The modulation of two motor behaviors by persistent sodium currents in *Xenopus laevis* tadpoles

Erik Svensson, Hugo Jeffreys, and Wen-Chang Li

School of Psychology and Neuroscience, University of St Andrews, St Andrews, Fife, United Kingdom

Submitted 19 September 2016; accepted in final form 22 March 2017

**NEW & NOTEWORTHY** We have characterized persistent sodium currents in three groups of spinal neurons and their role in shaping spiking activity in the *Xenopus* tadpole. We then attempted to evaluate the role of persistent sodium currents in regulating tadpole swimming and struggling motor outputs by using low concentrations of the persistent sodium current antagonist riluzole.

*Xenopus* tadpole; motor behavior; spinal cord; sodium currents; riluzole

**TRANIENT SODIUM CURRENTS** 

Persistent sodium currents in lower vertebrates and in mammals (Grillner and El Manira 2015; Grillner and Jessell 2009; Kiehn 2016; Roberts et al. 2012), and *I*\textsubscript{NaP} has been identified in many spinal neurons (Benedetti et al. 2016; Hu et al. 2002; Miles et al. 2005; Tazerart et al. 2008; Theiss et al. 2007; Tong and McDearmid 2012; Zhong et al. 2007). A low concentration of riluzole blocks *I*\textsubscript{NaP} (Urbani and Belluzzi 2000) and is used to treat amyotrophic lateral sclerosis (ALS) (Bellingham 2011; Benedetti et al. 2016; Devlin et al. 2015; Jenkins et al. 2014; Quinlan et al. 2011), which is associated with hyperactivity of spinal motor neurons and upper motor neurons in the primary motor cortex (Caballero-Hernandez et al. 2016). It is necessary to understand how *I*\textsubscript{NaP} modulates motor behaviors in a trackable spinal circuit and to assess the actions of riluzole (Benedetti et al. 2016).

*Xenopus* tadpole spinal and hindbrain circuits controlling swimming and struggling have been mapped using paired whole cell recordings (Berkowitz et al. 2010; Roberts et al. 2010). Tadpole swimming central pattern generator (CPG) comprises descending interneurons (dINs), commissural interneurons (cINs), ascending interneurons (aINs), and motoneurons. Among them, dINs provide the phasic excitation to drive other types of rhythmic neurons while their own firing is sustained by rebound firing following mid-cycle inhibition or NMDA receptor (NMDAR)-dependent pacemaker properties (Li et al. 2006, Li et al. 2010; Soffe et al. 2009). When the tadpole skin is stimulated repetitively, two types of interneurons are recruited [excitatory commissural interneurons (e-cINs) and repetitive firing descending interneurons (dINrs)], but the dIN activity is suppressed (Li et al. 2007b; Li 2015).

Tadpole neurons involved in swimming and struggling display different types of firing properties in response to depolarizing current pulses (Li et al. 2007a; Li et al. 2007b; Sautois et al. 2007; Winlove and Roberts 2012). dINs fire a single spike at the onset of depolarizing step currents, whereas other rhythmic neurons show repetitive firing, often with a delay caused by A-type potassium currents (Li 2015). The properties of *I*\textsubscript{NaT} have been characterized in dissociated tadpole spinal neurons and sensory neurons and sensory interneurons in situ (Dale 1995; Winlove and Roberts 2012). We analyzed sodium currents in the neurons involved in tadpoles swimming and struggling in situ. We report *I*\textsubscript{NaP} in the Rohon-Beard neurons (primary sensory neurons, RB), the excitatory descending interneurons (dINs), and other rhythmic neurons in tadpole
swimming and struggling (non-dINs) (Roberts et al. 2008). We have used the \(I_{\text{NaP}}\) antagonist riluzole at 1 \(\mu\)M to investigate its role in tadpole swimming and struggling.

**METHODS**

All experiment procedures were approved by the local Animal Welfare and Ethics Committee and comply with UK Home Office regulations. Human chorionic gonadotropin injections were carried out to induce mating between pairs of adult *Xenopus*. Tadpoles at stage 37/38 (Nieuwkoop and Faber 1956) were anesthetized using 0.1% MS-222 (3-aminobenzoic acid ester; Sigma, Irvine, UK) and then immobilized using 12.5 \(\mu\)M \(\alpha\)-bungarotoxin (Tocris, Bristol, UK) and mounted onto a Sylgard stage for dissections (Moult et al. 2013). The saline contained (in mM) 127 NaCl, 3 KCl, 2 CaCl\(_2\), 2.4 NaHCO\(_3\), 1 MgCl\(_2\), and 10 HEPES, with \(pH\) adjusted to 7.4. Fine dissections were carried out to expose muscle clefts for recording motor nerve activities using a glass suction electrode and neuronal somata in the caudal hindbrain and rostral spinal cord for whole cell recordings (between the 5th rhombomere segments and the 7th posterior spinal cord). Intracellular signals were amplified with an Axon Multiclamp 700B, digitized with a Power 1401 mkII data acquisition interface, and sampled with Signal (version 5; CED, Cambridge, UK).

