Transience BK outward current enhances motoneurone firing rates during *Drosophila* larval locomotion

Dimitrios Kadas, Stefanie Ryglewski and Carsten Duch

Institute of Neurobiology, Johannes Gutenberg University of Mainz, Mainz, Germany

**Key points**
- We combine *in situ* electrophysiology with genetic manipulation in *Drosophila* larvae aiming to investigate the role of fast calcium-activated potassium currents for motoneurone firing patterns during locomotion.
- We first demonstrate that *slowpoke* channels underlie fast calcium-activated potassium currents in these motoneurones.
- By conducting recordings in semi-intact animals that produce crawling-like movements, we show that *slowpoke* channels are required specifically in motoneurones for maximum firing rates during locomotion.
- Such enhancement of maximum firing rates occurs because *slowpoke* channels prevent depolarization block by limiting the amplitude of motoneurone depolarization in response to synaptic drive. In addition, *slowpoke* channels mediate a fast afterhyperpolarization that ensures the efficient recovery of sodium channels from inactivation during high frequency firing.
- The results of the present study provide new insights into the mechanisms by which outward conductances facilitate neuronal excitability and also provide direct confirmation of the functional relevance of precisely regulated *slowpoke* channel properties in motor control.

**Abstract** A large number of voltage-gated ion channels, their interactions with accessory subunits, and their post-transcriptional modifications generate an immense functional diversity of neurones. Therefore, a key challenge is to understand the genetic basis and precise function of specific ionic conductances for neuronal firing properties in the context of behaviour. The present study identifies *slowpoke* (*slo*) as exclusively mediating fast activating, fast inactivating BK current (*I\(_{CF}\*) in larval *Drosophila* crawling motoneurones. Combining *in vivo* patch clamp recordings during larval crawling with pharmacology and targeted genetic manipulations reveals that *I\(_{CF}\*) acts specifically in motoneurones to sculpt their firing patterns in response to a given input from the central pattern generating (CPG) networks. First, *I\(_{CF}\*) curtails motoneurone postsynaptic depolarizations during rhythmical CPG drive. Second, *I\(_{CF}\*) is activated during the rising phase of the action potential and mediates a fast afterhyperpolarization. Consequently, *I\(_{CF}\*) is required for maximal intraburst firing rates during locomotion, probably by allowing recovery from inactivation of fast sodium channels and decreased potassium channel activation. This contrasts the common view that outward conductances oppose excitability but is in accordance with reports on transient BK and Kv3 channel function in multiple types of vertebrate neurones. Therefore, our finding that *I\(_{CF}\*) enhances firing rates specifically during bursting patterns relevant to behaviour is probably of relevance to all brains.

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**Corresponding author** C. Duch: Institute of Neurobiology, Johannes Gutenberg University of Mainz, Col.- Kleinmann Weg 2, 55099 Mainz, Germany. Email: cduch@uni-mainz.de

**Abbreviations** AHP, afterhyperpolarization; CPG, central pattern generating network; CS, Canton-S; CTX, charybdotoxin; eag, *ether-a-go-go*; GFP, green fluorescent protein; MN, motoneurone; RNAi, RNA interference; SK, small conductance Ca\(^{2+}\) activated K\(^{+}\) channel; slo, *slowpoke*. DOI: 10.1113/JP271323
Introduction

The generation of rhythmic motor patterns, such as breathing, walking and flying, relies upon activity in central pattern generating (CPG) networks (Kiehn & Kullander, 2004; Grillner et al. 2005; Marder et al. 2005). Although CPGs play critical roles in generating the timing of patterned motoneurone (MN) firing output, complex complements of ionic mechanisms exist in MNs that shape synaptic input from the CPG far beyond simple threshold summation (Kiehn, 1991; Kiehn et al. 2000; Heckmann et al. 2003). Therefore, knowing how MN ionic currents sculpt motor output is fundamental to understanding the control of movement in all animals.

