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Research Paper

Two oscillatory patterns induced by depolarization in tectal neurons of *Xenopus*

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Abstract: In the present study, we used *in vitro* whole-cell patch-clamp technique to record and analyze oscillatory activity of neurons in the optic tectum of *Xenopus*. Two patterns of subthreshold oscillations were induced by long-term depolarizing current pulses. One of the oscillating patterns occurred without a slow inward current (SIC); the other was superimposed on the SIC. The subthreshold oscillations were induced by depolarization in 48% of the recorded neurons. Both the oscillations and the SIC were tetrodotoxin (TTX)-resistant, but neither occurred when the slices were immersed in Ca^{2+} free solutions. The evocation of the oscillations was voltage-sensitive: only when the initial membrane potentials of the neurons were held at -40 mV or -50 mV, 10 mV depolarization could induce the subthreshold oscillations. The amplitude and duration of the SIC depended on the level of the initial membrane potential. The subthreshold oscillations might play an important role in the physiological and behavioral functions of frogs, e.g. pattern discrimination, prey recognition, avoiding behavior *etc*., furthermore, these oscillations might play roles in the integration of neural activity in both mammals and non-mammalian vertebrates.

Key words: oscillations; slow inward current; depolarization; patch-clamp; tectal neurons; *Xenopus*

视顶盖神经元的两种振荡模式

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摘 要：本文旨在应用离体全细胞膜片钳技术，记录和分析爪蟾视顶盖神经元阈下振荡活动。长时程的去极化电流可以诱发两种模式的阈下振荡，一种振荡是叠加在慢内向电流(slow inward current, SIC)之上的，另一种振荡则不伴有 SIC。在本实验中，去极化能诱发阈下振荡的细胞占所记录神经元的 48%。振荡活动和 SIC 都是河豚毒素耐受的，而在无钙溶液中浸泡的脑片未记录到振荡和 SIC。振荡活动的诱发依赖膜电位初始水平和变化幅度，只有当神经元的钳制电压从 -40 mV 、-50 mV 或更低的初始水平去极化 10 mV 时，才能诱发阈下振荡。SIC 的幅度和时程依赖于膜电位的初始水平。本文报导的阈下振荡可能在蛙的生理和行为功能(如模式辨认，猎物识别和逃避行为等)方面起重要作用，进而也有可能在哺乳类和非哺乳类脊椎动物的神经活动整合中发挥作用。

关键词：阈下振荡；慢内向电流；去极化；膜片钳；视顶盖神经元；非洲爪蟾
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Neurons in the central nervous system show two distinct modes of activity. One mode is determined by the integration of excitatory and inhibitory inputs and subserves communication within neuronal networks. The other is characterized by intrinsically triggered membrane potential (current) oscillations. The oscillations have been recorded in several types of neurons in mammals using intracellular and/or whole-cell patch-clamp recording techniques[1-3], but similar results have not been reported in *Xenopus*.

Oscillating has been proposed to play a role in coordinat-

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ing the activity of different neurons and/or neural circuits. However, neither the cellular mechanism nor the biological functions of these oscillations are well understood[1,3]. Similar subthreshold oscillations might be expected to play an analogous role in non-mammalian vertebrates, which might provide relatively simple and useful model systems for studying these activities. However, though depolarizing step commands from a -70 mV holding potential have been found to generate a fast inward current followed by a fast outward current in the neuron of frog optic tectum, no oscillatory activity has been recorded[4]. In the present patch-clamp experiments, we found that when the membrane potentials of neurons in *Xenopus* tectal slices were depolarized by stepwise injections of long-term (60 s) direct current (DC) pulses, tetrodotoxin (TTX)-resistant subthreshold oscillations and/or slow inward current (SIC) could be elicited. To clarify the mechanisms responsible for these currents, we investigated the effects of changing two divalent cations (Ca^{2+} and Sr^{2+}) in the external solution on the oscillations and SIC.

1 MATERIALS AND METHODS

1.1 *Slice preparation*

In vitro experiments were carried out on layer VI in slice preparations from the adult *Xenopus* optic tectum using a modification of the blind patch-clamp method described by Blanton *et al*.[5] or using infrared differential interference contrast (IR-DIC) image and water immersion objective system under visible condition[6]. Briefly, under ethyl m-aminobenzoate (MS-222) anesthesia, *Xenopus* was placed in a chilled oxygenated solution of sucrose saline (4 ºC). The brain was removed and embedded in low-melting point agars. Para-sagittal sections (300 μm thick) of the optic tectum were cut with a vibratom. The slices were kept in an interface chamber with oxygenated ice-cold sucrose saline (4 ºC) for at least 40 min and then transferred to a chamber superfused with standard saline (in mmol/L: NaCl, 111; KCl, 2.5; $MgCl_2$, 1.5; $NaHCO_3$, 15; dextrose, 4.0; $CaCl_2$, 2.5) bubbled with 95 % O_2 and 5% CO_2 for more than 40 min at room temperature (20-23 ºC). The solution perfused continuously by gravity at a speed about 1.0-2.0 mL/min. Unless otherwise noted, TTX (0.5 μmol/L) was added into the perfusion saline to block Na^+-mediated action potentials.

