specially marked) and two identical objects. The mice were acclimatized

to the open field box for 3 days, with daily 10-minute sessions. Subsequently, during the training phase, 24 hours after the last acclimatization session, the mice were allowed to explore two identical objects for 10 minutes, and the investigation time for each object was recorded. After a 1-hour interval, one of the objects was moved diagonally opposite to the other, and the mice were allowed to explore the objects again for 5 minutes. The investigation time for each object was then recorded. Object exploration time was defined as the duration in which a mouse's nose touched or was oriented towards the object, and came within 2 cm of it. The NLR discrimination index, which reflects spatial recognition memory, was calculated as (novel location investigation time − familiar location investigation time) / (novel location investigation time + familiar location investigation time).

For the NOR test, the experimental apparatus consisted of an open field box (25 cm L × 25 cm W × 25 cm H) and two objects of different shapes but made of the same material. The acclimatization and training phases were identical to those of the NLR test, conducted over the first 4 days. On the fifth day, 24 hours after the training phase, one of the objects was replaced with a new object, and the mice were allowed to explore the two different objects for 5 minutes. The investigation time for each object was recorded and the NOR discrimination index was defined as in the NLR test.

Electrophysiology

Brain slices containing mPFC were prepared for whole-cell recordings from group, SI or SI + EE mice during PD 70–84. Mice were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and decapitated, and then brains were dissected quickly and placed in an ice-cold solution containing below substances (in mM): 75 sucrose, 87 NaCl, 3 KCl, 1.5 $CaCl_2$, 1.3 $MgCl_2$, 1 NaH_2PO_4, 26 $NaHCO_3$, 20 glucose equilibrated with 95% O_2–5% CO_2. Coronal slices (220 μm thickness) were prepared with a vibratome (Leica VT1200S, Leica, Germany), and then incubated in a chamber with artificial cerebrospinal fluid (aCSF) containing below substances (in mM): 124 NaCl, 3 KCl, 1 NaH_2PO_4, 1.3 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, and 20 glucose, 295–305 mOsm, equilibrated at 32°C with 95% O_2–5% CO_2). Slices were incubated for at least one hour before recording. Following incubation, the slices were transferred to a recording chamber, where the submerged slices were perfused with aCSF (32°C) saturated with mixed gas at a flow rate of 2 mL per min. Standard recordings were obtained using Multiclamp 700B amplifier and Digidata 1550B (Molecular Devices, Axon Instruments, CA, USA). Glass electrodes (3IN thin-wall GL1.5 OD/1.12 ID, TW150-3, WPI) were pulled into pipettes with a vertical two-stage puller (PC-10, NARISHIGE), with resistance ranging between 1.5 to 2 mOhm when filled with internal solution.

For whole-cell recordings, pyramidal neurons were visually identified based on their shape and prominent apical dendrite, patch pipettes were filled with a K-based internal solution was used (in mM): 120 K-gluconate, 10 KCl, 10 HEPES, 0.5 EGTA, 4 Mg-ATP, and 0.3 Na-GTP, 5 Phosphocreatine-Na_2, pH 7.2–7.4, 270–280 mOsm. Before current-clamp recording, series resistance was monitored and canceled using a bridge circuit and the pipette capacitance was compensated. After forming the whole-cell current-clamp configuration, the recorded cells were given 10 min for stabilization of their resting membrane potentials (RMP). A current step protocol (from − 200 to 500 pA, with a 50-pA increment; inter-pulse interval, 15 s)

was then performed. The fast after-hyperpolarization (fAHP) magnitude was measured as the lowest voltage attained during hyperpolarization subtracted from the resting membrane potential recorded before the stimulus used to induce the action potential. The half-width was valued from the 200-pA current injected step, and neurons without action potential in this step will not be counted. The Series resistance was monitored throughout the experiment using a 5 mV hyperpolarizing step. Recordings were terminated if the series resistance varied by more than 20% during the course of the experiment. All holding potentials were corrected for liquid junction potential. Data were low-pass filtered at 1 kHz and digitized at a sampling frequency of 10 kHz for on-line and later off-line analysis (Clampfit 10.7; Molecular Devices, CA, USA). Drugs of AmmTX3 (1 nM, 5659, TOCRIS) were added in the regular aCSF to block Kv4 channels.

All chemicals used in the patch clamp were purchased from Sinopharm Chemical Reagent Co., Ltd., except as noted.