The present study combines in situ patch clamp physiology with genetic manipulation in Drosophila to pinpoint the function of Ca$^{2+}$ activated K$^+$ currents in identified larval MNs. In central neurones, the proposed roles of these currents range from spike (Sah & Faber, 2002; Faber & Sah, 2003) and plateau potential termination (Lovell & McCobb, 2001) to the regulation of EPSP amplitude in dendrites (Iansek & Redman, 1973). Drosophila MNs display both transient ($I_{CF}$) and sustained ($I_{CS}$) Ca$^{2+}$ activated K$^+$ currents (Srinivasan et al. 2012; Ryglewski & Duch, 2009; Lee et al. 2008). In a three-step process, we specifically address the role of transient Ca$^{2+}$ activated K$^+$ current ($I_{CF}$) in the somatodendritic domain of larval crawling MNs. First, we identify the gene underlying $I_{CF}$ by genetic manipulation. Second, we determine the role of $I_{CF}$ for MN intrinsic excitability by comparing firing responses, action potential shape, and after-hyperpolarizations of mutant and control MNs. Third, the role of $I_{CF}$ for sculpting MN firing patterns during behaviour is addressed by patch clamp recordings during crawling-like motor patterns.

The Drosophila genes that could potentially encode $I_{CF}$ are the BK channel slowpoke ($slo$), SK (small conductance Ca$^{2+}$ activated K$^+$ channel) and eag (ether-à-go-go). In muscle, slo underlies $I_{CS}$ (Singh & Wu, 1989; Elkins & Ganetzky, 1986; 1998; Salkoff & Wyman, 1981). Sequence data predict that SK encodes a small conductance $I_{CS}$, although functional data are not available. $Eag$ (Kv11 in vertebrates) mutations affect Ca$^{2+}$ activated and Ca$^{2+}$ independent K$^+$ currents in Drosophila larval muscle (Zhong & Wu, 1991) and in larval MNs (Srinivasan et al. 2012), although no specific K$^+$ current is eliminated in eag null mutants.

Our data show that slo underlies all $I_{CF}$ in larval crawling MNs but plays no role for $I_{CS}$. Although mediating an outward current, slo channels increase MN excitability. Indeed, slo is required in MNs for maximum intraburst firing rates during locomotion. Quantitative analysis of firing rates, synaptic drive potentials, spike shape and after-hyperpolarization (AHP) reveal two specific functions of $I_{CF}$ in Drosophila MNs. First, $I_{CF}$ curtails synaptic drive potential amplitude in MNs, thus avoiding depolarization block during bursting. Second, by mediating a fast AHP, $I_{CF}$ ensures sufficient sodium channel de-inactivation at high intraburst firing rates. Therefore, Drosophila MN slo (BK) channel properties are tuned to support maximal MN firing responses to synaptic drive from the CPG during locomotor behaviour.

Methods

Animals

Drosophila melanogaster were reared in standard plastic vials with foam stoppers on a yeast–cornmeal–syrup–agar diet at 25°C under a 12:12 h light/dark photocycle. Wandering third-instar larvae of both sexes were used for all of the experiments. Canton-S (CS) larvae were used as wild-type controls. Two slo mutants were used to study BK channel function, slo1 (#4587; Bloomington Stock Center, Bloomington, IN, USA) and slo2 (kind gift from Dr N. S. Atkinson, The University of Texas at Austin, Austin, TX, USA). slo1 carries a gamma ray-induced inversion mutation between 96A17 and 96F5-8 on the third chromosome, resulting in loss of function. Northern blot analysis showed that two transcripts of 5.8 kb and 11 kb, which are detected by slo probes in CS, are missing in slo1 (Atkinson et al. 1991). slo1 is not molecularly characterized, although it is proposed to cause a null allele because the Ca$^{2+}$-activated fast K$^+$ current ($I_{CF}$) is completely eliminated in larval muscle fibres (Singh & Wu, 1990).

