

# SK channels modulate the excitability and firing precision of projection neurons in the robust nucleus of the arcopallium in adult male zebra finches

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**Abstract: Objective** Motor control is encoded by neuronal activity. Small conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channels (SK channels) maintain the regularity and precision of firing by contributing to the afterhyperpolarization (AHP) of the action potential in mammals. However, it is not clear how SK channels regulate the output of the vocal motor system in songbirds. The premotor robust nucleus of the arcopallium (RA) in the zebra finch is responsible for the output of song information. The temporal pattern of spike bursts in RA projection neurons is associated with the timing of the acoustic features of birdsong. **Methods** The firing properties of RA projection neurons were analyzed using patch clamp whole-cell and cell-attached recording techniques. **Results** SK channel blockade by apamin decreased the AHP amplitude and increased the evoked firing rate in RA projection neurons. It also caused reductions in the regularity and precision of firing. RA projection neurons displayed regular spontaneous action potentials, while apamin caused irregular spontaneous firing but had no effect on the firing rate. In the absence of synaptic inputs, RA projection neurons still had spontaneous firing, and apamin had an evident effect on the firing rate, but caused no significant change in the firing regularity, compared with apamin application in the presence of synaptic inputs. **Conclusion** SK channels contribute to the maintenance of firing regularity in RA projection neurons which requires synaptic activity, and consequently ensures the precision of song encoding.

**Keywords:** SK channels; excitability; firing precision; robust nucleus of the arcopallium; zebra finches

## 1 Introduction

Small-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channels (SK channels), widely expressed throughout the nervous system in mammals, are insensitive to changes of membrane potential<sup>[1–4]</sup>. SK channels have three subtypes, SK1, SK2 and SK3, all of which are expressed in the mammalian

brain<sup>[1]</sup>. These channels are activated by rises in intracellular  $\text{Ca}^{2+}$  concentration and are specifically blocked by the bee toxin apamin. SK channels contribute to the afterhyperpolarization (AHP) following the action potential<sup>[5]</sup>. In the central nervous system, SK channels are important in controlling the firing frequency of neurons, regulating dendritic excitability, synaptic transmission and synaptic plasticity<sup>[6–9]</sup>. In rat globus pallidus neurons, blockade of SK channels by apamin reduces the AHP amplitude, leading to an increased firing rate. Apamin also causes variation of the firing threshold, which results in impaired firing

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precision. Timing and frequency are important parameters in temporal encoding, thus, blockade of SK channels may influence neuronal encoding activity<sup>[10]</sup>. In hippocampal CA1 neurons, SK2 overexpression decreases long-term potentiation and weakens hippocampus- and amygdala-dependent learning, compared to wild-type littermates<sup>[11]</sup>. Therefore, SK channels also play a critical role in learning and memory.

Songbirds learn their songs by imitation and auditory feedback<sup>[12]</sup>, which makes them an excellent model for studying the neural modulation of a complex learned behavior<sup>[13]</sup>. The song system is composed of a discrete set of interconnected nuclei, which underlie song learning and production<sup>[14,15]</sup>. These nuclei and their connections can be divided into two pathways: the vocal motor pathway (VMP) that contributes to song production, and the anterior fore-brain pathway (AFP) that is necessary for song learning and plasticity<sup>[16,17]</sup>. The robust nucleus of the arcopallium (RA) in the VMP receives input from the AFP as well, via the lateral magnocellular nucleus of the anterior nidopallium (LMAN) in the AFP<sup>[16,18,19]</sup>. Therefore, the RA, as a premotor nucleus, occupies an important position in the song system, integrating information from both pathways.

RA projection neurons are responsible for transmitting song information to midbrain and brainstem vocal and respiratory structures<sup>[20]</sup>. The temporal pattern of spike bursts in RA projection neurons is associated with the timing of the acoustic features of birdsong. The precise timing of individual spikes is closely associated with stereotypic behavior, which suggests that the song is represented in the RA by a temporal code<sup>[21,22]</sup>. In the zebra finch, apamin-sensitive neurons have been identified in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA)<sup>[23]</sup>, suggesting that SK channels participate in the activities of neurons in the song system. Although it is known that the RA controls the output of song information encoding, the involvement of SK channels is unclear. The present study tested whether and how SK channels modulate the excitability and firing precision of RA projection neurons in adult male zebra finches.

## 2 Materials and methods

**2.1 Drugs** Apamin, *DL*-2-amino-5-phosphonovaleric acid (APV), 6,7-dinitroquinoxaline-2,3-dione (DNQX) and picrotoxin were from Sigma-Aldrich, St. Louis, MO. Apamin is a noncompetitive selective antagonist of SK channels, and resisted washout from our slice preparation. Unless indicated otherwise, all solutions were continuously bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