Qualitative Polymerase Chain Reaction (qPCR)

Frozen hippocampal tissues were lysed in TRIzol and homogenized using the MagNALyser (Roche, United States). The RNA concentration was quantified, and its purity was assessed based on an OD260/OD280 value. Subsequently, reverse transcription was performed using the primers listed in Table 1. All reactions were conducted in a 5 μL system consisting of 1 μL of cDNA, 2.2 μL of UltraSYBR Mixture, 0.1 μL of primer, and 1.6 μL of purified water, using a LightCycler 480 instrument (Roche, United States) and 384-well plates. The relative expression levels of target genes were quantified with 2-ΔΔCt, and the measured mRNA abundance was normalized to 18S rRNA.

Table 1
The primers used for the Q-PCR assessment in the study.

Genes	Forward primer	Reverse primer	Size(bp)
Kv4.1	TGTGCTTCGAGATGTGTGGTT	ACCAACGTCAAATAGCTGACTC	126
Kv4.2	CCGAGGGGAGCAAATCACC	TAGTCCACACAGCTAACCAGG	178
Kv4.3	ATGGAGGGGTCTAAGGCGTC	TGGGCTGTGGTAAGTAATGGA	149

Western blot

mPFC tissues were homogenized in ice-cold RIPA buffer (Beyotime, Shanghai, China), supplemented with a protease inhibitor cocktail (Beyotime, Shanghai, China), using a glass Teflon homogenizer and incubated on ice for 10 min. Homogenates were centrifuged at 12,000 g for 12 minutes at 4°C, after which supernatants were collected. The protein concentration of each sample was determined prior to loading onto the gel, and 40 μg of protein per sample was loaded onto each lane. The membrane was blocked in 5% skim milk, in 0.05% Tween-20 in PBS (PBST) at RT, and then incubated overnight at 4°C with the primary antibody (Kv4.2, 1:300, 21298-1-Ig, Proteintech), and then reacted with the secondary antibodies (HRP-conjugated affinity purified goat anti-rabbit IgG (H + L), 1:10000, SA00001-2, Proteintech)

for 2 hours at RT. The protein bands were detected in a fluorescence scanner (Odyssey Infrared Imaging System, LI-COR Biotechnology, Lincoln, NE, United States) by automatic exposure and the gray value of the immunoreactive protein band was analyzed with the Image J software (version 1.52a; National Institutes of Health). GAPDH (Rabbit polyAb, 1:5000, 10494-1-AP, Proteintech) served as an internal protein control. The results were obtained from three independent replications of the experiment.

Statistical analysis

GraphPad Prism version 8 (GraphPad Software, San Diego, CA, United States) was used to conduct statistical analyses. Unpaired or paired student's t-test was used to compare two groups. One-way ANOVA followed by Turkey's post hoc test was used for assessing effects within more than two groups in electrophysiological and behavioral experiments. Data are presented as mean ± SEM. $p < 0.05$ was considered as statistically significant for all results.

Results

Environmental enrichment improved the impaired recognition memory in adult mice following social isolation

We performed the NLR and NOR tests in 10-week-old mice to detect the recognition memory (Fig. 1B). The discrimination index demonstrated that SI-reared mice largely failed to recognize new location or new objects, contrary to group-reared mice, and EE improved the recognition memory both in NLR and NOR tests (Fig. 1C, NLR: one-way-ANOVA: $F_{(2, 43)} = 15.80$; $p < 0.0001$; Fig. 1D, NOR: one-way-ANOVA: $F_{(2, 43)} = 5.215$; $p = 0.0094$). These results suggested that socially isolated mice have impaired spatial and object recognition memory, which was restored by enriched environment.

Environmental enrichment regulated the abnormal Kv4.2 channel in the mPFC of adult mice following social isolation

Kv4 is the A–type potassium channel which mediates the excitability of pyramidal neurons in the cortex, has been shown to be involved in neural plasticity and cognitive function. The results of real-time qPCR analysis showed that the mRNA expression levels of Kv4.2, but not Kv4.1 or Kv4.3, were found to be elevated in the mPFC of SI-reared mice compared with group mice, which was reversed in mice that were exposed to an enriched environment (Fig. 2A-C, Kv4.2: one-way-ANOVA: $F_{(2, 21)} = 4.664$; $p = 0.0211$). Western blot analysis also revealed that EE was able to restore the expression of Kv4.2 in the SI-reared mice back to the same level as in the group-reared mice (Fig. 2D, one-way-ANOVA: $F_{(2, 14)} = 9.947$; $p = 0.0021$). These findings further support the notion that EE can reverse the dysregulation of Kv4.2 expression induced by SI and suggest a potential mechanism by which EE may exert its beneficial effects on recognition memory.