To restrict slo-RNAi (stocks 6723 and 104421; VDRC, Vienna, Austria) knockdown to two identified MNs per ventral nerve cord hemisegment, expression was driven under the control of the even-skipped promoter (RN2-GAL4; Fujioka et al. 2003). UAS-GFP was included for identification of the MNs (RN2-GAL4, UAS-CD8-GFP). One of the two MNs targeted by RN2-GAL4 expression innervates muscle 1 with big type I terminal boutons and is known as MN1-Ib. The second MN innervates multiple dorsal muscles via the intersegmental nerve, has small type I terminal boutons, and is known as MNISN-Is (Hoang & Chiba, 2001). MN1-Ib and MNISN-Is are also named after their embryonic identities as aCC and RP2, respectively (Fujioka et al. 2003). After expression of green fluorescent protein (GFP), aCC and RP2 can readily be distinguished by their distinct morphologies (Choi et al. 2004). In all RNA interference (RNAi) experiments, inclusion of extra Dicer-2 (UAS-Drc2; #24650; Bloomington Stock Center; Dietzl et al. 2007) was used to increase knockdown strength as reported previously (Ryglewski et al. 2012; Hutchinson et al. 2014). However, RN2 is a relatively weak driver during the third instar. Therefore, we employed a Flippase strategy to express the UAS-RNAi constructs in a mosaic fashion.
under the control of the particularly strong actin-GAL4 promoter. In this scheme, the Flippase removes a stop cassette to activate the strong driver, actin-GAL4. If the weak eye promoter (RN2 in this example) is used to drive the Flippase, the strong actin promoter is activated in only a small subset of eye expressing neurons, thus causing GFP and RNAi expression in some aCC and RP2 MNs (Hartwig et al. 2008; line courtesy of Dr S. Sanyal, BIOGEN-Idec, Cambridge, MA, USA). For all RNAi experiments, we crossed homozygous female w; RN2-GAL4, UAS-CDB-GFP; Act-FRT-stop-FRT-GAL4, UAS-FLP flies with male w; UAS-slo-RNAi; UAS-Drc2 flies. Because this scheme creates genetic mosaics, non-GFP labelled aCC MNs were used as internal controls to evaluate RNAi knockdown efficacy. In these experiments, as well as in recordings from homozygous slow mutant animals, the identity of non-GFP labelled aCC neurones was confirmed by intracellular dye labelling with rhodamine dextran (D3308; Molecular Probes, Carlsbad, CA, USA) via the patch pipette.

**Larval preparation**

Third-instar larvae were dissected in normal saline (composition in mM: 128 NaCl, 2 KCl, 1.8 CaCl₂, 4 MgCl₂, 5 Hepes and ~35 sucrose depending on the osmolality of the solution; pH was adjusted to 7.25 with 1 M NaOH). Larvae were pinned dorsal side up in silicone elastomere (Sylgard, Dow Corning, Wiesbaden, Germany) lined Petri dishes with minute pins through the mouthhooks and the tail. Animals were dissected along the dorsal mid-line, and the dorsal cuticle and muscles were stretched laterally and pinned down with two minute pins on each side. After removal of the gut and oesophagus, the ventral nerve cord was exposed, mounted onto an upright fixed stage Axioskop 2 FS plus fluorescence microscope (Carl Zeiss, Oberkochen, Germany), and viewed with a 40× water immersion objective. When constantly perfused with saline, ~20% of the larval preparations spontaneously produced crawling-like motor patterns as characterized by peristaltic waves of rhythmic contractions that propagate from posterior to anterior (Fox et al. 2006). Experiments comply with the policies and regulations on animal experimentation (Drummond, 2009).

**Electrophysiology**

To facilitate access to aCC MNs with the patch pipette, the ganglionic sheath was focally removed with a large patch pipette (tip resistance 1 MΩ) filled with 1% protease in normal saline. Patch pipettes were pulled from borosilicate glass (outer diameter 1.5 mm, inner diameter 1.0 mm without filament; World Precision Instruments, Sarasota, FL, USA) with a vertical pipette puller (PC-10; Narishige International Ltd, London, UK). After protease treatment, the preparation was rinsed with 5 ml of normal saline for 2 min before aCC was approached with a second patch pipette (tip resistance 6–8 MΩ) for whole cell patch clamp recordings. Recording electrodes contained internal solution of composition (in mM): 140 potassium gluconate, 2 MgCl₂, 2 Mg-ATP, 10 Hepes, 11 EGTA and glucose to adjust osmolality to 300 mOsm kg⁻¹ (pH 7.25).