**2.2 Slice preparation** Adult male zebra finches (*Taeniopygia guttata*) were obtained from a commercial supplier and housed in group cages. All animal experimental procedures were performed in accordance with the national guidelines and approved by South China Normal University Animal Care Committee. Brain slices were prepared as previously described<sup>[24]</sup>. Briefly, birds were anesthetized with 10% chloral hydrate and then rapidly decapitated. The brain was dissected into ice-cold, oxygenated (95% O<sub>2</sub> and 5% CO<sub>2</sub>) slice solution, consisting of (in mmol/L): 62.5 NaCl, 5 KCl, 28 NaHCO<sub>3</sub>, 10 glucose, 1.3 MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.26 NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, and 248 sucrose<sup>[25,26]</sup>. Standard artificial cerebrospinal fluid (ACSF) consisted of (in mmol/L): 125 NaCl, 2.5 KCl, 25 NaHCO<sub>3</sub>, 1.27 NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, 1.2 MgSO<sub>4</sub>·7H<sub>2</sub>O, 2 CaCl<sub>2</sub>, and 25 glucose, and the osmolarity was adjusted with sucrose to 350 mOsm<sup>[25]</sup>. Coronal brain slices (250–300 μm thick) containing the RA were cut with a vibrating microtome (NVSML1, WPI, Sarasota, FL) and collected in standard ACSF (37°C). Slices were allowed to recover in the holding chamber for at least 1 h and cooled to room temperature before recordings.

**2.3 Electrophysiological recordings** Electrophysiological recordings were performed at room temperature (24–28°C) in oxygenated ACSF. The RA and the surrounding tissues were distinguished under a BX51WI microscope with a DIC-IR video camera (Olympus, Japan). Recording pipettes were fabricated from borosilicate glass (Sutter Instruments, Novato, CA) using a Flaming-Brown puller (model P-97, Sutter Instruments) and were filled with pipette solution containing (in mmol/L): 120 KMeSO<sub>4</sub>, 5 NaCl, 10 HEPES, 2 EGTA, 2 ATP, and 0.3 GTP (pH 7.2–7.4, 340 mOsm). The recording pipettes, which had resistances of 4–7 MΩ, were positioned using an integrated

motorized control system (Sutter Instruments). Whole-cell and cell-attached recordings were made using standard techniques.

The two cell types in the RA, glutamatergic projection neurons and GABAergic interneurons, were distinguished according to their distinct electrophysiological properties. The former displayed regular spontaneous firing, and could be induced to generate a time-dependent inward rectification by hyperpolarizing current injection, while the latter displayed no spontaneous firing in the resting state. Moreover, a depolarizing current injection induced a higher firing rate and a less regular frequency in interneurons than in projection neurons<sup>[27,28]</sup>.

**2.4 Data analysis** All numerical data are presented as mean  $\pm$  SEM. Measurements of firing rate, interspike interval (ISI), intercurrent interval (ICI) and spike threshold were made with Clampfit 9.2 software (Axon Instruments, Union City, CA). The coefficient of variation (CV) was determined as the value of standard deviation/mean. The AHP amplitude was quantified as the difference between spike threshold and the lowest point of the AHP. Data were graphed using OriginPro 8 (OriginLab, Northampton, MA). All statistics were calculated using Excel and OriginPro 8. The two-tailed paired-sample *t*-test was used to evaluate differences between groups. Differences were considered statistically significant when  $P < 0.05$ .

### 3 Results

#### 3.1 SK channel blockade reduces the AHP amplitude and increases the firing rate of RA projection neurons

To test whether SK channels contribute to the AHP and regulate excitatory activity in RA projection neurons in adult male zebra finches, we made plots of injected currents from 0 to 200 pA for 500 ms at 50 pA steps and 10 s intervals, against mean firing rate (Fig. 1A). Apamin (100 nmol/L), a specific antagonist of SK channels, caused an increase in the evoked firing rate ( $P < 0.05$ ,  $n = 10$ ) (Fig. 1B), as a result of which apamin reduced the amplitude of the AHP. The ISI was shortened because the AHP depth was decreased (Fig. 1C). The average AHP amplitude was reduced after 100 nmol/L apamin application, in particular