Environmental enrichment restored intrinsic excitability of pyramidal neurons in the mPFC of SI-reared mice

To determine the effects of Kv4.2 expression alterations on pyramidal neurons in the mPFC, we then tested the intrinsic excitability of pyramidal neurons via a series of somatic positive current steps. The reduced firing number of action potential (AP) was found in pyramidal neurons recorded from SI-reared mice compared with group-housed mice, while EE significantly increased intrinsic excitability of pyramidal neurons in mice following SI (Fig. 3B, two-way-ANOVA: factor of life history, $F_{(2, 45)} = 5.742$, $p = 0.0060$; factor of current steps, $F_{(2.34, 105.3)} = 654.6$, $p < 0.0001$; interaction of two factors: $F_{(20, 450)} = 3.154$, $p < 0.0001$). This indicated that pyramidal neurons in the mPFC of SI mice fired less APs compared with other two groups. Notably, we observed that EE attenuated the amplitude of fAHP (Fig. 3C) in SI-reared mice (Fig. 3D, one-way-ANOVA: $F_{(2, 45)} = 5.762$; $p = 0.0059$). Generally, fAHP is largely modulated by voltage-gated potassium channels and limits firing frequency. The large fAHP found in SI-reared mice shown that Kv channel was strongly activated during AP depolarization, which consistent with the results of abnormal expression of Kv4.2. Given that Kv4.2 is known to affect the AP half-width, accordingly, we found that the AP half-width reduced in SI-reared mice compared with other two groups (Fig. 3E & F, two-way-ANOVA: factor of life history, $F_{(2, 45)} = 3.368$, $p = 0.0434$; factor of current steps, $F_{(3.144, 141.5)} = 67.99$, $p < 0.0001$; interaction of two factors: $F_{(16, 360)} = 1.960$, $p = 0.015$). Taken together, our results suggest that EE may increase intrinsic excitability of pyramidal neurons in the mPFC of SI-reared mice by upregulating Kv channels, particularly Kv4.2.

Blocking Kv4.2 channels using the scorpion toxin AmmTX3 increased intrinsic excitability of pyramidal neurons in the mPFC of SI-reared mice

We performed a series of current-clamp recordings to assess the effects of acute blockade of Kv4.2 channels via incubation of scorpion toxin AmmTX3 in the mPFC of SI-reared mice. Consistent with previous reports [25, 31], AmmTX3 strongly increased firing number of pyramidal neurons (Fig. 4A & B, two-way-ANOVA: factor of drug, $F_{(1, 7)} = 9.282$, $p = 0.0187$; factor of current steps, $F_{(1.56, 10.92)} = 207.7$, $p < 0.0001$; interaction of two factors: $F_{(3.593, 25.15)} = 3.864$, $p = 0.0164$), which suggested that blocking Kv4.2 channels increased the intrinsic excitability of pyramidal neurons in the mPFC of SI-reared mice. Accordingly, the effects of SI on fAHP and AP half-width were also reversed by AmmTX3 treatment (Fig. 4C, paired t-test: $t_{(7)} = 3.891$; $p = 0.006$; Fig. 4D, two-way-ANOVA: factor of drug, $F_{(1, 7)} = 13.23$, $p = 0.0083$; factor of current steps, $F_{(8, 56)} = 10.44$, $p < 0.0001$; interaction of two factors: $F_{(8, 56)} = 2.567$, $p = 0.0185$). Most importantly, the actions of this drug were very similar to that observed in the SI + EE mice: firing number and relative half-width (AP_2 /AP_1) were increased by ~20% and ~15% in both conditions in response to step-up currents from 250 to 500 pA. While amplitude of fAHP was decreased by 42% after AmmTX3 and by 39% in SI + EE mice. Combined with our previous data on expression levels of Kv4.2, these findings strongly imply that the Kv4.2-mediated A-type current contributes to the increased intrinsic

excitability of pyramidal neurons in the mPFC of SI-reared mice and that its abnormality can be reversed by EE.

Discussion

The present study demonstrates that SI lead to a reduction in the intrinsic excitability of pyramidal neurons in the mPFC and deficits of recognition memory. Specifically, we have found that the abnormal expression of Kv4.2 channels contribute to the reduction in the firing activity of pyramidal neurons. Furthermore, EE regulates Kv4.2 channels, normalizes the intrinsic excitability of pyramidal neurons and restores the behavioral deficits following SI. Our findings suggest that Kv4.2 channels may present a promising therapeutic target for impaired recognition memory.