1.2 *Patch-clamp recording*

Patch-clamp electrodes (diameter: ~2 μm) were pulled from borosilicate glass capillaries by a vertical microelectrode-puller (PP-830, Narishige). Tectal neurons were recorded (Fig. 1) under whole-cell voltage- or current-clamp mode with the electrodes (2-4 MΩ) filled with internal solution containing (in mmol/L): Cs-gluconate, 100; NaCl, 5; EGTA, 10; $MgCl_2$, 5; HEPES, 2; ATP, 2 and GTP, 0.3, pH 7.4. The membrane potential usually was held at -50 mV. Depolarizing currents were delivered through the patch-clamp pipettes. Membrane potentials were depolarized in 60 s by +10 mV or +50 mV incremental steps from various initial membrane potentials. Test solutions were applied by bath perfusion. Whole-cell currents were filtered at 1 kHz and recorded using a PC-2B or PC-2C amplifier (Biochemical and Biophysical Institute, Wuhan, China). Signals were digitally sampled at 3-5 kHz and analyzed using pClamp10 or MINI Analysis software package 6.0.

1.3 *Statistic analysis*

Significant differences between two groups were determined using Student's *t*-test and $P<0.05$ was considered to be significant.

2 RESULTS

After the tectal neurons were voltage clamped, some neurons generated large amplitude spontaneous Na^+-mediated transient inward currents, which interfered with the measurements of the relatively low amplitude oscillatory activity. To block the spontaneous Na^+- mediated inward currents or action potentials, the experiments were performed under the presence of TTX (0.5 μmol/L) in the external solution. However, TTX-resistant spontaneous miniature postsynaptic currents (mPSCs) could still be recorded (Fig. 2*A*, left bottom trace), which has been reported previously[7], so we would not describe them further here.

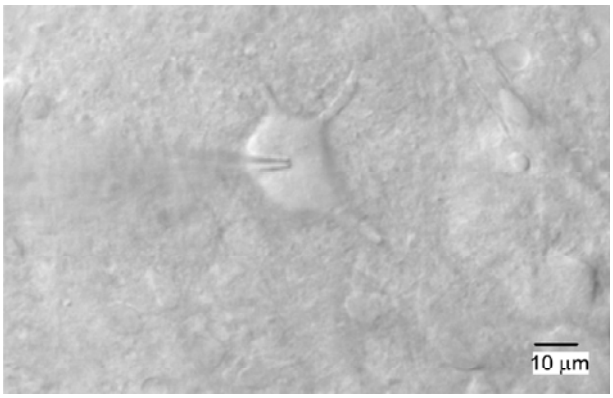

Fig. 1. Infrared differential interference contrast (IR-DIC) image of a live tectal neuron in a slice of *Xenopus* tectum for electrophysiological recording. Scale bar, 10 μm

Spontaneous oscillations were never observed in neurons voltage clamped near their resting membrane potentials (~-50 mV); but approximately half of the sampled neurons responded promptly with oscillatory activities during injection of a family of voltage step commands (Fig. 2*A*). Of the 25 sampled tectal neurons, 12 generated subthreshold current oscillations with or without SIC following depolarizing current injections. Under current-clamp condition, depolarization also induced subthreshold oscillations of membrane potential (Fig. 2*B*). So the damped oscillations of currents or of potentials could be elicited respectively with both voltage- or current-clamp methods (Fig. 2).

2.1 Differences of resting potentials between oscillating and non-oscillating neurons

None of the 25 neurons exhibited oscillations in response to -10 mV hyperpolarizations, regardless of the initial holding potential (Fig. 2). We compared the resting membrane potentials of oscillating and non-oscillating neurons. The mean resting membrane potentials were (-50.5±8.8) mV and (-60.8±4.8) mV in oscillating and non-oscillating neurons, respectively ($P < 0.01$, $n = 11$) (Fig. 3).