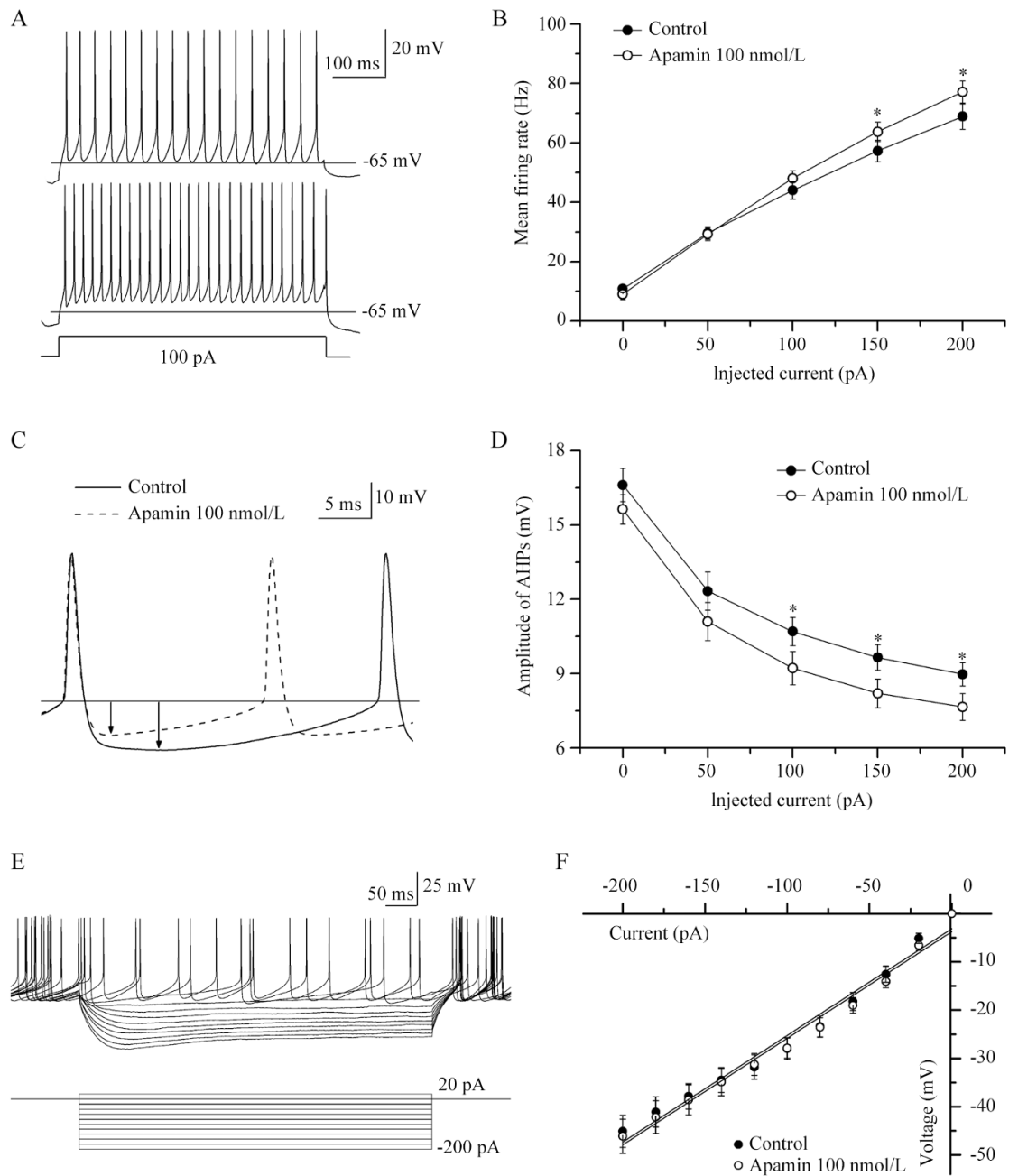
at 100, 150 and 200 pA ( $P < 0.05$ ,  $n = 8$ ) (Fig. 1D). These results implied that SK channels contribute to the excitability of RA projection neurons and might regulate the output of birdsong.

On the other hand, to test whether SK channels also contribute to activity below the membrane potential in RA projection neurons, we compared the current-voltage (*I-V*) relationships in control and 100 nmol/L apamin treatment groups (Fig. 1E). Apamin had no influence on the slope of the *I-V* curve by linear fit (Fig. 1F), suggesting that SK channels were activated only by  $\text{Ca}^{2+}$  influx when the membrane potential was depolarized in RA projection neurons.