**Neuron identification and grouping.** The sensory RB neurons were initially visually identified by their large round somata and location on the dorsal edge of the spinal cord. Further RB identification was by their wide action potential and typical firing pattern (Winlove and Roberts 2012) in whole cell recordings. We grouped neurons rhythmically active during fictive swimming as dINs and non-dINs (motoneurons, commissural interneurons, ascending interneurons, repetitive firing descending interneurons), which have similar firing properties to current injections (Li et al. 2007a; Sautois et al. 2007). dINs and non-dINs were identified by their responses to light dimming, which triggers swimming activity. It is possible to distinguish dINs and non-dINs by recording extracellular action potentials with a loose-patch electrode. The dINs have monophasic action potentials, and the non-dINs have biphasic action potentials (Soffe et al. 2009).

Because sensory interneurons are not active during swimming (Li et al. 2004; Li et al. 2007a; Sillar and Roberts 1988), our screening method using loose-patch recordings should have systematically excluded them.

**Current-clamp recordings.** Whole cell recording pipettes were filled with a solution containing (in mM) 100 K-glucuronate, 2 MgCl\(_2\), 10 EGTA, 10 HEPES, 3 Na\(_2\)ATP, and 0.5 NaGTP with 0.1% neurobiotin (Vector Laboratories, Burlingame, CA; \(pH\) adjusted to 7.4). The inclusion of neurobiotin allowed the revealing of neuronal anatomy after whole cell recordings in some recordings (Li and Moult 2012). Current-clamp recordings of spiking properties were performed in bridge mode, and stimulations in all cases were done from membrane potential set at −60 mV by injecting slow DC currents using the Multiclamp 700B controller. Microperfusion of riluzole (Tocris) was done by positioning a glass pipette with a tip opening of ~10 \(\mu\)m more than 30 \(\mu\m\) upstream to the recorded soma (Li and Moult 2012). A gentle pressure was applied inside the pipette by compressing a connected 50-ml syringe for 100 \(\mu\)l (~200 Pa) to eject riluzole. The pipette was moved >200 \(\mu\m\) away from the preparation, combined with gentle suction (approximately −100 Pa) to stop gravity-driven leakage when not in use.

**Voltage-clamp recordings of sodium currents.** After identification of neurons, the preparation was bath perfused with a solution containing (in mM) 35 NaCl, 40 NMDG, 3 KCl, 10 CaCl\(_2\), 2.4 NaHCO\(_3\), 1 MgCl\(_2\), 10 HEPES, 40 tetraethylammonium (TEA), 1.4 4-aminopyridine (4-AP), and 0.15 CdCl\(_2\), and \(pH\) was adjusted to 7.4. The neurons were patched with an electrode filled with a pipette solution in which 100 mM K-glucuronate was replaced with 100 mM cesium methanesulfonate to block potassium channels from the inside. Liquid junction potential was 5.7 mV, calculated using the Clampex 10.2 junction potential formula. This was corrected during all recordings. Leak currents were subtracted during experiments, and serial resistance compensation was done for 70%. Serial resistances accepted for voltage-clamp recordings were between 10 and 20 \(M\Omega\).

Decay time constants were measured by double-exponential curve fitting of the recovery phase of sodium currents using the curve fitting function in Signal (Fig. 1C). The peak amplitude of \(I_{\text{NaP}}\) was measured by extrapolating a single-exponential curve fit to the slow persistent component, where \(I_{\text{NaT}}\) was expected to have closed (Fig. 1F). The surface area was calculated from the capacitance of the whole cell configuration and with the assumption that neurons had a specific capacitance of 1 \(\mu\F/cm^2\) (Winlove and Roberts 2012). The current densities were then calculated by dividing \(I_{\text{NaP}}\) (pA) with the calculated cell surface (\(\mu\m^2\)). Statistical significance was examined using the Kruskal-Wallis or Friedman test with Dunn’s post hoc test when the data distribution was not normal or sample size was small. When the data were normally distributed, ANOVA or Student’s \(t\)-tests were carried out. Means are given with SE.

**RESULTS**

\(I_{\text{NaP}}\) and \(I_{\text{NaT}}\) in tadpole spinal neurons. Previous studies have not been able to show the presence of any \(I_{\text{NaP}}\) in tadpole spinal neurons (Dale 1995; Winlove and Roberts 2012). To reinvestigate this, we applied a voltage ramp (from −80 to 20 mV over 200 ms) to RB neurons. The slowly rising depolarization will inactivate the \(I_{\text{NaT}}\) and activate the \(I_{\text{NaP}}\). The ramp induced an inward current that started to open at about −40 mV and had a peak of −133.38 ± 42.12 pA at around −10 mV (\(n = 8\); Fig. 1, A and B). This shows that the RB neurons possess \(I_{\text{NaP}}\).