Alterations in neuronal excitability have been observed in rodents that were socially isolated at both neonatal and stages [11, 32, 33]. We previously reported that SI increased intrinsic excitability of medium spiny neurons (MSNs) in nucleus accumbens (NAc) [33]. The SI-reared mice at the stage increased the excitatory inputs to fast-spiking parvalbumin-expressing (PV) interneurons and thus increased the firing of PV interneurons [32]. The increased intrinsic excitability of PV interneurons was still observed in the mPFC of mice in the adult mice following SI [12], together with increased inhibitory synaptic inputs onto a subtype of layer 5 pyramidal cells (prominent h-current cells) [11], which were in accordance with our findings that a reduction in intrinsic excitability of pyramidal neurons in the mPFC. By contrast, EE is a well-established paradigm for attenuating the negative effects during periods of social isolation. EE has been shown to increase the intrinsic excitability of hippocampal CA1 pyramidal neurons [34] and granule-cells [35], which is in line with our findings that the reduction excitability of pyramidal neurons resulting from SI can be reversed by EE. These results highlight the sensitivity of neuronal excitability to environmental factors and suggest that it may represent a promising therapeutic target.

Kv4.2 is a subunit of the A-type potassium channel, which modulate neuronal excitability in various brain regions [36]. We found that the reduced expression of Kv4.2 channels and the decreased intrinsic excitability of pyramidal neurons in the mPFC in SI-reared mice. Furthermore, EE regulated Kv4.2 channels and normalized the activity of pyramidal neurons in the mPFC, which was blocked by the scorpion toxin AmmTX3 (the inhibitor of Kv4 channel). These results indicate that the changes of Kv4.2 channels contribute to the alterations in neuronal excitability. However, the mechanism underlying the changes of Kv4.2 expression levels or function remain to be examined in future studies. Although our data demonstrate a critical role of EE modulation of Kv4.2 channels in the mPFC in mediating recognition memory following SI, it is certainly true that the expression of Kv4.2 is widespread throughout the nervous system [37], thus, it is possible that Kv4.2 plays important roles in other parts of the brain. We are particularly curious to see whether other subtypes of Kv4 channels may be impacted by SI, although no significant differences in mRNA and protein levels were detected in the mPFC of SI-reared mice.

Neuronal intrinsic excitability and the synaptic connections among neurons produce behavior and cognition. Plasticity of neuronal excitability facilitates learning and memory, while aberrant modulation of

this process being linked to the development of neurological symptoms in multiple diseases [38]. Previous work showed that alterations in PV neuronal excitability in the mPFC resulting from acute neuroinflammation may mediate neuronal recruitment and confer a beneficial outcome on recognition memory [39]. The rats with head injury exhibited impairment in novel object recognition memory, which was accompanied by an increase in intrinsic excitability in layer 2/3 neurons within the mPFC [40]. Correspondingly, we found that socially isolated mice showed the impaired recognition memory and the decreased intrinsic excitability of pyramidal neurons in layer 5 within the mPFC. Furthermore, the evidences concerning Kv4.2 channel alterations in the pathophysiology of recognition memory deficits following SI were emerging. More importantly, the abnormal phenomenon could be reversed by EE treatment. Additionally, it is well established that EE modulates hippocampal neurogenesis and behavior, resulting in an increase in newborn neurons (data not shown) and enhanced hippocampus-dependent cognition [29, 41], including the recognition memory [42, 43]. Considering the major contribution of newborn neurons in recognition memory [44, 45], Cell-specific expression and function of Kv4.2 require a further detection in future studies.

Conclusions

In summary, our study highlights the importance of neuronal excitability and synaptic plasticity in shaping behavior and cognition, and the critical role of Kv4.2 channels in regulating these processes. Our findings suggest that alterations in Kv4.2 channels may contribute to the deficits of recognition memory, particularly in response to SI. Moreover, our study adds to the growing body of evidence suggesting that EE may represent a promising therapeutic strategy for mitigating the adverse effects associated with SI. Further research is needed to fully elucidate the underlying mechanisms of Kv4.2 in regulating neural and behavioral functions, which may ultimately lead to the development of novel treatments for neurological and psychiatric disorders.

Abbreviations

SI	Social isolation
mPFC	Medial prefrontal cortex
EE	Environmental enrichment
PND	Postnatal day
NLR	Novel location recognition
NOR	Novel object recognition
aCSF	Artificial cerebrospinal fluid
RMP	Resting membrane potentials
fAHP	Fast after-hyperpolarization
AP	Action potential
MSNs	Medium spiny neurons
NAc	Nucleus accumbens
PV	Parvalbumin-expressing

Declarations

Ethics approval and consent to participate

The animal study protocol was approved by the Committee of Ningbo University on the Ethics of Animal Experiments (NBU2021160, March 2021).

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author with reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Experimental design, XQ and QS. Conduct of experiments, QS, YB, LX, JW and WY. Analysis and interpretation of data, QS, JH, YH and JS. Writing original manuscript, QS and XQ. Reviewing and editing, JS, XG and XQ. Funding acquisition, XQ, JH, JS and XG. All authors read and approved the final manuscript.