2.2 Two oscillation patterns

Two patterns of subthreshold oscillations were observed. In the first pattern (n=3), damped oscillations were induced by the depolarization without evoking any SIC (Fig. 4*A*, top trace) and the autocorrelogram (Fig. 4*A*, bottom trace) showed the first peak at 59.5 ms, i.e. the dominant frequency was ~16.8 Hz. In this case, the rhythm index could be calculated and was ~0.54, indicating that the oscillatory activity was considerably high[8]. The second pattern (n=9) was a transient current followed by single or multiple intermittent SICs. A set of sinusoidal oscillatory currents was superimposed on either the decay phase of

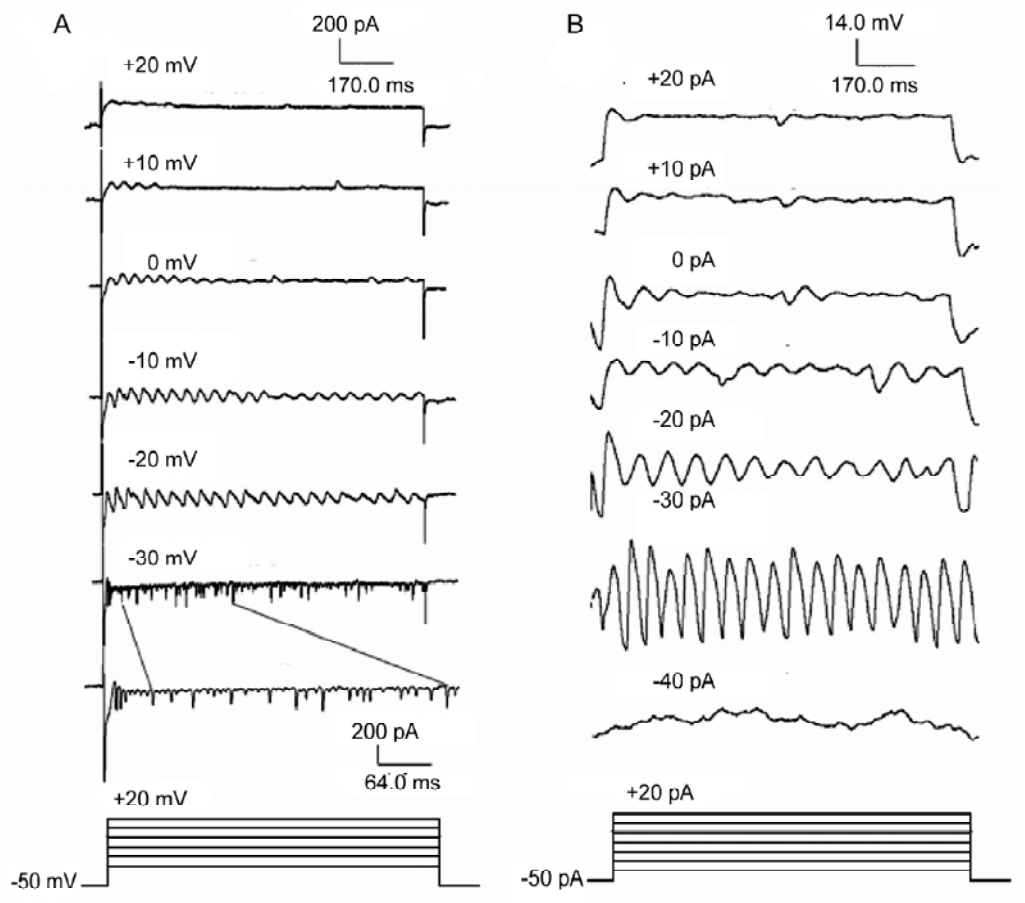

Fig. 2. Voltage dependence of subthreshold oscillations. Depolarizing current was injected through recording electrode in stepwise (increment=10 mV in voltage-clamp or 10 pA in current-clamp) and holding potential at -50 mV, a sinusoid oscillations of membrane current or potential could be induced under voltage- (*A*) or current- (*B*) clamp conditions. Numbers above each trace indicate depolarizing membrane potential (in mV) or injected current (in pA). All traces were recorded from the same neuron in an identical experiment. When the membrane potential was depolarized from -50 mV to -30 mV, no oscillatory activities could be elicited. Instead, the miniature postsynaptic currents (mPSCs) appeared (left bottom trace, the time scale is expanded to show the detail of mPSCs).

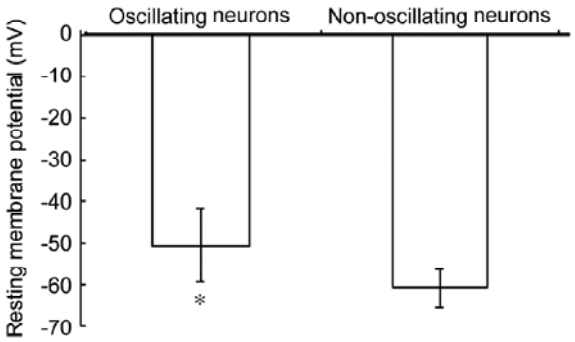

Fig. 3. Histogram of resting potentials in two groups of tactal neurons. Mean±SD, $^*P$ <0.01 *vs* non-oscillating neurons, n=11.

the SIC or the baseline after the SIC had subsided (Fig. 4*B* and 4*C*).