To quantify \(I_{\text{NaP}}\) properties relative to \(I_{\text{NaT}}\), we used voltage steps rather than ramps in RB neurons, dINs, and non-dINs. Voltage steps ranged from −80 to 30 mV in 10-mV increments. The recovery phase of sodium currents was best fitted with a two-exponential curve, suggesting the presence of a fast-decaying (\(I_{\text{NaT}}\)) and slowly decaying component (\(I_{\text{NaP}}\)) (Fig. 1C). To estimate the size of both currents, we carried out a single-exponential fitting of the latter phase of the currents and extrapolated the size of \(I_{\text{NaP}}\) when the combined currents peaked (Fig. 1, C and F). \(I_{\text{NaT}}\) was calculated by subtracting the \(I_{\text{NaP}}\) component from the combined peak currents (Fig. 1, C and D). The peak \(I_{\text{NaT}}\) in RB neurons was −3,103.38 ± 586.25 pA (\(n = 8\); Fig. 1D) at steps to −10 mV. The \(I_{\text{NaP}}\) in dINs was −1,516 ± 296.5 pA at steps to 10 mV (\(n = 7\)), and that in non-dINs was −979.8 ± 185.86 pA at steps to 10 mV (\(n = 6\); Fig. 1D). The peak decay constant for \(I_{\text{NaT}}\) was to steps to −20 mV in RB neurons (0.89 ± 0.21 ms, \(n = 8\)) and at steps to −10 mV in dINs (1.00 ± 0.17 ms, \(n = 7\)) and non-dINs (1.08 ± 0.20 ms, \(n = 7\)), which is shown in Fig. 1E. There was no difference in the peak decay time constants for the \(I_{\text{NaT}}\) between neuron types (\(P > 0.05\)).

The ratio between the \(I_{\text{NaP}}\) and \(I_{\text{NaT}}\) amplitudes did not differ between RB neurons (0.19 ± 0.025, \(n = 8\)) and dINs (0.21 ± 0.042, \(n = 7\); \(P > 0.05\)) or non-dINs (0.33 ± 0.076, \(n = 6\); \(P > 0.05\); Fig. 1G). The current density for the \(I_{\text{NaT}}\) was higher in 8 RB neurons (2.02 ± 0.35 pA/\(\mu\m^2\)) than in 7 dINs (1.13 ± 0.17 pA/\(\mu\m^2\); \(P > 0.05\)) and 6 non-dINs (0.66 ± 0.058 pA/\(\mu\m^2\); \(P < 0.01\); Fig. 1H). There was no difference in the current density of \(I_{\text{NaP}}\) among the three neuron groups (RB...
neurons: $0.37 \pm 0.086 \text{pA/um}^2$; dINs: $0.21 \pm 0.03 \text{pA/um}^2$; non-dINs: $0.25 \pm 0.084 \text{pA/um}^2$; $P > 0.05$; Fig. 1D). These results show that all three neuron groups express both fast-inactivating $I_{NaP}$ and a slowly inactivating $I_{NaP}$.

**Properties of $I_{NaP}$.** We further analyzed the properties of $I_{NaP}$ in these neurons on the basis of voltage-step experiments. The $I_{NaP}$ in RB neurons started to activate at steps to $-40 \text{mV}$, and maximum current was achieved at steps to $-10 \text{mV}$ ($552.00 \pm 91.17 \text{pA}$; Fig. 2, A and D; $n = 8$). Steps to more depolarized voltages reduced the amplitude, and steps to $30 \text{mV}$ generated a current of $-170.12 \pm 31.52 \text{pA}$ (Fig. 2, A and D; $n = 8$). The decay time constant was also voltage dependent and had its maximum of $17 \pm 4.7 \text{ms}$ at steps to $-10 \text{mV}$ (Fig. 2E).

Also, in the dINs the $I_{NaP}$ first activated at steps to $-40 \text{mV}$. The maximum $I_{NaP}$ was generated at steps to $-10 \text{mV}$ ($-311.43 \pm 49.90 \text{pA}$, $n = 7$; Fig. 2, B and D). Steps to more depolarized levels generated smaller $I_{NaP}$ (Fig. 2, B and D; $n = 7$). The decay constant for the $I_{NaP}$ in dINs had its peak at steps to $-10 \text{mV}$ with a decay constant of $9.9 \pm 1.5 \text{ms}$ (Fig. 2E).

In the non-dINs, the $I_{NaP}$ also first activated at steps to $-40 \text{mV}$. However, the $I_{NaP}$ in the non-dINs had its peak at steps to $0 \text{mV}$ ($-323.83 \pm 94.88 \text{pA}$; Fig. 2, C and D; $n = 6$). The amplitude of $I_{NaP}$ decreased at more positive steps (Fig. 2, C and D; $n = 6$). The decay constant of $4.27 \pm 0.80 \text{ms}$ at steps to $0 \text{mV}$ in non-dINs ($n = 6$) was faster than that in 8 RB neurons and 7 dINs (both $P < 0.05$, Student’s unpaired t-test; Fig. 2E). No difference was found between the decay time constants between RB neurons and dINs ($P > 0.05$).