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Figures

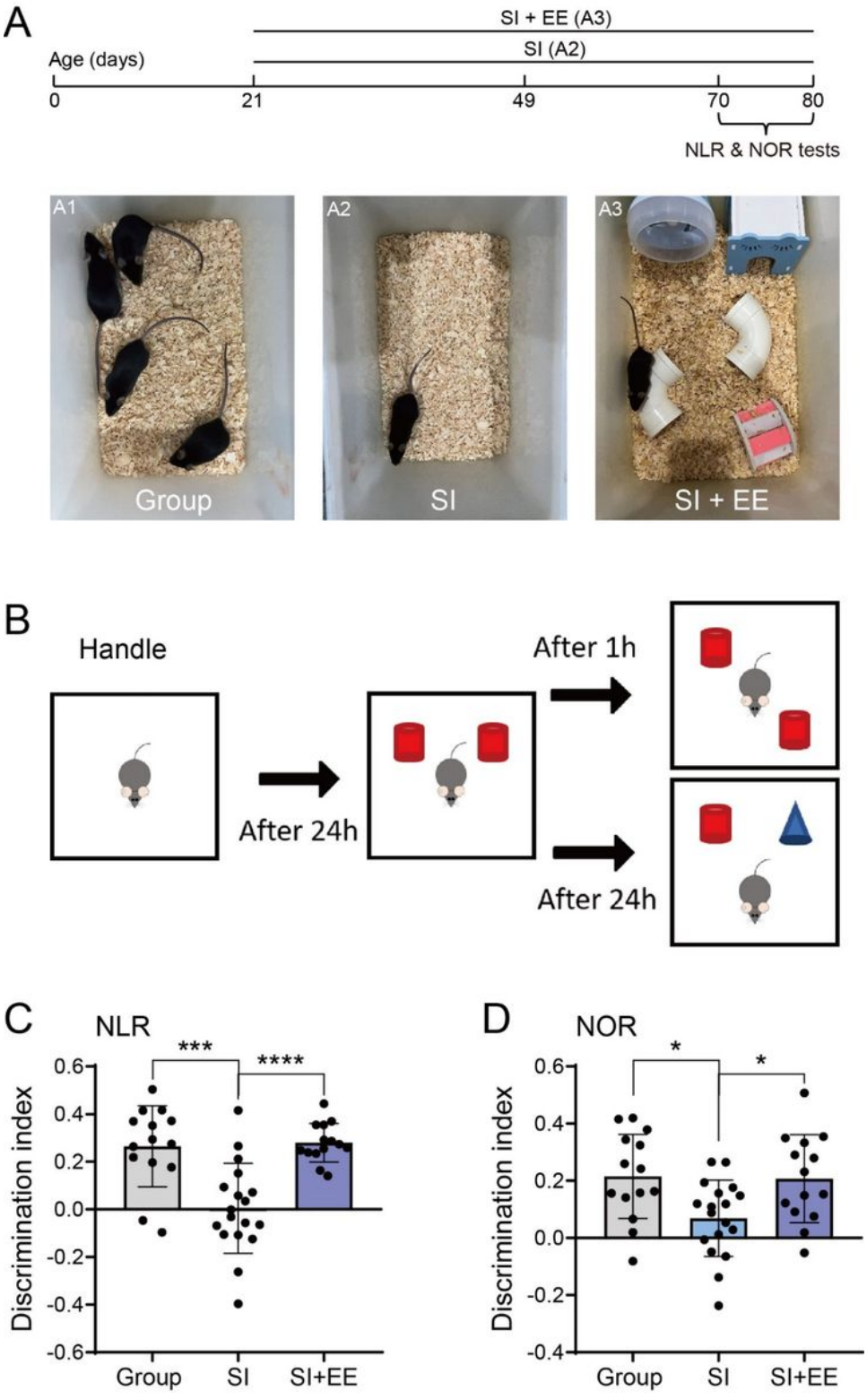

Figure 1

The impaired recognition memory in SI-reared mice was improved by EE.

(A) Timeline for the behavioral tests and setup of the group, social isolation (SI) and enriched environment (EE). (B) Diagram of the experimental process of NLR and NOR tests. (B) EE improved the impaired spatial recognition memory in SI-reared mice (****p< 0.0001, Tukey's *post hoc* test following

one-way ANOVA). (C) EE improved the impaired object recognition memory in SI-reared mice (*p = 0.0263, Tukey's *post hoc* test following one-way ANOVA) (Group: n = 14; SI: n = 18; SI+EE: n = 14).