All recordings were conducted under constant saline flow (~2 ml/min) at 24°C with an Axopatch 200B amplifier (Molecular Devices). Data were digitized with a Digidata 1322A (Molecular Devices) at a sampling rate of 10 kHz. Before approaching the cell, the offset potential was nulled manually. Upon obtaining a gigahm seal, fast capacitive artefacts were compensated manually in patch mode at a holding potential of −70 mV. Whole cell configuration was achieved with gentle negative pressure. Recordings were carried out with serial resistances between 10 and 15 MΩ. Whole cell capacitance was determined and compensated using the Axopatch 200B C-slow dial. Voltage errors caused by series resistance were predicted at 90%, and compensated for by 40–50% at a time constant of 2 μs. In voltage clamp mode, input resistance was calculated from the linear slope of the I–V relationship at subthreshold command potentials and subtracted offline. In current clamp, input resistance was calculated identically but not corrected.

We restricted our recordings to aCC MNs from the abdominal segments 2–4 because differences in aCC input resistance and firing properties have been reported between thoracic and abdominal segments and suggested for the most posterior abdominal segments 7 and 8 (Srinivasan et al. 2012). We did not observe differences in input resistance or firing responses of aCC between abdominal segments 2–4. Total outward currents were recorded in TTX (10⁻⁶ M) containing saline to block transient Na⁺ currents. K⁺ outward currents were induced by voltage steps from −90 to +20 mV in 10 mV increments from a holding potential of −90 mV. Transient currents were inactivated by pre-pulses of 200 ms duration to −20 mV immediately followed by command potentials from −90 to +20 mV in 10 mV increments. Ca²⁺-dependent K⁺ currents were blocked by bath application of 300 μM cadmium. Voltage-dependent transient K⁺ current (Ih) was isolated by offline subtraction of non-inactivating from total currents. Voltage-dependent delayed rectifier-like current was isolated by combining bath application of cadmium and aforementioned pre-pulse protocol. I_CSF and I_CSF were isolated by offline subtraction (see Results).

Because charybdotoxin (CTX; Alomone Labs, Jerusalem, Israel) is reportedly a highly specific BK channel blocker in adult (Elkins et al. 1986) and larval muscle (Singh, Wu, 1990), as well as in larval neurones (Pym et al. 2006), we intended to isolate BK current...
pharmacologically. Subtraction of outward current after bath application of CTX (200 nM) from total outward current revealed CTX-sensitive current. Intrinsic excitability of MNs was determined in current clamp mode by somatic square pulse and somatic ramp current injections. At a resting membrane potential of −60 mV, square pulse currents of 400 ms duration were applied in 10 pA increments from −40 to +100 pA. Ramp current injections were applied from −40 to 220 pA (final values at the end of each ramp) in 20 mV increments with linearly rising current amplitudes throughout the 400 ms duration of each ramp. MN firing patterns during crawling-like movements were recorded in current clamp in gap-free acquisition mode. Larval movements were monitored visually. Larval locomotion was not induced. Therefore all recordings shown are spontaneously occurring motor patterns.

**Burst parameters measured during crawling-like locomotor patterns**

Burst duration was defined as the time from the first to the last action potential in a burst. Mean intraburst firing frequency was defined as number of action potentials in a burst divided by burst duration. Maximum intraburst frequency was measured as 1 divided by shortest interval between two subsequent action potentials in a burst. Bursts occurred on top of large excitatory drive potentials (Figs. 4–6). Drive potential amplitude was measured as the difference between the mean membrane potential during the last 50 ms preceding a burst and the maximum subthreshold depolarization during that burst. Half-amplitude drive potential duration was measured as the time between the depolarizing and the hyperpolarizing phases at half-amplitude.

**Results**

**Slowpoke underlies transient Ca \(^{2+}\)-dependent K\(^+\) current**

To unravel the genetic basis of transient Ca\(^{2+}\)-activated K\(^+\) outward current (\(I_{\text{CF}}\)) in larval *Drosophila* MNs, we employed *in situ* voltage clamp recordings from identified MNs in controls and in mutant animals. We chose aCC because numerous electrophysiological studies have described K\(^+\) outward currents in this motoneuron (Choi et al. 2004, Srinivasan et al. 2012; Schaefer et al. 2010). \(I_{\text{CF}}\) was not eliminated in *eag\(^{29}\)* null mutants (not shown), nor after SK RNAi knockdown in aCC (not shown). Therefore, we next tested *slo\(^4\)* and *slo\(^2\)*mutants.