Finally, we tested the action of riluzole (1, 10, and 20 $\mu$M) on $I_{NaP}$ in spinal neurons. All three concentrations of
riluzole significantly reduced the amplitude of $I_{NaP}$ (Fig. 2). A concentration of 1 μM riluzole reduced the $I_{NaP}$ to 71.7 ± 15.0% of control (Fig. 2F; n = 2 dINs, 2 non-dINs, and 4 RB neurons; $P < 0.01$), 10 μM riluzole reduced $I_{NaP}$ to 44.1 ± 12.7% of control (n = 6 RB neurons; $P < 0.05$), and 20 μM riluzole reduced $I_{NaP}$ to 25.7 ± 6.0% of control (n = 6 RB neurons; $P < 0.01$). The effect of riluzole recovered completely or partially after washout. We also tested the action of 1, 10, and 20 μM riluzole on the $I_{NaT}$. Riluzole at 1 μM reduced $I_{NaT}$ significantly to 74.7 ± 15.9% of control (Fig. 2, H and I; n = 2 dINs, 2 non-dINs, and 3 RB neurons; $P < 0.01$). Higher doses of riluzole (10 and 20 μM) significantly reduced the amplitude of $I_{NaT}$ (Fig. 2I). At 10 μM riluzole, $I_{NaT}$ was reduced to 46.8 ± 12.2% of control (n = 6 RB neurons; $P < 0.05$), and at 20 μM riluzole, $I_{NaT}$ was reduced to 28.8 ± 8.2% of control (n = 6 RB neurons; $P < 0.05$).

These results show that all three groups of neuron express $I_{NaP}$, but the properties of the current vary, and 1 μM riluzole can reduce the amplitude of $I_{NaP}$ and $I_{NaT}$. $I_{NaP}$ in non-dINs peaks at steps to 0 mV, whereas the current in RB neurons and dINs peaks at steps to −10 mV. There are also differences in the decay time constants, where non-dINs decay significantly faster than the dINs and non-dINs.

Effect of 1 μM riluzole on neuronal firing properties. Riluzole is known to affect many other aspects of neuronal func-

Fig. 2. Properties of $I_{NaP}$ in the 3 neuron groups and the effect of riluzole. A–C: sodium currents in an RB neuron (A), a dIN (B), and a non-dIN (C) in response to voltage step to −40 (black traces), −10, or 0 mV (red traces showing the maximal $I_{NaP}$) and to 30 mV (blue traces). D: averaged I-V curves for the $I_{NaP}$ in RB neurons, dINs, and non-dINs. E: decay time constants of $I_{NaP}$ at steps to different membrane potentials in RB neurons, dINs, and non-dINs. F: effect of 1 μM riluzole on $I_{NaP}$ in an RB neuron. G: blocking effects of 1, 10, and 20 μM riluzole on $I_{NaP}$. H: effect of 1 μM riluzole on $I_{NaT}$ in an RB neuron. I: effect of 1, 10, and 20 μM on $I_{NaT}$. *$P < 0.05$; **$P < 0.01$.
tion, especially at concentrations much higher than 1 μM (Bellingham 2011; Urbani and Belluzzi 2000). Having demonstrated that riluzole at 1 μM could weaken $I_{\text{NaP}}$, we next tested 1 μM riluzole in current-clamp mode so that we could monitor its effects on neuronal firing in the three groups of neurons. Riluzole at 1 μM did not alter the resting membrane potential, which was −62.15 mV in control, −61.32 in riluzole, and −60.77 mV after washout ($n = 11$; $P > 0.05$).

The dINs typically fire single action potentials in response to a depolarizing current pulse. However, hyperpolarizing pulses on top of depolarization to mimic inhibitory synaptic inputs generate reliable rebound spikes in dINs (Li et al. 2006) (Fig. 3A). Microperfusion of 1 μM riluzole reduced the number of rebound spikes from 10 in control to 1.33 ± 0.76 (from 10 hyperpolarizing pulses; Fig. 3, A1 and A2; $P < 0.001$). The rebound firing recovered to 7 ± 1.32 spikes after washout (Fig. 3A3).

**Fig. 3.** Effects of riluzole on firing properties of the 3 different neuron groups. A1: effect of 1 μM riluzole on the rebound firing in a dIN. A2: bar chart summarizing the reduction of rebound spikes in dINs ($n = 6$). A3: subthreshold depolarization and firing at threshold level in a dIN in control (left), 1 μM riluzole (**$P < 0.01$; middle), and wash (right). B1: effect of riluzole on an RB neuron with slow, repetitive firing. B2: bar chart showing the reduction of spikes by riluzole ($n = 3$). B3: subthreshold depolarization and firing at threshold level in an RB neuron in control (left), 1 μM riluzole (middle), and wash (right). C1: effect of riluzole on the repetitive spiking in a non-dIN. C2: reduction of number of spikes by riluzole ($n = 9$). C3: subthreshold depolarization and firing at threshold in a non-dIN in control (left), 1 μM riluzole (middle), and wash (right). D: effect of riluzole on the spike thresholds in RB neurons ($n = 6$), dINs ($n = 4$), and non-dINs ($n = 5$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. E: lack of effects of riluzole on spike amplitudes in RB neurons, dINs, and non-dINs. F: lack of effects of riluzole on input resistances in RB neurons, dINs, and non-dINs.
excitability, in the presence of riluzole (Fig. 3D). We next analyzed neuronal firing reliability during swimming, defined as the percentage of cycles with neuronal spiking. However, we did not identify any change in firing reliability in 1 μM riluzole (Fig. 4D; n = 7 non-dINs; related samples Friedman’s 2-way ANOVA by ranks, P = 0.82).