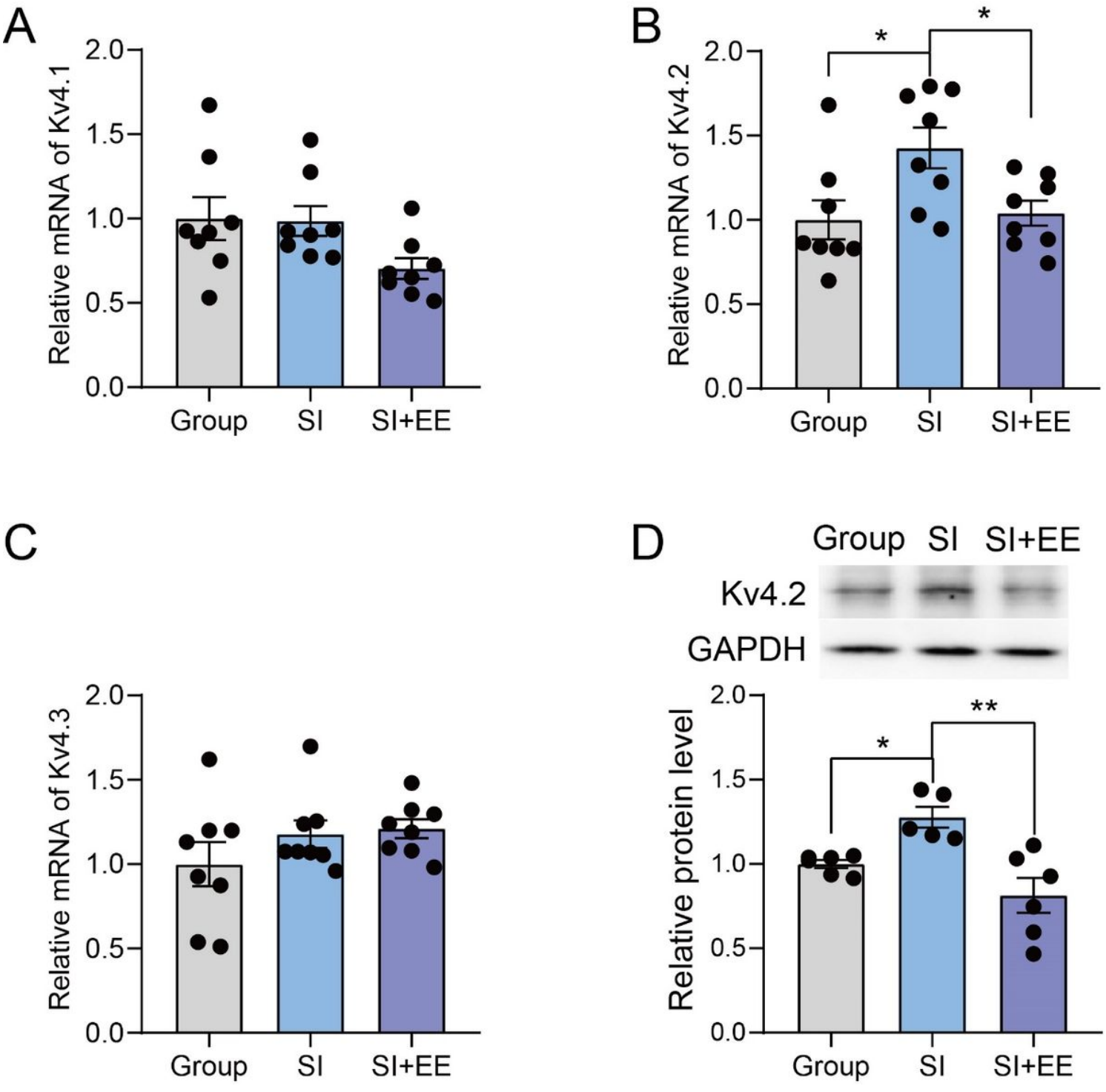

Figure 2

The abnormally increased expression of Kv4.2 in SI-reared mice was reversed by EE.

(A-C) The mRNA expression levels of the Kv4 channel (Kv4.1, Kv4.2, Kv4.3) in the mPFC. EE regulated abnormally increased expression of Kv4.2 in SI-reared mice (*p = 0.0442, Tukey's *post hoc* test following

one-way ANOVA) (n = 8 for each group). (D) Protein bands of Kv4.2 in the mPFC, GAPDH severed as the loading control. EE brought the expression of Kv4.2 in the SI-reared mice back to the same level as in the group-reared mice (**p = 0.0015, Tukey's *post hoc* test following one-way ANOVA) (Group: n = 6; SI: n = 5; SI+EE: n = 6).