2.3 Dependence of oscillatory characteristics on the membrane potentials

The duration of the train of oscillations depended partially on the magnitude of depolarization, that is, larger magnitudes of depolarization evoked longer trains of oscillatory activity (Fig. 4*C*). The cycle interval (5-20 s) and duration (0.5-10.0 s) of oscillations varied among neurons. Changing the magnitude of the depolarization in a restricted range (between +10 and +50 mV) did not significantly alter the mean oscillatory frequency [(12.13±0.80) Hz, n=4]. From the example illustrated in Fig. 4*B*, it can be seen that when the membrane of the neuron was hyperpolarized initially at -90 mV and then depolarized to -40 mV (a +50 mV depolarization), oscillations with longer duration and larger amplitude were elicited, compared with those elicited by depolarization from -50 mV to -40 mV (Fig. 4*C*).

The amplitude of the oscillatory activity was voltage-sensitive. The responses of a tectal neuron initially clamped at different levels and then depolarized by +10 mV were shown in Fig. 5*B*. As it can be seen from the figure, when the membrane potential was initially clamped at -40 mV or -50 mV, the 10 mV depolarization induced subthreshold oscillations and the SIC. The same magnitude of depolarization from more positive membrane potentials evoked smaller amplitude oscillations (Fig. 5*B*, traces 3 and 4). If the neuron was clamped initially at more depolarized membrane potentials (e.g. > -30 mV), the 10 mV depolarizing pulse no longer induced oscillations and induced smaller SICs (Fig. 5*B*, traces 1 and 2). The amplitude of the oscillations increased or decreased with time, and then ceased abruptly or dampened slightly, as long as the membrane potential was not more positive than -30 mV.

In contrast with the changes in the amplitude of the subthreshold oscillations, the oscillatory frequency was relatively stable, that is, pooled data did not reveal any significant differences in frequency between the neurons initially clamped at -40 mV and -50 mV [(16.6±0.9) Hz *vs* (16.9±1.2) Hz, $P > 0.05$, $n = 5$]. Similar results were also obtained from other 9 neurons.

2.4 SICs and their variance with the membrane potentials

SICs could be induced in some tectal neurons. Usually, a train of oscillatory activities was superimposed on the decay phase of the SIC and/or on the baseline after the SIC ended (Fig. 4*B*). The depolarizing pulse induced a SIC and a short period of oscillations. However, some of the oscillating neurons exhibited a second and even a third SIC with oscillations, which could last for several seconds during the period of depolarization (Fig. 4*C*). For the example illustrated in Fig. 4*D*, when the membrane potential was depolarized from -50 mV to -40 mV, the response was an initial SIC with oscillations, and then more than 10 SIC-like downward inflections, which were irregular in the duration and the interval of the cycles. No oscillatory activity was superimposed on these later SIC-like downward inflections.

The SICs produced by a +10 mV depolarizing pulse rapidly reached their maximal value and then returned slowly to baseline with an approximately double exponential decay. The amplitude of SIC was defined as the difference between the membrane current level immediately preceding the depolarizing pulse and the current value at the peak of the SIC. The amplitudes of SICs were voltage-sensitive and decreased with increasing level of the initial membrane potential. Examples of SIC elicited by +10 mV depolarization from various initial membrane potentials were shown in Fig. 5.

2.5 Effects of bivalent ions on oscillations and SIC

We examined the effects of the bivalent ions Ca^{2+} and Sr^{2+} on the oscillations and SIC. Removal of Ca^{2+} from the extracellular media resulted in a complete disappearance of both the SIC and the oscillations (Fig. 5*A*). The same effects of Ca^{2+} removal were observed in 4 independant experiments. Since Sr^{2+} is a substitute for Ca^{2+} in activating regenerative responses[9], we examined the effects of substitution of Sr^{2+} (Fig. 5*C*) for Ca^{2+} (Fig. 5*B*) on the subthreshold oscillations and the SIC. Replacement of Ca^{2+} by Sr^{2+} resulted in a decrease in the frequency of the oscillatory events. Moreover, the continuous oscillatory activities were aborted at a plateau of SIC (Fig. 5*C*). Furthermore, in some neurons with the Sr^{2+}-containing solution,