The other motor output from the tadpole spinal/hindbrain circuit is struggling, during which neurons fire multiple spikes on each struggling cycle (Li et al. 2007b). In seven tadpoles, repetitive skin stimulation of the rostral trunk skin was used to induce fictive struggling (Li et al. 2007b). Because we have shown that riluzole application could reduce repetitive firing of non-dINs at rest, we analyzed whether riluzole could weaken non-dIN firing during struggling. The struggling rhythms became irregular in two tadpoles when 1 μM riluzole was bath applied. In the other five tadpoles, fictive struggling persisted throughout riluzole application (Fig. 4C). There was a decrease in the number of spikes per struggling cycle (n = 5 non-dINs; P < 0.05, paired t-test; Fig. 4E). During struggling with higher frequencies, there can be fewer spikes on each cycle because burst duration is shorter. However, there was no change in struggling frequencies in the presence of riluzole (Fig. 4F), suggesting the reduction of neuronal spiking during struggling may be a direct effect of 1 μM riluzole on the recorded neuron.

**DISCUSSION**

\( I_{\text{NaP}} \) in spinal/hindbrain circuits. \( I_{\text{NaP}} \) has been widely identified in spinal circuits controlling locomotion (Dai and Jordan 2011; Heckman 2000; 1996), whereas others have reported the gene- ration of \( I_{\text{NaP}} \) in preBötzinger complex neurons critical for generating breathing rhythms (Del Negro et al. 2002; Del Negro et al. 2005; Koizumi and Smith 2008; Pace et al. 2007). We have identified \( I_{\text{NaP}} \) in the sensory RB neurons and in all groups of neurons involved in rhythmic swimming and struggling activity. The majority of \( I_{\text{NaP}} \), with other persistent inward currents, may be located on dendrites (Houngsaard and Kiehn 1993; Lee and Heckman 2000; 1996), whereas others have reported the generation of \( I_{\text{NaP}} \) in the proximal axon (Astman et al. 2006; Osorio et al. 2010). This may explain why clear \( I_{\text{NaP}} \) was not recorded in the isolated *Xenopus* tadpole spinal neurons (Dale 1995), where neurons lose axons and most dendrites during the dissociation process. This study also shows that the activation of \( I_{\text{NaP}} \) will require stepping the membrane potential above −40 mV, peaking at around 0 mV. The absence of \( I_{\text{NaP}} \) in the RB neurons in a previous study may be a result of using depolarizing voltage steps below −40 mV (Winlove and Roberts 2012).

**Role of \( I_{\text{NaP}} \) in regulating spiking activity.** In this study we show that three groups of neuron in the tadpole display \( I_{\text{NaP}} \) but that the properties of the current differ slightly between the neuron types. The \( I_{\text{NaP}} \) in all neuron groups started to be activated at voltage steps to −40 mV, which is below the threshold for the action potential in all three neuron types (Figs. 1A, 1B, 2E, and 3D) (Li 2015). This suggests that depolarization from synaptic currents or current injections may just need to depolarize the membrane potential to the level to activate \( I_{\text{NaP}} \). The activation of \( I_{\text{NaP}} \) then will depolarize the membrane potential further to trigger spiking. Therefore, \( I_{\text{NaP}} \) can play a role in setting the spike threshold, defined as the
highest depolarization before spiking in this study. Indeed, increased $I_{NaP}$ current density is correlated with more negative spike threshold (Bellingham 2013; Kuo et al. 2006). This was supported by the observation that riluzole reduced the amplitude of $I_{NaP}$ and significantly decreased the excitability of all three groups of neurons. One difference between the $I_{NaP}$ in the three neuron groups was that $I_{NaP}$ peaked at voltage steps to $-10$ mV in RB neurons and dINs, whereas the peak in non-dINs was at steps to 0 mV. The decay time constant also differed in that RB neurons had the slowest decay and non-dIN $I_{NaP}$ decayed the fastest. We do not know how much these differences can be explained by the space-clamping issues, which depend on the anatomy of neurons and the distribution of ion channels. One explanation can be that the different groups of neurons express different type of sodium channel isoforms. Non-dINs in the tadpole spinal cord and hindbrain typically show repetitive firing to depolarizing current injections, and they have a higher $I_{NaP}/I_{NaT}$ ratio than the dINs and RB neurons (Fig. 3, C1 and C2) and the fastest $I_{NaP}$ decay time constant. These properties of $I_{NaP}$ may help to shape their repetitive firing, as suggested in the 11- to 19-day-old rat ventral horn interneurons in the lumbar cord region (Theiss et al. 2007). The potential of $I_{NaP}$ in setting neuronal firing thresholds has also been reported in the commissural interneurons and motoneurons of neonatal mouse spinal cord (Zhong et al. 2007).