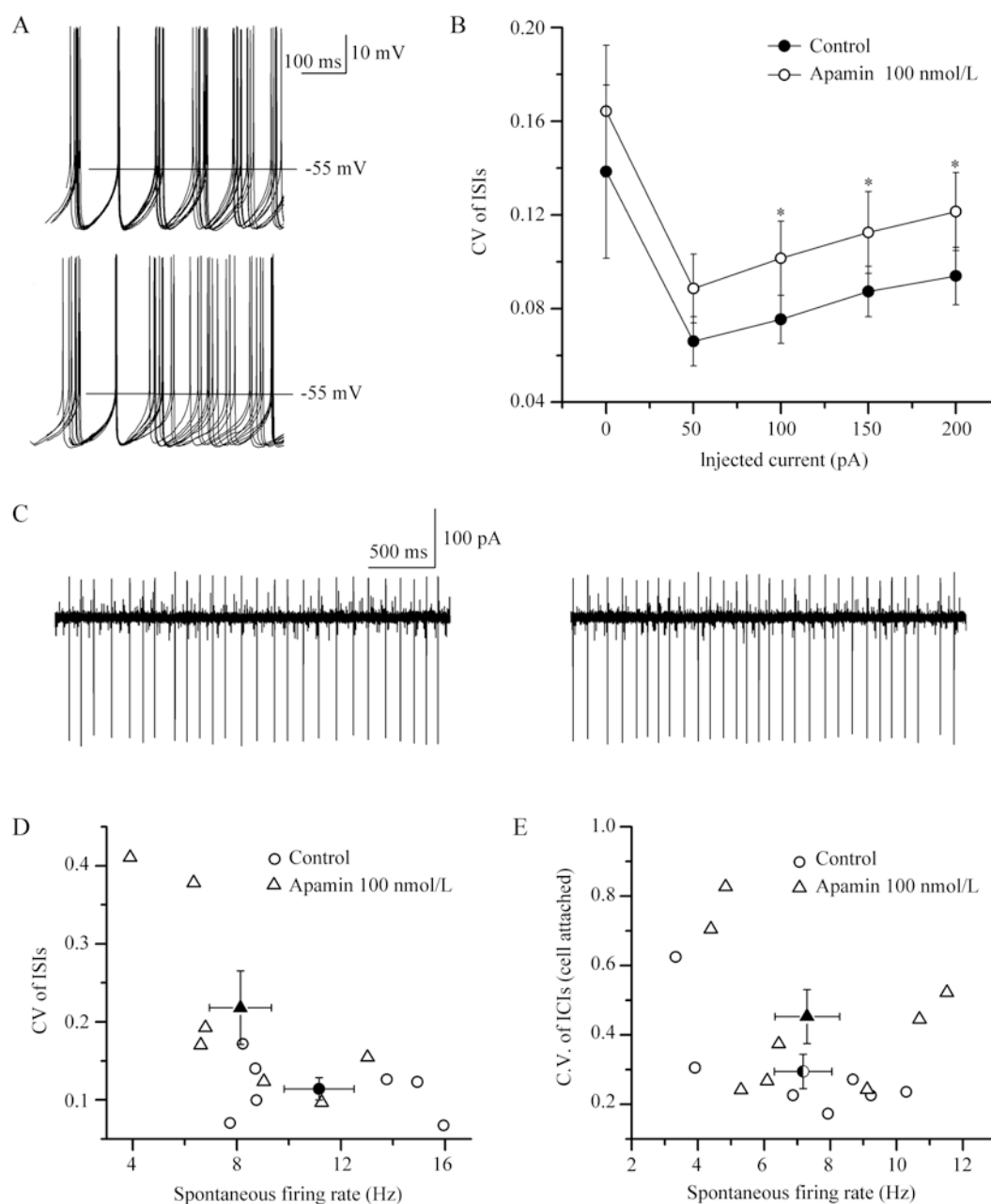
#### 3.2 SK channel blockade disrupts firing regularity of RA projection neurons

The AHP, an essential part of the action potential, allows the recovery of ion channels from inactivation in each cycle. The spontaneous firing became more irregular after 100 nmol/L apamin (Fig. 2A). Apamin significantly increased the CV of ISIs induced by  $\geq 100$  pA current injection ( $P < 0.05$ ,  $n = 10$ ) (Fig. 2B). The CV of ISIs of spontaneous firing (no current injection) was higher than that of evoked firing in control solutions. Apamin did not cause a significant increase in the CV of ISIs of spontaneous firing ( $0.1385 \pm 0.037$  in control vs  $0.1643 \pm 0.0282$  in apamin,  $n = 10$ ) (Fig. 2B).

The production of spontaneous action potentials is more complex than that by current injection. To test the effects of apamin on the spontaneous firing of each neuron, we made plots of mean firing rate against the CV of ISIs. Spontaneous firing was recorded for at least 80 s. The results showed that apamin (100 nmol/L) had no significant effect on the mean spontaneous firing rate or the CV of ISIs ( $n = 7$ ) (Fig. 2D). Besides, we found that spontaneous firing rates gradually declined with time in controls, which might be because during whole-cell recording, the electrode tip inevitably broke part of the cell membrane, weakening the cell and reducing the spontaneous firing rate. To confirm the effect of apamin on the spontaneous activity of RA projection neurons, we recorded spontaneous currents in the cell-attached voltage-clamp configuration (Fig. 2C). On average, apamin had no effect on the mean spontaneous current firing rate ( $7.18 \pm 0.87$  Hz in control vs  $7.30 \pm$



**Fig. 1.** Blockade of SK channels reduces the amplitude of afterhyperpolarizations (AHPs) and increases the firing rate in RA projection neurons. **A:** Sample traces of firing in response to 100 pA current injection (500 ms) in control ACSF (upper) and with apamin (lower). The firing rate was increased by 100 nmol/L apamin, from 34.00 Hz in control to 53.36 Hz in apamin. **B:** Plot of firing frequency *versus* injected current for a sample of 10 neurons. Apamin application caused an increase in firing rate, but a small decrease without current injection (i.e., spontaneous firing rate) ( $*P < 0.05$ ,  $n = 10$ ). **C:** The AHP amplitude was reduced by 100 nmol/L apamin. Action potentials evoked by 100 pA current injection are overlaid to show the change of AHP by apamin application. The arrows show the depth of AHP. The spikes in control are shown as a solid line, and those in apamin as a dashed line. **D:** Plot of AHP amplitude *versus* injected current. Apamin caused a significant decrease in AHP ( $*P < 0.05$ ,  $n = 8$ ). **E:** Sample traces in response to hyperpolarizing current injection, from -200 pA to 10 pA for 600 ms at 20 pA steps and 2 s intervals. **F:** *I-V* relationships in control and 100 nmol/L apamin groups. The slope of the *I-V* curve was not changed after apamin application,  $0.221 \pm 0.010$  9 in control and  $0.220 \pm 0.009$  8 in apamin ( $n = 10$ ). The average in control is shown as filled circles, and that in apamin as open circles.