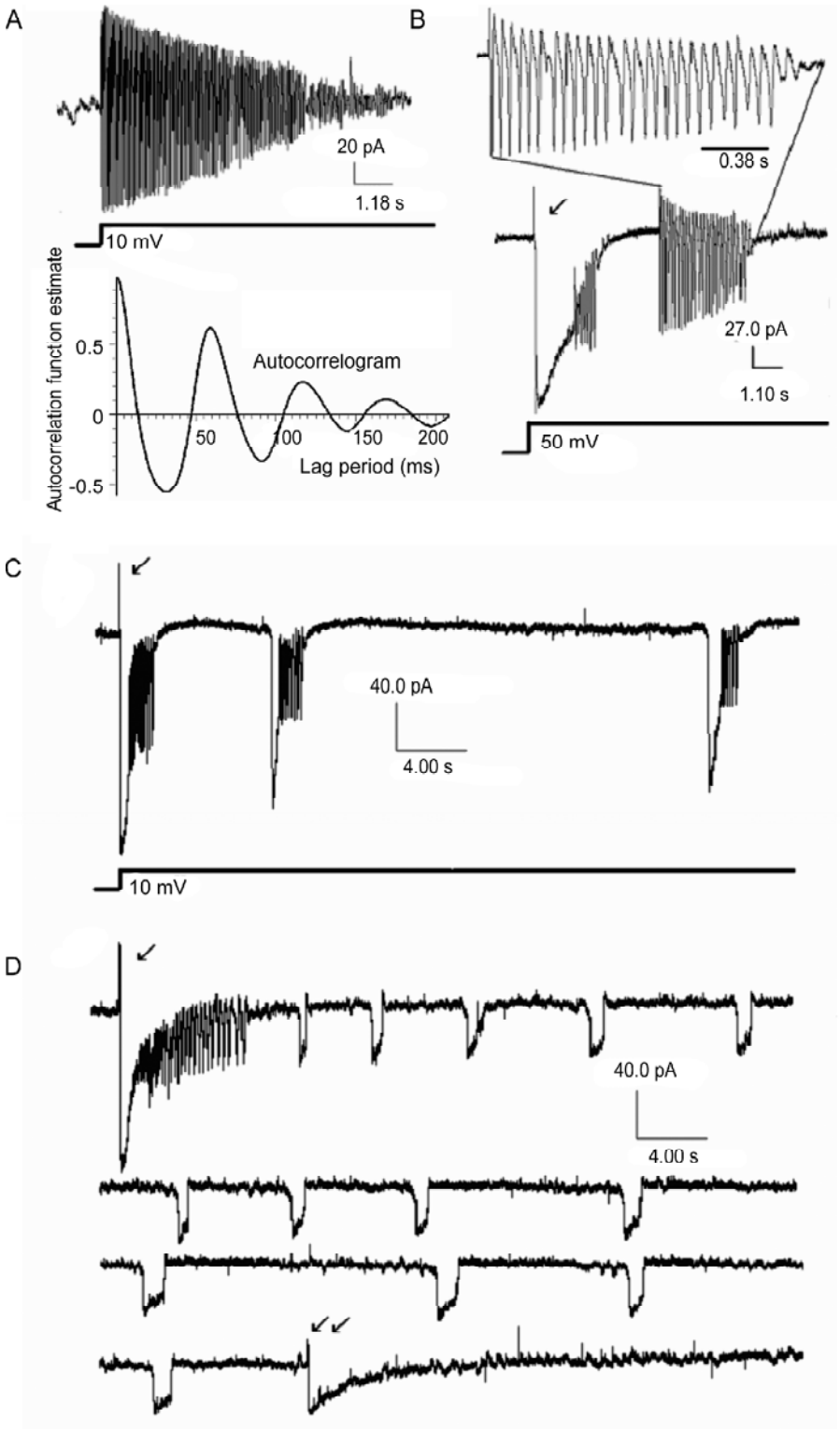

Fig. 4. Voltage-activated subthreshold current oscillations in the tectal neurons of *Xenopus*. *A*: Oscillations induced by depolarizing the membrane potential from -50 to -40 mV (top trace) and the corresponding autocorrelogram (bottom trace). In this case, there was no SIC after the depolarization stimulus. *B*: Depolarization of membrane potential (from -90 to -40 mV, 60 s duration) induced a transient spike (indicated by an arrow), a slow inward current (SIC) as well as two trains of oscillatory activities. *C*: In addition to a transient spike (indicated by an arrow), the depolarizing pulse (from -50 to -40 mV, 60 s duration) induced three consecutive SIC(s) and three trains of subthreshold oscillations. *D*: When the neuron was depolarized by injection of 10 mV direct current (DC) pulse in 60 s, an initial SIC and a train of oscillations were induced, followed by 13 SIC(s) with various duration and interval of cycles. The SICs lasted until the next DC pulse appeared (indicated by double arrows).