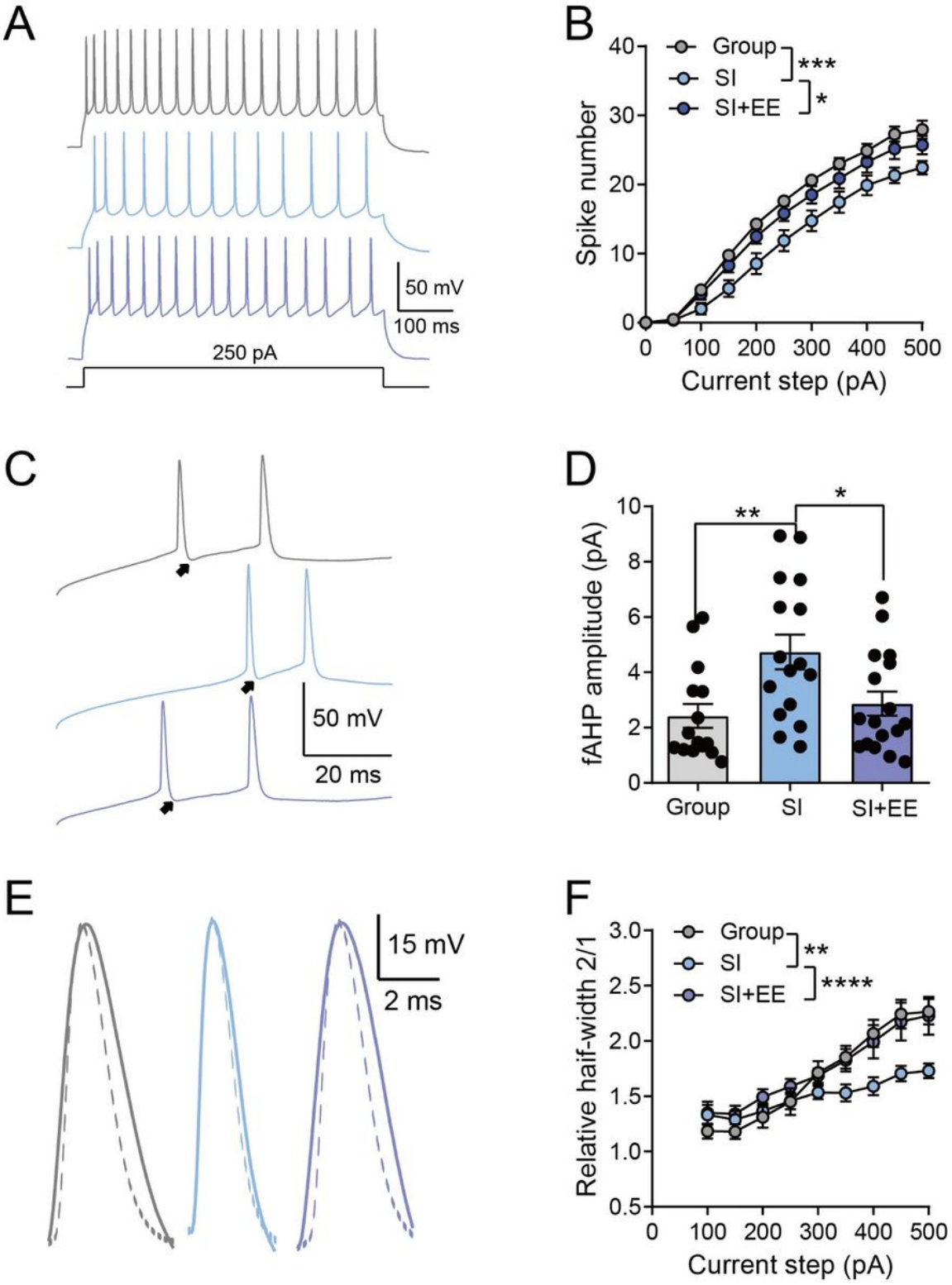

Figure 3

The decreased intrinsic excitability of pyramidal neurons in the mPFC of SI-reared mice was attenuated by EE.

(A) Typical traces showed action potentials (AP) elicited by a depolarizing 250 pA current step of pyramidal neuron in the mPFC. (B) Summarized current-firing curves showed that spike number was significantly decrease in SI-reared mice (n = 16 neurons from 6 mice) compared with group-housed mice (n = 17 neurons from 6 mice) and SI+EE mice (n = 15 neurons from 5 mice). (C) Representative traces of AP from pyramidal neuron responding to a depolarizing current and black dotted arrow showed the portion of the trace analyzed to determine the magnitude of fAHP. (D) The effect of SI on fAHP was reversed by EE. *p = 0.03, **p = 0.0076, Tukey's *post hoc* test following one-way ANOVA. (E) Representative trace of AP waveforms in pyramidal neuron and the dotted line showed the first AP (AP_1) and solid line showed the second AP (AP_2). (F) The effect of SI on relative half-width (AP_2 / AP_1) was reversed by EE. **p = 0.0033, ****p < 0.0001, Tukey's *post hoc* test following two-way ANOVA.