Role of $I_{NaP}$ in regulating tadpole swimming and struggling. $I_{NaP}$ is not thought to be essential in the generation of respiratory-related rhythm because riluzole microinjection in the mouse preBötzinger complex did not perturb respiratory frequency in a slice preparation (Pace et al. 2007), although $I_{NaP}$ plays a role in the pacemaker bursting of some neurons therein (Del Negro et al. 2002; Koizumi and Smith 2008). $I_{NaP}$ also contributes to the induced oscillations in some rodent spinal
interneurons (Bouhadfane et al. 2013; Tazerart et al. 2008; Ziskind-Conhaim et al. 2008). $I_{\text{Na}P}$ may help to stabilize the fictive locomotion rhythms in neonatal rats (Tazerart et al. 2007) and is suggested to have an essential role in the generation of neonatal mouse locomotion pattern (Zhong et al. 2007) or in generating the rat masticatory movements (Brocard et al. 2006).

Does $I_{\text{Na}P}$ play a role in the generation of tadpole swimming rhythms? The generation of swimming rhythms relies on rebound/pacemaker firing in dINs (Li 2011). In this report we have shown that riluzole shortened swimming episodes. It is known that tonic depolarization in dINs during swimming is needed in dIN rebound firing (Li and Moul 2012). $I_{\text{Na}P}$ has been suggested to amplify excitatory synaptic inputs and prolong membrane depolarization and firing (Lee and Heckman 1996). The activation of $I_{\text{Na}P}$ can enhance NMDAR-mediated depolarization in dINs during normal swimming and contribute to the maintenance of swimming rhythms. dINs in tadpoles also show large membrane oscillations when their NMDARs are activated (Li et al. 2010). The activation threshold for $I_{\text{Na}P}$ is within the oscillation voltage range, suggesting $I_{\text{Na}P}$ should play a role in the NMDAR-dependent oscillations, although further direct experiments on $I_{\text{Na}P}$ and NMDAR interactions are needed. Furthermore, the activation of $I_{\text{Na}P}$ in dINs at membrane potentials below their firing threshold may facilitate their rebound firing following inhibition. Indeed, our results show riluzole has decreased dIN rebound firing reliability (Fig. 3, A1 and A2). However, the firing reliability of rhythmic neurons during swimming is not affected by 1 μM riluzole. Firing of dINs as a cell group during swimming is very robust due to the extensive electrical coupling among them (Li et al. 2009). Reliable dIN firing can in turn sustain non-dIN firing on each swimming cycle (Soffe et al. 2009). Because the brain stem dINs play critical roles in the maintenance of tadpole swimming, the shortening of swimming may be a result of reduced activity in a small number of dINs, which can alter swimming activity (Moul et al. 2013) but missed in our recordings. Another confounding factor is that whole cell recordings can alter the cytoplasmic ionic composition and lead to rundown of certain ion channel currents, which may cause mismatching changes in neuronal spiking in whole cell recordings and network activities such as swimming. We also cannot exclude the possibility that swimming is shortened due to the presence of some subtle, unobserved effects from riluzole application.

Tadpole struggling rhythms involve high-frequency repetitive firing of spinal and hindbrain neurons (non-dINs), whereas dINs appear to play a minor role because their firing is weak (Li 2015; Li et al. 2007a). $I_{\text{Na}P}$ has been shown previously to promote repetitive firing in rat ventral horn neurons (Theiss et al. 2007a). Riluzole has similar effect on non-dIN repetitive firing evoked either by current injection (Fig. 3C) or during struggling (Fig. 4, C and E). Therefore, the depression of non-dIN repetitive firing by riluzole should affect the generation of struggling rhythms. Meanwhile, RB neurons need to be activated repetitively to evoke struggling rhythms (during repetitive skin stimulation), and they can be another target for riluzole. Riluzole reduced the number of spikes CPG neurons fire on each struggling cycle. This may result indirectly from weakened RB outputs to the CPG circuit and directly from suppression of CPG excitability by riluzole. However, the frequency of struggling rhythms was not affected by riluzole at 1 μM (Fig. 4F), although we still do not fully understand what controls struggling frequencies. The lack of effect could be due to the limited block of $I_{\text{Na}P}$ by riluzole at 1 μM. Increasing riluzole concentration will affect chemical synaptic transmission (Bellingham 2011; Mohammadi et al. 2001), calcium (Huang et al. 1997), potassium (Cao et al. 2002), and transient sodium channels (Kuo et al. 2006) and make the results very difficult to interpret. Therefore, we do not know if swimming or struggling rhythms could persist through a full blockade of $I_{\text{Na}P}$ unless a more specific way to block $I_{\text{Na}P}$ becomes available.

In this study, we have revealed the wide expression of $I_{\text{Na}P}$ in all groups of tadpole spinal neurons. Although we have reported some effects of 1 μM riluzole on tadpole swimming and struggling behavior, it is difficult to evaluate how $I_{\text{Na}P}$ shapes the swimming and struggling outputs because of the lack of specificity of riluzole blockade of $I_{\text{Na}P}$ and its indiscriminate targeting of all neuronal types with $I_{\text{Na}P}$ expression. To genetically modify $I_{\text{Na}P}$ currents targeting specific types of neurons in the motor rhythm generation circuits may provide more definitive insights into the $I_{\text{Na}P}$ modulation of motor outputs (Brocard et al. 2016).