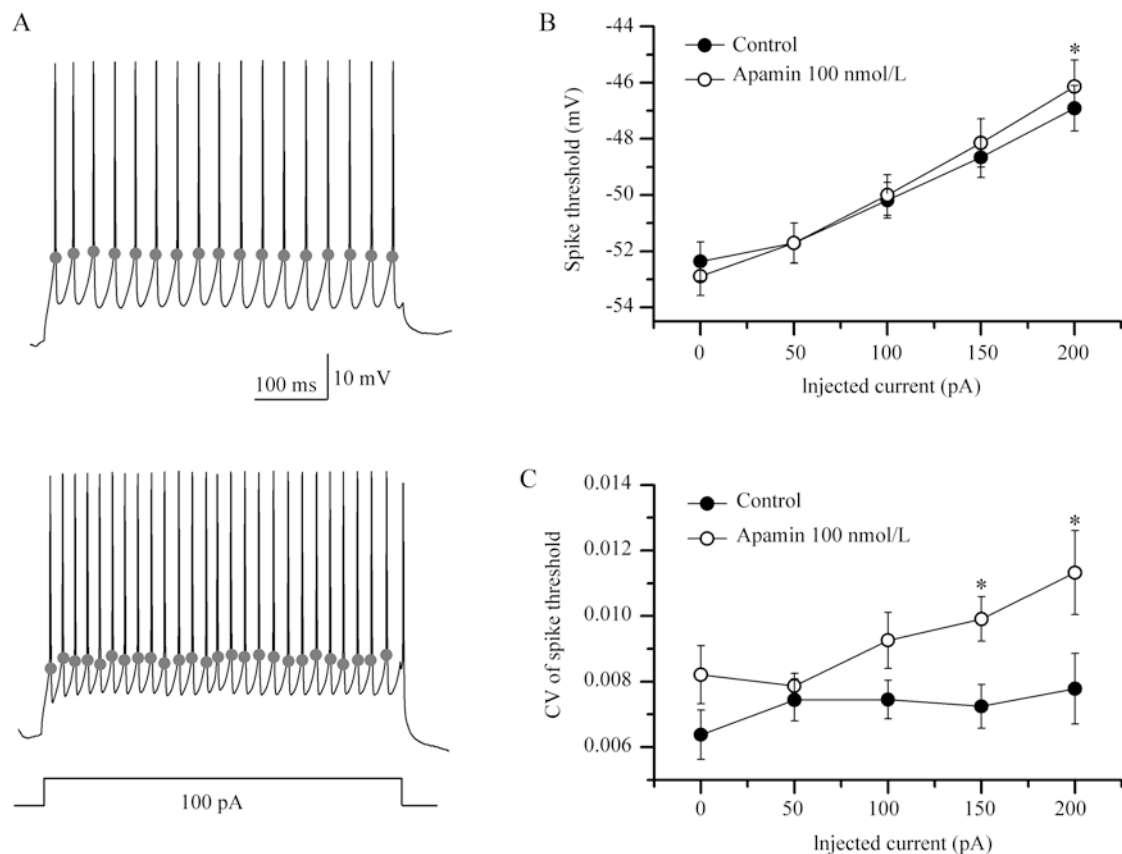


**Fig. 2.** SK channels maintain the regularity of firing in RA projection neurons. **A:** Ten traces of spontaneous action potentials recorded in the whole-cell configuration are aligned and superimposed to show the regularity of firing. In control solution (upper), the action potentials repeated in a regular manner. In 100 nmol/L apamin (lower), the spontaneous firing became irregular. **B:** SK channel blockade disrupted the firing regularity of evoked action potentials. The average coefficient of variation (CV) of interspike intervals (ISIs) induced by  $\geq 100$  pA current injection was increased by 100 nmol/L apamin ( $*P < 0.05$ ,  $n = 10$ ). **C:** Examples of spontaneous currents recorded in the cell-attached voltage-clamp configuration, in control (left) and in 100 nmol/L apamin (right). **D:** Measurements of spontaneous firing recorded in the whole-cell configuration, showing no statistically significant difference in mean spontaneous firing rate and CV of ISIs ( $n = 7$ ). The spontaneous firing of each RA cell was recorded for 80–120 s. Individual cells are shown as open circles in control and triangles in apamin, and the average of each group is shown as a filled circle in control and triangle in apamin. **E:** Measurements of spontaneous currents recorded in the cell-attached configuration, showing no difference in mean spontaneous firing rate ( $n = 8$ ), but a marked increase in the CV of ICIs ( $P < 0.05$ ,  $n = 8$ ). Individual cells are shown as open circles in control and triangles in apamin, and the average in apamin is shown as a filled triangle. An open circle is covered by the filled circle, so the average in control is shown as a half-filled circle.

0.96 Hz in apamin,  $n = 8$ ). On the contrary, the CV of ICIs was distinctly increased from  $0.2946 \pm 0.0495$  in controls to  $0.4532 \pm 0.0777$  in the apamin group ( $P < 0.05$ ,  $n = 8$ ) (Fig. 2E). These results suggest that SK channels enhance the regularity of spontaneous activity in RA projection neurons.