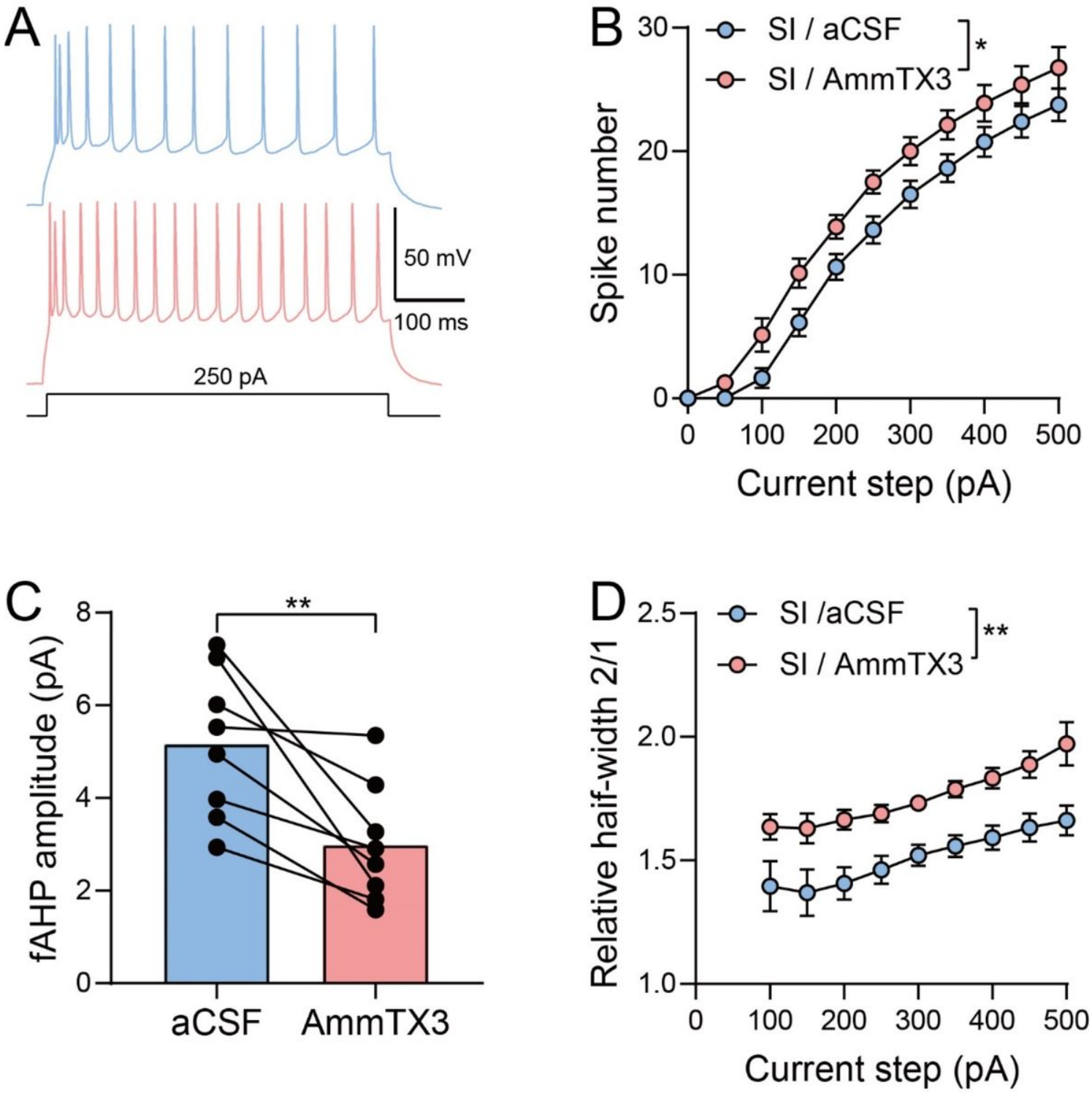

Figure 4

The decreased intrinsic excitability of pyramidal neurons in the mPFC of SI-reared mice was attenuated by blocking Kv4.2 channels using AmmTX3.

(A) Typical traces showed action potentials (AP) elicited by a depolarizing 250 pA current step of pyramidal neuron before and after AmmTX3 incubation. (B) Summarized current-firing curves showed that spike number was significantly increased in SI mice after AmmTX3 incubation (n = 8 neurons from 4

mice). (C) The effect of SI on fAHP was reversed by AmmTX3 (n = 8 neurons from 4 mice). $^{**}p = 0.006$, paired t-test. (D) The effect of SI on relative half-width (AP_2 / AP_1) was reversed by AmmTX3. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, Tukey's *post hoc* test following two-way ANOVA.