**ACKNOWLEDGMENTS**

We thank Drs. Steve Soffe and Alan Roberts for helpful discussions. Present address for E. Svensson: Department of Neuroscience, Functional Pharmacology, Uppsala University, 751 24 Uppsala, Sweden.

**GRANTS**

This work was financially supported by Biotechnology and Biological Sciences Research Council Grant BB/LO011X/1.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

E.S., H.J., and W.-C.L. performed experiments; E.S., H.J., and W.-C.L. analyzed data; E.S., H.J., and W.-C.L. interpreted results of experiments; E.S., H.J., and W.-C.L. prepared figures; E.S. drafted manuscript; E.S. and W.-C.L. edited and revised manuscript; E.S., H.J., and W.-C.L. approved final version of manuscript.

**REFERENCES**

Astman N, Gutnick MJ, Fleidervish IA. Persistent sodium current in layer 5 neocortical neurons is primarily generated in the proximal axon. *J Neurosci* 26: 3465–3473, 2006. doi: 10.1523/JNEUROSCI.4907-05.2006.

Bean BP. The action potential in mammalian central neurons. *Nat Rev Neurosci* 8: 451–465, 2007. doi: 10.1038/nrn2148.

Bellingham MC. A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis: what have we learned in the last decade? *CNS Neurosci Ther* 17: 4–31, 2011. doi: 10.1111/j.1755-5949.2009.00116.x.

Bellingham MC. Pre- and postsynaptic mechanisms underlying inhibition of hypoglossal motor neuron excitability by riluzole. *J Neurophysiol* 110: 1047–1061, 2013. doi: 10.1152/jn.00587.2012.

Benedetti L, Giliardi A, Rottoli E, De Maglie M, Prosperi L, Pereg O, Baruscelli M, Bucci A, Del Giacco L, Francolini M. $I_{\text{Na}P}$ selective inhibition reverts precocious inter- and motorneurons hyperexcitability in the Sod1-G93R zebrafish ALS model. *Sci Rep* 6: 24515, 2016. doi: 10.1038/srep24515.
Grillner S, El Manira A. Devlin AC, Burr K, Borooah S, Foster JD, Cleary EM, Geti I, Vallier L, Persistent sodium current in mammalian central neurons. J Physiol 574: 819–834, 2006. doi:10.1113/jphysiol.2006.107094.

Lee RH, Beckman CJ. Influence of voltage-sensitive dextrin conductances on bistable firing and effective synaptic current in cat spinal motoneurons in vivo. J Neurophysiol 76: 2107–2110, 1996.

Lee RH, Beckman CJ. Adjustable amplification of synaptic input in the dendrites of spinal motoneurones in vivo. J Neurosci 20: 6734–6740, 2000.

Li WC. Generation of locomotion rhythms without inhibition in vertebrates: the search for pacemaker neurones. Int. Comp. Biol. 51: 879–889, 2011. doi:10.1093/icb/icr021.

Li WC. Selective gating of neuronal activity by intrinsic properties in distinct motor rhythms. J Neurosci 35: 9799–9810, 2015. doi:10.1523/JNEUROSCI.0323-15.2015.

Li WC, Cooke T, Sautois B, Soffe SR, Borisuyk R, Roberts A. Axon and dendrite geometry predict the specificity of synaptic connections in a functioning spinal cord network. Neuronal Dev 2: 17, 2007a. doi:10.1186/1749-8104-2-17.

Li WC, Moul PR. The control of locomotor frequency by excitation and inhibition. J Neurosci 32: 6220–6230, 2012. doi:10.1523/JNEUROSCI.2689-2011.

Li WC, Roberts A, Soffe SR. Locomotor rhythm maintenance: electrical coupling among premotor excitatory interneurones in the brainstem and spinal cord of young Xenopus tadpoles. J Physiol 587: 1677–1693, 2009. doi:10.1113/jphysiol.2008.166942.

Li WC, Roberts A, Soffe SR. Specific brainstem neurones switch each other into pacemaker mode to drive movement by activating NMDA receptors. J Neurosci 30: 16609–16620, 2010. doi:10.1523/JNEUROSCI.3695-10.2010.

Li WC, Sautois B, Roberts A, Soffe SR. Reconfiguration of a vertebrate motor network: specific neuron recruitment and context-dependent synaptic plasticity. J Physiol 27: 12267–12276, 2007b. doi:10.1523/JNEUROSCI.3694-07.2007.

Li WC, Soffe SR, Roberts A. Dorsal spinal interneurons forming a primitive, cutaneous sensory pathway. J Neurophysiol 92: 895–904, 2004. doi:10.1152/jn.00024.2004.

Li WC, Soffe SR, Wolf E, Roberts A. Persistent responses to brief stimuli: feedback excitation among brainstem neurones. J Neurosci 26: 4026–4035, 2006. doi:10.1523/JNEUROSCI.4727-05.2006.

Miles GB, Dai Y, Brownstone RM. Mechanisms underlying the early phase of spike frequency adaptation in mouse spinal motoneurones. J Physiol 566: 519–532, 2005. doi:10.1113/jphysiol.2005.086033.