SK channel blockade reduced AHP depth and increased the firing rate, but some voltage-gated  $\text{Na}^+$  channels may not completely recover from inactivation before the start of the next action potential. If so, the threshold of the next action potential would be variable and unstable. To test this hypothesis, we analyzed the change of spike threshold after 100 nmol/L apamin application.

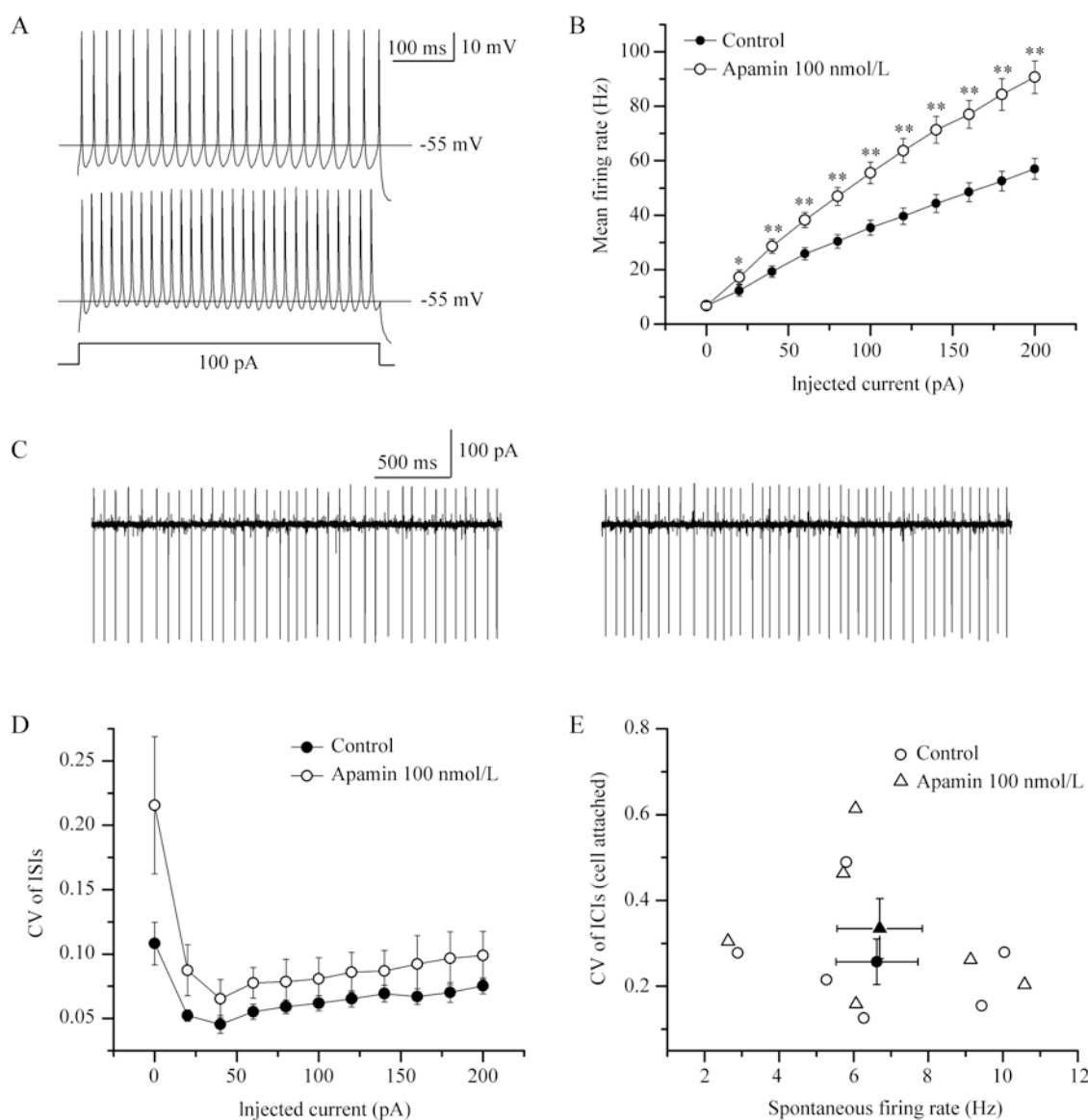
Apamin caused a frequency-dependent depolarization in spike threshold and increased the threshold variability in the whole-cell configuration (Fig. 3A). The average spike threshold by 200 pA current injection was increased by 100 nmol/L apamin ( $P < 0.05$ ,  $n = 8$ ) (Fig. 3B). But the spontaneous spike threshold had a small hyperpolarization, probably resulting from the decrease of spontaneous firing rate. Apamin also led to a frequency-dependent increase in the average CV of the evoked spike threshold ( $P < 0.05$ ,  $n = 8$ ) (Fig. 3C). The variability of spike threshold determines the regularity of spike firing. These results indicate that SK channels ensure the precision of spike threshold and firing regularity by modifying the AHP.