Voltage clamp recordings from the somata of aCC in TTX (10\(^{-6}\) M) containing saline from representative wild-type controls (Fig. 1A–J, left) and *slo\(^4\)* mutant (Fig. 1K–T, right) animals revealed prominent voltage and Ca\(^{2+}\)-dependent outward K\(^+\) currents upon voltage steps from −90 mV to +20 mV in 10 mV increments. Ca\(^{2+}\) inward current amplitudes as recorded from the aCC soma are negligible as long as K\(^+\) outward currents are not blocked pharmacologically (Worrell & Levine, 2008). Total transient K\(^+\) current amplitudes (measured as the peak current between 2 and 10 ms after voltage step onset) were slightly larger in CS controls than in *slo* mutant MNs (Fig. 1A, K and U). Voltage-dependent K\(^+\) currents were isolated by bath application of cadmium (1B control, L *slo\(^4\)* and V). Ca\(^{2+}\)-dependent K\(^+\) current was obtained by subtraction of voltage-dependent from total outward current (1C control and M *slo\(^4\)*). Note that the transient component, \(I_{\text{CF}}\), was missing in *slo\(^4\)* mutants (Fig. 1C and M, red arrows), although *slo\(^4\)* mutant MNs clearly displayed sustained Ca\(^{2+}\)-dependent K\(^+\) current (\(I_{\text{CS}}\)) (Fig. 1M). All transient current was inactivated by pre-pulses to −20 mV, leaving sustained K\(^+\) current (1D control and N *slo\(^4\)*). Total transient K\(^+\) current was then isolated by subtraction of sustained from total current (1E control and O *slo\(^4\)*). In controls, maximal transient K\(^+\) current was significantly larger compared to *slo\(^4\)* mutants (Fig. 1U). Voltage-dependent transient K\(^+\) current (1F, H control, P, R *slo\(^4\)*) was obtained by subtraction of sustained outward current from total outward current, both measured in 300 \(\mu\)M cadmium. Voltage-dependent transient K\(^+\) current amplitudes were not statistically different in controls and *slo\(^4\)* mutants (Fig. 1F, P and V). Finally, \(I_{\text{CF}}\) was obtained by subtraction of voltage-dependent transient from total transient current (Fig. 1G, I control, Q and S *slo\(^4\)*). This left a fast activating, fast inactivating BK-like current in controls (\(I_{\text{CF}}\)) that was completely eliminated in *slo\(^4\)*mutants (Fig. 1Q, S and W). Therefore, *slo* specifically mediates \(I_{\text{CF}}\) in larval aCC MNs.

CTX-sensitive current as obtained by subtraction of currents before and after bath application of CTX (200 nM) displayed a transient component qualitatively similar to \(I_{\text{CF}}\) (Fig. 11 and J). However, in wild-type controls CTX-sensitive current also displayed a small sustained component (Fig. 1J), indicating some unspecific action. Accordingly, a small CTX-sensitive current was also observed in *slo\(^4\)* mutants (Fig. 1T). Therefore, CTX blocks \(I_{\text{CF}}\) as reported previously for larval (Singh & Wu, 1990) and adult muscle (Elkins et al. 1986), as well as embryonic MNs (Pym et al. 2006), although at least at 200 nM CTX also exerts some non-specific blockade of additional outward currents. Consistent with CTX blocking \(I_{\text{CF}}\) and an additional transient current component, transient CTX-sensitive current is larger than \(I_{\text{CF}}\) in wild-type controls (Fig. 1W).

We did not attempt to quantify time constants of current activation and inactivation because *in vivo* voltage clamp recordings from these *Drosophila* MNs suffer
from space clamp limitations (total somatodendritic length > 2500 μm) that affect the setting time of the measurements. Because space clamp generally improves with the number of channels blocked pharmacologically, current activation in distal compartments may also differ between subsequent measurements from the same cell under different pharmacological conditions. Although our quantification of the different transient K\(^+\) current amplitudes contained some inevitable inaccuracies as a result of neurone size and in vivo analysis, the absence of transient Ca\(^{2+}\)-dependent K\(^+\) current in mutants demonstrated that slo underlies I\(_{CF}\).