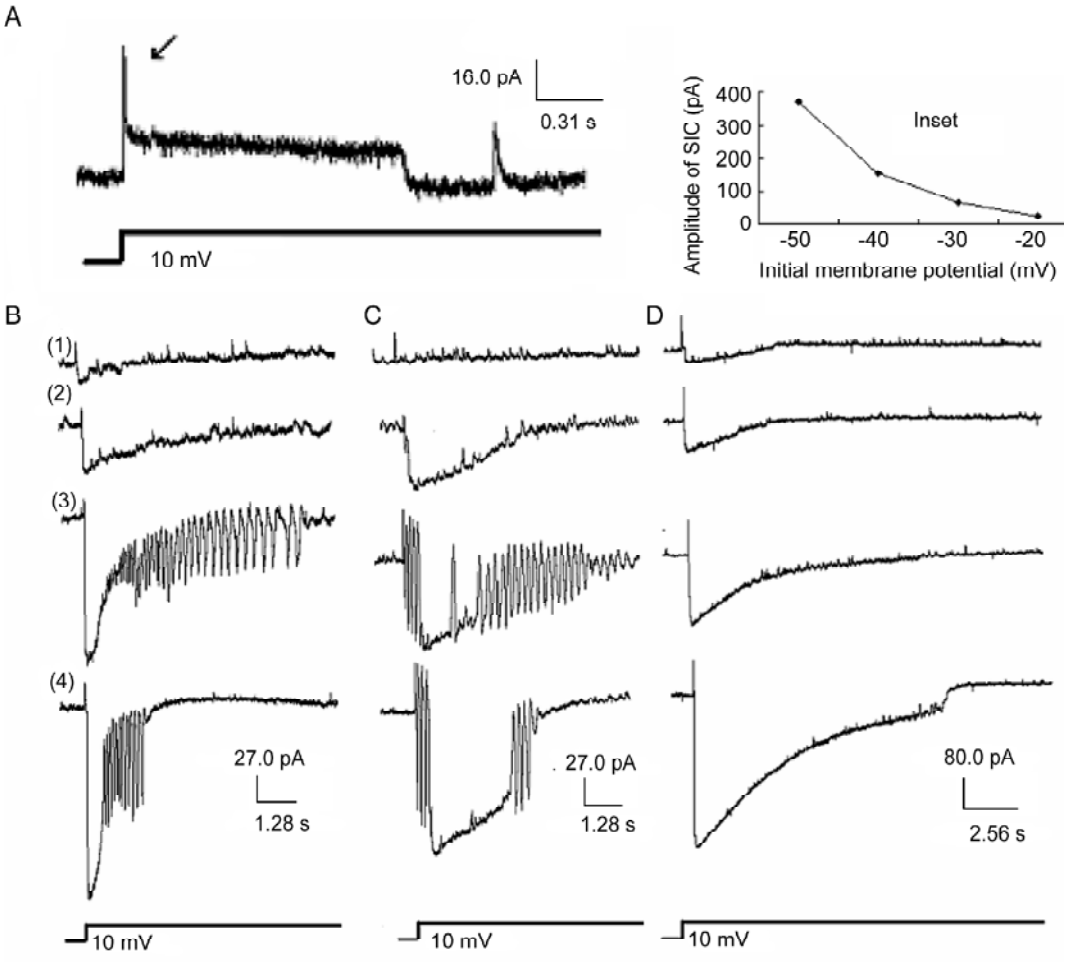

Fig. 5. Ca^{2+}-dependent mechanisms are involved in the generation of the SIC and subthreshold oscillations. *A*: A depolarizing voltage pulse (from -50 to -40 mV, 60 s duration) did not induce a SIC or subthreshold current oscillations in the Ca^{2+}-free bath solution. *B*: The slice was superfused in normal saline (control). *C*, *D*: Two types of neurons superfused with Sr^{2+}-containing bath solution. The inset on the top of *D* showed that the amplitude of SIC in this neuron decreased in approximate linearity with the increase of the initial holding potential under the same amplitude (+10 mV) of depolarization. Traces (1) to (4) in both panels follow depolarizations of 10 mV from various initial membrane potentials: (1) -20 mV, (2) -30 mV, (3) -40 mV, (4) -50 mV. Note that the vertical scale in *D* is different from those in panels *B* and *C*.

depolarization induced the SIC, but not the subthreshold oscillations (Fig. 5*D*). The most significant difference between SIC(s) with and without oscillatory activities was that the amplitude of SIC without oscillatory activities in the presence of Sr^{2+} was significantly larger and the duration longer (Fig. 5*D*). In this neuron, the amplitude of SIC decreased in approximate linearity with the increase of the initial holding potential (Fig. 5*D*, *inset*), under the same amplitude (+10 mV) of depolarization.

After the washout of Sr^{2+} with normal saline (2.5 mmol/L Ca^{2+}) for more than 10 min and during the period of washout, the characteristics of the SIC and the subthreshold oscillations did not return to pre-Sr^{2+} treatment levels; that is, compared to the control, the amplitude and frequency of oscillations as well as the amplitude of SIC remained significantly reduced (data not shown). These results suggest that the effects of Sr^{2+} on oscillations and SIC last a long period (at least >10 min). These results also may reflect the difficulty in totally eliminating the Sr^{2+} from the slices within the 10 min washout period. In addition, intracellular diffusion of Cs^{+} ions from the recording pipette could also progressively reduce oscillatory activities (see discussion).