**Fig. 3.** SK channels enhance the precision of spike threshold to ensure regularity of firing. **A:** Examples of action potential traces induced by 100 pA current injection (500 ms) from an RA projection neuron recorded in the whole-cell configuration before (upper) and after (lower) 100 nmol/L apamin application. Thresholds are shown as grey dots. **B:** Effect of 100 nmol/L apamin on spike threshold. The threshold of evoked spikes had a frequency-dependent depolarization ( $*P < 0.05$ ,  $n = 8$ ), but the threshold of spontaneous firing (no current injection) had a small hyperpolarization ( $n = 8$ ), which was related to the decline of spontaneous firing rate as shown in Fig. 2D. **C:** Effect of 100 nmol/L apamin on the precision of spike threshold. Apamin caused a frequency-dependent increase in the coefficient of variation (CV) of evoked spike threshold ( $*P < 0.05$ ,  $n = 8$ ).

**3.3 Effect of apamin on neurons without spontaneous synaptic inputs** In songbirds, the RA receives dual synaptic inputs from the higher vocal center (HVC) and

the LMAN. Both are excitatory glutamatergic, but have distinct postsynaptic properties. The HVC-RA input is largely mediated by the  $\alpha$ -amino-3-hydroxy-5-methyl-4-



**Fig. 4.** Blockade of SK channels increases the firing rate and decreases the regularity of firing in the absence of synaptic inputs. **A:** Sample traces of firing in response to 100 pA current injection (500 ms) in control ACSF (upper) and with 100 nmol/L apamin (lower) in the presence of 50  $\mu$ mol/L APV, 20  $\mu$ mol/L DNQX and 150  $\mu$ mol/L picrotoxin. The firing rate was clearly increased by apamin, from 42.73 Hz in control to 58.61 Hz in apamin. **B:** In the presence of 50  $\mu$ mol/L APV, 20  $\mu$ mol/L DNQX and 150  $\mu$ mol/L picrotoxin, 100 nmol/L apamin induced an increase in the firing rate (\* $P$  < 0.05, \*\* $P$  < 0.01,  $n$  = 6). **C:** Examples of spontaneous currents recorded in the cell-attached voltage-clamp configuration in the presence of APV, DNQX and picrotoxin, in control (left) and in 100 nmol/L apamin (right) groups. **D:** Apamin had no effect on the coefficient of variation (CV) of interspike intervals (ISIs) in the presence of APV, DNQX and picrotoxin ( $n$  = 6). **E:** Measurements of spontaneous currents recorded in the cell-attached configuration in the presence of APV, DNQX and picrotoxin showed no difference in mean spontaneous firing rate or the CV of ICIs ( $n$  = 6). Individual cells are shown as open circles in control and triangles in apamin, and the average of each group is shown as a filled circle in control and a filled triangle in apamin.

isoxazolepropionic acid receptor (AMPA), whereas the LMAN-RA input is almost completely mediated by the N-methyl-D-aspartic acid receptor (NMDAR)<sup>[29–32]</sup>. The LMAN is the last output nucleus of the anterior forebrain pathway that is necessary for song learning and plasticity<sup>[15–17]</sup>. RA projection neurons also receive glutamatergic inputs from the axon collaterals of other RA projection neurons and GABAergic inputs from local interneurons<sup>[19]</sup>. These synaptic inputs may affect the neuronal responses to apamin.

To test whether the effect of SK channel blockade on RA projection neuronal firing was dependent on synaptic activity, we made plots of depolarizing current from 0 to 200 pA for 500 ms at 20 pA steps and 10 s intervals, against the mean firing rate or CV of ISIs in the presence of 50  $\mu\text{mol/L}$  APV (an NMDAR blocker), 20  $\mu\text{mol/L}$  DNQX (a non-NMDAR blocker) and 150  $\mu\text{mol/L}$  picrotoxin (a GABA receptor blocker) (Fig. 4). Apamin (100 nmol/L) caused a notable increase in the average firing rate when the neurons were injected with  $\geq 40$  pA currents ( $P < 0.01$ ,  $n = 6$ ) (Fig. 4B), but had no significant effect on the CV of ISIs ( $n = 6$ ) (Fig. 4D). Furthermore, to confirm the effect of apamin on spontaneous activity in the absence of synaptic inputs, we recorded spontaneous currents in the cell-attached voltage-clamp configuration in the presence of 50  $\mu\text{mol/L}$  APV, 20  $\mu\text{mol/L}$  DNQX and 150  $\mu\text{mol/L}$  picrotoxin (Fig. 4C). On average, apamin had no effect on the mean spontaneous current firing rate ( $6.62 \pm 1.1$  Hz in control,  $6.7 \pm 1.15$  Hz in apamin,  $n = 6$ ) or the CV of ICIs ( $0.257 \pm 0.0529$  in control,  $0.3345 \pm 0.0704$  in apamin,  $n = 6$ ) (Fig. 4E). These results demonstrated that the effect of postsynaptic SK channels on controlling the firing rate is independent of synaptic activity, but the effect on firing precision may be influenced by such inputs.