Figure 1. Loss of slo selectively eliminates transient Ca\(^{2+}\)-dependent K\(^+\) current in larval MNs
Voltage clamp recordings of K\(^+\) outward currents from aCC MNs in a representative control (CS, left) and a representative slo null mutant (slo\(^{−}\), right) animal. A–G, stepwise isolation of transient voltage and Ca\(^{2+}\)-dependent
Slowpoke does not contribute to $I_{CS}$ in larval MNs

To test rigorously whether $slo$ also contributed to sustained outward currents, aCC MN $K^+$ currents were recorded in TTX containing saline by command voltage steps from $-90$ to $+20$ mV from a holding potential of $-20$ mV to inactivate all transient current (Fig. 2). Representative recordings from aCC in CS controls (Fig. 2A–C) and in $slo^4$ mutants revealed no differences in total sustained current (Fig. 2A control and $D$ slo$^4$), voltage-dependent sustained current isolated by bath application of 300 $\mu$M CdCl$_2$ (Fig. 2B control and $E$ slo$^4$) or in $I_{CS}$ (Fig. 2C control and $F$ slo$^4$) as obtained by subtraction of voltage-dependent current from total current. Quantification in $I/V$ plots revealed no statistically significant differences in total (Fig. 2G), voltage-dependent (Fig. 2H) or Ca$^{2+}$-dependent (Fig. 2I) sustained $K^+$ currents between aCC recorded in CS or in slo$^4$ animals. Therefore, $slo$ underlies specifically $I_{CS}$ but does not contribute to $I_{CS}$ or any other $K^+$ current in aCC.

$slo$-mediated $I_{CF}$ increases MN excitability

To test whether $slo$-mediated $I_{CF}$ affected MN excitability, we next measured the firing responses of aCC to somatic current injections in controls and in slo mutants in current clamp mode. Square pulse current injection (see methods) were delivered to aCC in CS (Fig. 3A, $n = 12$), slo$^4$ (Fig. 3B, $n = 8$) and slo$^4$ (Fig. 3C, $n = 8$) larvae. For clarity, only selected sweeps are shown in the representative recordings for each genotype (Fig. 3A–C). In controls and in both slo mutants, aCC responded with tonic firing to somatic square pulse current injections and showed no obvious frequency adaptation at any given pulse amplitude. Quantification using current–firing response plots demonstrated that MN responses to square pulse somatic current injections were not significantly affected in slo mutants (Fig. 3D). Data were found to fit well with a shifted Boltzmann function (correlation coefficient$>0.9$ for each data set). No statistically significant differences were observed between any of the three groups (CS, slo$^1$, slo$^4$). Therefore, at least for square pulse current injections into the soma of aCC, $I_{CF}$ had no effect on the firing responses.

To provide currents physically more equivalent to the gradual depolarizations probably occurring in response to synaptic input from the CPG network, we repeated these experiments with ramp current injections (see Methods). Representative recordings of wild-type controls (CS) (Fig. 3E, $n = 12$), as well as both slo mutants (Fig. 3F, slo$^1$, $n = 8$; Fig. 3G, slo$^4$, $n = 8$) showed that aCC MNs responded with increasing firing rates through the duration of each ramp. Firing threshold was at $-28 \pm 4$ mV in controls, and no statistically significant difference was observed in either slo mutant (slo$^1$, $-29 \pm 4$ mV; slo$^4$, $-30 \pm 5$ mV). By contrast, increases in firing frequency throughout the rising ramps appeared smaller in both slo mutants compared to the control. Quantification (Fig. 3H) demonstrated that slo mutants showed decreased firing rates for all current injection amplitudes resulting in firing above 30 Hz. The more depolarized the membrane potential and the higher the firing rate, the larger its reduction in the absence of slo (Fig. 3H). The underlying membrane voltages in response to larger amplitude current injections were not significantly different between slo mutants (slo$^4$, maximum $4.625 \pm 8.9$ mV at $180 \mu$A; slo$^4$, maximum $5.0 \pm 6.89$ mV at $180 \mu$A current injection amplitude) and CS (maximum $6.625 \pm 5.9$ mV at $180 \mu$A current injection amplitude). These data indicate that $I_{CF}$ was required for...
maximum MN firing rates in response to ramp current injections.