3 DISCUSSION

To our knowledge, this is the novel report showing the subthreshold oscillations in the tectal neurons of *Xenopus*. Subthreshold oscillations are trains of sinusoid variations in membrane potential or current, often independent of

action potential. High- and/or low-threshold oscillations have been described in several mammalian cell groups including the dorsal root ganglia[2,10], the mesencephalic nucleus of the trigeminal nerve[11,12], the nucleis mediodorsalis thalami (MDT)[13], the lateral and basolateral nuclei of the amygdala[14] and the rat supraoptic nucleus[15]. However, neither the oscillations nor the SIC had been documented previously in *Xenopus*. In the present study, some of the tectal neurons in *Xenopus* were found to display oscillations with or without the accompanying SIC, when their membrane was depolarized by injection of depolarizing current through the recording pipette. However, neither the oscillations nor the SIC appeared spontaneously when the cells were voltage clamped near their resting potential (-50 mV) or when they were subjected to a 60 s hyperpolarizing pulse. The mean frequency of oscillatory activity was relatively slow in comparison with sensory neurons[2,10]. That is, while oscillatory frequency [(12.13±0.80) Hz, n = 9] of the tectal neurons is close to that of cortical neurons[1] and MDT neurons in guinea pig[13], it is much lower than that of dorsal root ganglion neurons[2,16]. While the neurons at different levels of the nervous system may have different characteristic frequencies, in the present study the low frequency of the oscillations might be due to the inclusion of Cs^+ in the internal solution. The internal Cs^+ might reduce the frequency of oscillations by blocking a sustained voltage-gated K^+ conductance[11,16]. On the other hand, Cs^+ also produces a slow depolarization in most neurons and can facilitate oscillations. Amir *et al*.[16] reported that as Cs^+ entered the cells during the first 5-20 min of a patch-clamp recording, 15 initially non-oscillating cells began to oscillate in response to depolarization. For comparison, we also performed experiments (data not shown) in which KCl was used in place of Cs-gluconate. No oscillations or SICs occurred in any of the five neurons. Cs^+ might allow the Ca^{2+} current to become regenerative by suppressing a K^+ outward current that normally cancels the effect of the Ca^{2+} (or Sr^{2+}) influx.

There are a few well-documented examples that demonstrate a close association between electrical oscillations and resonance in neurons. For example, in inferior olivary neurons with subthreshold oscillations, the peak of the resonance and the frequency of the oscillations coincide[17]. Also, Puil *et al*.[18] found that the production of resonance in thalamic neurons of guinea pig requires a low-threshold Ca^{2+} current (I_T), but not a hyperpolarization-activated cation current (I_h). The low-frequency resonance is TTX insensitive. Cs^+ completely blocks the rectification, but does not alter the low-frequency resonant hump. Our results in this study are consistent with these results mentioned above on the effects of TTX and Cs^+ on oscillations.

The oscillations vary with the resting potential of the recorded neurons. In this study, the mean resting potential in non-oscillating neurons is significantly lower than that in oscillating neurons [(-60.8±4.8) mV *vs* (-50.5±8.8) mV]. Similarly, the resting potentials of dorsal root ganglia neurons that exhibited spontaneous oscillations were more depolarized than those of neurons without spontaneous oscillations [(-49.4±6.4) mV *vs* (-60.5±6.5) mV, P<0.01][2]. Although spontaneous oscillations could be recorded in dorsal root ganglia neurons, the prevalence of oscillatory neurons depended on depolarization level[19]. Similarly, the mean resting potential of mesencephalic trigeminal neurons that had spontaneous oscillations was (-50.2±3.0) mV, whereas the mean resting potential of the neurons without spontaneous oscillations was (-54.2±2.5) mV[11]. Moreover, subthreshold membrane oscillations were evoked more commonly in neurons of the rat mesencephalic nucleus of the trigeminal nerve after postnatal day 7, while the mean resting potential is more positive after postnatal day 7[12]. Taken together, these results suggest that more positive resting membrane potentials facilitate the generation of oscillations.

Basically, oscillations could be divided into two types: TTX-sensitive [e.g. in dorsal root ganglia and mesencephalic trigeminal sensory neurons (Mes V)][2,12] and TTX-resistant (in thalamic, cortical and inferior olivary neurons)[18,20,21]. When the oscillations are TTX-sensitive, the depolarizing phase of the oscillations is generated by a voltage-dependent sodium conductance. A potassium conductance is responsible for the falling phase. For the TTX-resistant neurons, a Ca^{2+} conductance is responsible for the rising phase of the oscillations. In the *Xenopus* tectal neurons, both the oscillations and the SIC were TTX-resistant, suggesting that a Ca^{2+} rather than Na^+ current is responsible.