Mohammadi B, Krampf K, Moschref H, Dengler R, Bürker J. Interaction of the neuroprotective drug riluzole with GABAA and glycine receptor channels. Eur J Pharmacol 415: 135–140, 2001. doi:10.1016/S0014-2999(01)00847-0.

Moul PR, Cotrell GA, Li WC. Fast silencing reveals a lost role for reciprocal inhibition in locomotion. Neuron 77: 129–140, 2013. doi:10.1016/j.neuron.2012.10.040.

Nieuwkoop PD, Faber J. Normal Tables of Xenopus laevis (Daudin). Amsterdam: North Holland, 1956.

Osorio N, Catalá H, Meisler MH, Crest M, Magistretti J, Delmas P. Persistent Nav1.6 current at axon initial segments tunes spike timing of cerebellar granule cells. J. Physiol 588: 651–670, 2010. doi:10.1113/jphysiol.2010.183798.

Pace RW, Mackay DD, Feldman JL, Del Negro CA. Role of persistent sodium current in mouse preBötzinger complex neurones and respiratory rhythm generation. J Physiol 580: 485–496, 2007. doi:10.1113/jphysiol.2006.124602.

Qiu Y, Bieger EA, Schuster JE, Fu R, Siddique T, Beckman CJ. Altered postnatal maturation of electrical properties in spinal neurones in a mouse model of amyotrophic lateral sclerosis. J Physiol 589: 2245–2260, 2011. doi:10.1113/jphysiol.2010.200659.

J Neurophysiol • doi:10.1152/jn.00755.2016 • www.jn.org
Roberts A, Li WC, Soffe SR. How neurons generate behavior in a hatchling amphibian tadpole: an outline. *Front Behav Neurosci* 4: 16, 2010. doi:10.3389/fnbeh.2010.00016.

Roberts A, Li WC, Soffe SR. A functional scaffold of CNS neurons for the vertebrates: the developing *Xenopus laevis* spinal cord. *Dev Neurobiol* 72: 575–584, 2012. doi:10.1002/dneu.20889.

Roberts A, Li WC, Soffe SR, Wolf E. Origin of excitatory drive to a spinal locomotor network. *Brain Res Brain Res Rev* 57: 22–28, 2008. doi:10.1016/j.brainresrev.2007.06.015.

Sautois B, Soffe SR, Li WC, Roberts A. Role of type-specific neuron properties in a spinal cord motor network. *J Comput Neurosci* 23: 59–77, 2007. doi:10.1007/s10827-006-0019-1.

Sillar KT, Roberts A. A neuronal mechanism for sensory gating during locomotion in a vertebrate. *Nature* 331: 262–265, 1988. doi:10.1038/331262a0.

Soffe SR, Roberts A, Li WC. Defining the excitatory neurons that drive the locomotor rhythm in a simple vertebrate: insights into the origin of reticulospinal control. *J Physiol* 587: 4829–4844, 2009. doi:10.1113/jphysiol.2009.175208.

Tazerart S, Viemari JC, Darbon P, Vinay L, Brocard F. Contribution of persistent sodium current to locomotor pattern generation in neonatal rats. *J Neurophysiol* 98: 613–628, 2007. doi:10.1152/jn.00316.2007.

Tazerart S, Vinay L, Brocard F. The persistent sodium current generates pacemaker activities in the central pattern generator for locomotion and regulates the locomotor rhythm. *J Neurosci* 28: 8577–8589, 2008. doi:10.1523/JNEUROSCI.1437-08.2008.

Theiss RD, Kuo JJ, Heckman CJ. Persistent inward currents in rat ventral horn neurones. *J Physiol* 580: 507–522, 2007a. 10.1113/jphysiol.2006.124123.

Tong H, McDarmid JR. Pacemaker and plateau potentials shape output of a developing locomotor network. *Curr Biol* 22: 2285–2293, 2012. doi:10.1016/j.cub.2012.10.025.

Urbani A, Belluzzi O. Riluzole inhibits the persistent sodium current in mammalian CNS neurons. *Eur J Neurosci* 12: 3567–3574, 2000. doi:10.1046/j.1460-9568.2000.00242.x.

Waxman SG, Zamponi GW. Regulating excitability of peripheral afferents: emerging ion channel targets. *Nat Neurosci* 17: 153–163, 2014. doi:10.1038/nn.3602.

Winlove CI, Roberts A. The firing patterns of spinal neurons: in situ patch-clamp recordings reveal a key role for potassium currents. *Eur J Neurosci* 36: 2926–2940, 2012. doi:10.1111/j.1460-9568.2012.08208.x.

Zhong G, Masino MA, Harris-Warrick RM. Persistent sodium currents participate in fictive locomotion generation in neonatal mouse spinal cord. *J Neurosci* 27: 4507–4518, 2007. doi:10.1523/JNEUROSCI.0124-07.2007.

Ziskind-Conhaim L, Wu L, Wiesner EP. Persistent sodium current contributes to induced voltage oscillations in locomotor-related h9 interneurons in the mouse spinal cord. *J Neurophysiol* 100: 2254–2264, 2008. doi:10.1152/jn.90437.2008.

*J Neurophysiol* • doi:10.1152/jn.00755.2016 • www.jn.org