**The role of $I_{CF}$ on MN activity patterns during larval locomotion**

We next tested whether the role of $I_{CF}$ for increasing intrinsic MN excitability was reflected in MN activity patterns during larval locomotion (i.e. crawling motor behaviour). Forward crawling of *Drosophila* larvae comprises rhythmic waves of contractions that occur in a posterior to anterior peristaltic manner (Fox et al. 2006). Larvae often spontaneously produced crawling-like motor patterns with peristaltic intersegmental motor co-ordination when opened dorsally to allow access of the CNS with recording electrodes (Fig. 4A). In this preparation, bouts of co-ordinated crawling-like motor output can be monitored by extracellular recordings from the segmental nerve roots, each of which carries $\sim 30$ motor axons. For example, simultaneous extracellular recordings from the nerve roots in segments 6 and 4 revealed rhythmic bursts of MN activity with similar cycle periods in both segments (i.e. cycle period is a measure of the duration between two subsequent waves of peristaltic contractions as measured from a given segment), with a phase delay of segment 4 in relation to segment 6 of $\sim 15\%$ (Fig. 4B, see dashed lines). Thus, crawling-like motor patterns can be recorded from semi-intact larval preparations that allow for patch clamping onto identified MNs.

Representative somatic patch clamp recordings during bouts of crawling-like motor patterns (Fig. 4C–E) indicated shorter cycle periods in $slo$ mutant animals.
Quantification showed that the cycle period was significantly decreased in both slo¹ and slo⁴ mutants compared to wild-type controls (Fig. 4F). Because aCC burst duration was not altered (see below), the shorter cycle period in slo mutants resulted in significant increases in duty cycle compared to controls (Fig. 4G).

However, to unravel the specific function of slo for MN activity patterns, we measured burst duration, spikes

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**Figure 3. slo-mediated transient outward current is required for maximum MN firing rates**

A–C, representative sweeps of aCC firing responses to square pulse somatic current injections from −40 pA to 90 pA in 10 pA increments as recorded in a wild-type control (A, CS) and in two slowpoke mutants (B, slo¹; C, slo⁴). For clarity, only sweeps of 10, 30 and 80 pA current injection are shown. D, number of action potentials as evoked during the 400 ms current pulses plotted over current amplitude (mean ± SEM). No significant differences are obtained between the groups (ANOVA). E–G, aCC MN firing responses to somatic ramp current injections ranging from −40 pA to 220 pA in 20 pA increments. For clarity, only three sweeps are shown (E, CS; F, slo¹; G, slo⁴). H, number of action potentials as evoked during the 400 ms current ramp plotted over current amplitude (mean ± SEM). Both slo¹ and slo⁴ MNs show significantly lower excitability (ANOVA, Newman–Keuls posthoc comparison, *P < 0.5, **P < 0.01).
per burst and firing frequencies, as well as the duration and the amplitude of the depolarization potential (drive potential), during each wave of segmental excitation (Fig. 5). Activity patterns during representative drive potentials are depicted in Fig. 5A (CS), B (slo\(^4\)) and C (slo\(^1\)). In addition to the use of mutants, \(I_{\text{CF}}\) was acutely blocked by bath application of CTX to control for potential compensatory effects in the mutants (Fig. 5D). Note that the representative burst shape after CTX application (Fig. 5D) is intermediate compared to wild-type control (Fig. 5A) and slo mutants (Fig. 5B and C). This could either be a true difference between acute and permanent \(I_{\text{CF}}\) blockade (see Discussion) or a result of two problems with the pharmacological approach. First, although CTX blocks \(I_{\text{CF}}\) (Fig. 1J), it is not entirely specific (Fig. 1T and W). Second, bath application of CTX requires halting continuous perfusion of the preparation with fresh saline, which, in turn, strongly reduced the probability of

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**Figure 4.** Crawling-like locomotor patterns in controls and slo mutants