#### 4 Discussion

In this study, the contribution of SK channels to the modulation of the excitability and firing precision of RA projection neurons in zebra finches was investigated. The results showed that SK channels controlled the firing rate, even without synaptic inputs. SK channels also main-

tained the firing regularity and precision, which requires synaptic activity in the RA. These results are supported by the findings in rat globus pallidus (GP) neurons<sup>[10]</sup>. Both RA projection neurons and rat GP neurons have rhythmic spontaneous action potentials, even in the absence of synaptic inputs<sup>[10,33–38]</sup>. In the RA, SK channels contribute to the AHP, and provide time for  $\text{Na}^+$  channels to recover from inactivation<sup>[8,39]</sup>. They also ensure the firing regularity and precision in the presence of synaptic activity. RA projection neurons may encode birdsong information through changes in spike time and firing rate.

The present study showed that SK channel blockade caused a small reduction in the AHP amplitude of spontaneous action potentials, while the spontaneous firing rate was also reduced in the whole-cell configuration. This may be due to the lesion caused by rupturing part of the cell membrane. RA projection neurons fired action potentials autonomously without synaptic transmission. This may be induced by the activity of subthreshold-activated  $\text{Na}^+$  and  $\text{K}^+$  channels, as suggested in rat GP neurons. These ion channels bring the membrane potential to threshold on each cycle of oscillation<sup>[10,40]</sup>. After breaking the cell membrane in the whole-cell configuration, changes in intracellular constituents may disrupt the mechanism of membrane potential oscillation. Cell-attached recording provides a way to record spontaneous firing activity without breaking the membrane<sup>[41]</sup>. Therefore, the results from cell-attached recording suggest that SK channels maintain the regularity of spontaneous firing, which is dependent on synaptic activity.

In the absence of synaptic inputs, SK channels still contributed to the AHP, suggesting that the postsynaptic SK channels modulate the excitability of RA projection neurons. Recent studies in rats indicate that SK channels are located postsynaptically<sup>[7,42–44]</sup>. Blockade of SK channels has no influence on neurotransmitter release in hippocampal CA1 pyramidal neurons<sup>[6]</sup>. In songbirds, the activities of individual RA projection neurons probably encode a whole song precisely<sup>[45]</sup>. Precision in behavior is reflected in precision in neuronal control elements<sup>[22]</sup>. Thus, postsynaptic SK channels in the RA ensure that the



bird can produce a stereotyped song. In the presence of APV, DNQX and picrotoxin, however, the change of firing regularity was not statistically significant, suggesting that not only postsynaptic SK channels, but also synaptic activity may contribute to the firing regularity of RA projection neurons.

During the action potential, SK channels are activated by  $\text{Ca}^{2+}$  influx through voltage-gated  $\text{Ca}^{2+}$  channels<sup>[5,8,44]</sup>. Furthermore, it has been reported that  $\text{Ca}^{2+}$  influx through postsynaptic NMDARs also activates SK channels, especially in dendritic spines. SK channels re-polarize the membrane potential and drive  $\text{Mg}^{2+}$  to block the NMDAR channels. The  $\text{Mg}^{2+}$  lock limits NMDAR-dependent  $\text{Ca}^{2+}$  influx. Thus, SK channels and NMDARs form a  $\text{Ca}^{2+}$ -mediated negative feedback loop in dendritic spines<sup>[6,9,42,46-48]</sup>. In excitatory synaptic transmission, SK channels, activated by  $\text{Ca}^{2+}$  influx through NMDARs, contribute to the excitatory postsynaptic potential (EPSP).  $\text{Ca}^{2+}$  influx through NMDARs is essential for multiple forms of synaptic plasticity, including long-term potentiation and long-term depression<sup>[49-52]</sup>. The SK channel blocker apamin causes an increase in the EPSP, which requires NMDAR activity<sup>[7,42-44]</sup>. In the present study, SK channels modulated the excitability and maintained the regularity of firing in RA projection neurons of zebra finches. The NMDAR – SK channel negative feedback loop may have an effect on the complex process of song learning and plasticity in songbirds. Therefore, in addition to contributing to the precision of song encoding, it is possible that SK channels coordinate with NMDARs to modulate synaptic transmission and plasticity in the song system of songbirds.

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## Retraction Statement

Ya-Dan WEN, Hui-Ling ZHANG, Zheng-Hong QIN. Inflammatory mechanism in ischemic neuronal injury. *Neurosci Bull* 2006, 22(3): 171–182.

This review contains large amounts of material copied from three papers published in the *Journal of Neuropathology and Experimental Neurology*, *Progress in Neurobiology*, and *Neuroscience and Biobehavioral Reviews*. We hereby retract the paper from the journal, PubMed, Medline and other databases involved in China and abroad. We deeply apologize for the inconvenience caused to our readers.

Neuroscience Bulletin  
May 15, 2012

March 22, 2012

Dear Editor of Neuroscience Bulletin:

It has come to my attention that the review published in *Neuroscience Bulletin* 2006 May;22(3):171–182, entitled "Inflammatory mechanism in ischemic neuronal injury", contains large amounts of material copied from three references. This has violated scientific ethics and thus I ask you to withdraw this article and apologize to readers.

Sincerely,

Zheng-Hong Qin  
Professor of Pharmacology,  
Department of Pharmacology,  
Soochow University School of Pharmaceutical Science