In the present experiments, if TTX was added to the bath at a sufficient concentration to block Na^+ action potentials, the oscillations and/or SIC would still be produced by the depolarizing pulse. However, when a Ca^{2+}-free external solution was used, neither SIC nor oscillations could be generated. Furthermore, when Ca^{2+} in the bath solution was substituted with Sr^{2+}, the SIC still could be produced and its duration was prolonged, but the frequency and amplitude of the oscillations decreased markedly, and in some cases it would disappear. Thus, the SIC and oscillations are dependent on the presence of external Ca^{2+} or Sr^{2+}. In the mechanosensory neurons of the lamprey, a prolonged TTX-resistant Ca^{2+}-spike has been

recorded. If Sr^{2+} was substituted for Ca^{2+}, the neurons generated responses that were longer than the Ca-spike[22]. In intracellular recording experiments, Alvarez-Leefmans and Miledi[9] also reported that depolarizing pulses elicited prolonged regenerative depolarizing responses in frog motoneurons. The response was TTX-resistant and dependent on the presence of external Ca^{2+}. Sr^{2+} could be substituted for Ca^{2+}, but Sr^{2+}-dependent responses were significantly longer than the Ca^{2+}-dependent ones. In the present study, we found that the duration of the Sr^{2+} spike was slightly longer than that of the Ca^{2+}, which is consistent with the above results.

White *et al.*[23] reported that a brief depolarization of patch clamped dorsal root ganglion cells in the rat generated an inward current. If the neurons were superfused with a Ca^{2+}-free solution, the inward currents would decrease by 90%. These observations suggested that the inward current requires Ca^{2+} influx. Further observations argue that a Ca^{2+} current rather than a Ca^{2+}-activated cationic current constitutes the inward current. For example in the presence of Ni^{2+} (a T-type Ca^{2+}-channel blocker), the inward current is completely eliminated. The low-threshold, voltage- and Ca^{2+}-sensitive inward current is also important in generation of oscillatory behavior in neurons of the inferior olive[21] and other brain regions[24]. Recently, Pinado and Midtgaard[25] also reported that low-threshold calcium spikes (LTSs) were evoked and persisted in the presence of TTX but were antagonized by the blockers of T-type calcium channels, e.g. Ni^{+}. So, some properties of SIC are consistent with those of the inward current in dorsal root ganglion neurons of rats and the low-threshold calcium spike in granule cells of the turtle olfactory bulb. However, it remains to be determined whether the T-type or other calcium channels are involved in the SIC.

The mechanism of the oscillations and their role in neural integration issued presently are still controversial. Some authors consider that the rhythmic activity of a neuronal network can be driven by individual neurons intrinsically oscillating[1]. Izhikevich *et al.*[26] proposed that oscillations were important for selective communication via resonant bursts. Pyramidal neurons in the neocortex have two resonances, each with different voltage dependence. A 1-2 Hz resonance occurs near resting membrane potential and might support the thalamocortical delta-wave oscillations; whereas a 5-20 Hz resonance occurs at potentials more positive than -55 mV. The latter higher-frequency rhythms appear in the cortex during cognition[14, 15, 17]. Similar to the mammalian cortex, the optic tectum in amphibians plays an important role in neuronal processing of visual information. In the present study, the oscillatory frequency of the tectal neurons was around 16 Hz and is within the range of the higher-frequency rhythms in the cortex of the mammalians. Thus, the subthreshold oscillations reported in this study might play an important role in the physiological and behavioral functions of frogs, e.g. pattern discrimination, prey recognition, avoiding behavior *etc*. Further studies of this role might clarify the contributions of these oscillations to the integration of neural activity in both mammals and non-mammalian vertebrates.

Taken together, oscillatory model is as following: in the presence of I_{Na} blockers (TTX), subthreshold oscillations result from activation of a voltage-sensitive TTX-resistant Ca^{2+} conductance with rapid activation, inactivation and repriming kinetics, reciprocating with repolarization due to ohmic K^{+} leak. In *Xenopus*, voltage-gated K^{+} channels and K^{+} leak both might contribute to spike repolarization. The generation of subthreshold oscillations is a result of the successive mutual activation of a depolarizing current (such as a Ca^{2+} current) and a re- or hyperpolarizing current (such as a K^{+} current). First, depolarizing current is activated and depolarizes the neuron. The more depolarized voltage leads to passive repolarizing current or an activation of the hyperpolarizing current, and possibly an inactivation of the depolarizing current. The hyperpolarizing current hyperpolarizes the neuron, and the more negative voltage inactivates the hyperpolarizing current, and possibly de-inactivates the depolarizing current. Then the cycle restarts.

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