A, view onto ventral nerve cord with GFP labelled aCC MNs (white arrow marks right aCC in abdominal segment 5). White arrowheads mark segmental nerve roots 4 and 6 as used for monitoring motor output by extracellular recordings. B, extracellular recordings of nerve roots in segments 6 and 4 during a bout of crawling-like locomotor activity. Lower two traces depict instantaneous firing rates of extracellularly recorded motor bursts in both segments. Note that the rhythmic wave of MN activity propagates from posterior to anterior as is typical for crawling motor behaviour (indicated by dashed lines). C–E, representative recordings of aCC activity patterned locomotor activity in representative examples of CS (C), slo\(^1\) (D) and slo\(^4\) (E). D–E, quantification of cycle period and duty cycle taken from 20 consecutive bursts each from seven to nine animals of each genotype. F, cycle period, the time between the onsets of two consecutive burst in one segment, is significantly decreased in both slo mutants (Kruskal–Wallis ANOVA with post hoc U tests, \(* * * P < 0.001\)). G, duty cycle is significantly increased in both slo mutants.
crawling. Therefore, we had only a small time window (1–2 min) during which the animal still performed locomotor patterns and CTX action had started (3 min after bath application). Therefore, we cannot be sure to have fully blocked all $I_{CF}$ in these experiments. Consistent with the latter, in CTX, drive potential amplitude and firing rates were reduced to intermediate values compared to controls and slo mutants (Fig. 5A–D).

Neither the duration of the burst (Fig. 5E), nor that of the synaptic drive potential (Fig. 5F) or the duration of the drive potential at half-amplitude (Fig. 5G), was significantly altered in slo mutants or after bath application of CTX. Pairwise comparison of burst duration before and after CTX application showed no significant differences (Fig. 5K), and burst durations before or after CTX were similar to wild-type or slo mutant ones (Fig. 5E and K). This indicated that $I_{CF}$ was not required for burst or plateau potential termination and that the duration of rhythmically re-occurring bursts of synaptic drive to MNs from the CPG network was not altered in slo mutants or by acute $I_{CF}$ blockade. By contrast, drive potential amplitude was significantly increased by ~40% in both slo mutants compared to control (Fig. 5H).

Although slo mutant MNs displayed significantly more depolarized membrane potentials (~15 mV) during each wave of rhythmic synaptic excitation (Fig. 5A–C and H), intraburst firing frequencies were significantly lower in slo mutants compared to control (Fig. 5A–C and I). This was in accordance with the reduced MN firing responses to somatic ramp current injection (Fig. 3E–H). Because the burst duration remained unaltered, whereas intraburst firing frequencies were significantly decreased, the number of action potentials per burst (Fig. 5J), and thus presumably muscle force production during each segmental contraction, was significantly reduced in slo mutants. Accordingly, the number of spikes per MN burst was also significantly reduced by acute $I_{CF}$ blockade after bath application of CTX during crawling (Fig. 5L). Drive potential amplitude was also significantly increased by CTX bath application (Fig. 5D), although

![Image](image_url)

**Figure 5.** $I_{CF}$ dampens synaptic drive to MNs but is required for high intraburst firing rates during crawling

A–C, selective enlargements of representative bursts of MN activity during ongoing locomotion in a wild-type control (A, CS), a slo<sup>+</sup> mutant (B), a slo<sup>−</sup> mutant (C) and a wild-type control in 200 nM CTX (D). Dashed lines in (A) and (B) depict burst duration, synaptic drive potential amplitude, drive potential duration and the duration of the drive potential at half-amplitude. E–G, in slo mutants, neither burst duration, nor the total and the half-amplitude durations of the synaptic drive potential differs from wild-type control. H, amplitude of the synaptic drive potential is significantly increased in both slo mutants. I and J, both the mean intraburst firing rate ($\bar{f}$), as well as the number of spikes per burst ($N$) are significantly decreased in both slo mutants. K, burst duration in wild-type controls is not different before and after bath application of CTX. L, number of spikes per burst is significantly reduced by bath application of CTX during ongoing locomotor activity of wild-type controls. E–L, bars depict 25%, 50% and 75% quartiles and error bars indicate the 10% and 90% values (**$P < 0.001$; **$P < 0.01$; *$P < 0.05$; Kruskal–Wallis ANOVA with post hoc U tests).
to a lesser extent (10 ± 3 mV) compared to slo mutants (Fig. 5B and C).

The increased synaptic drive potential amplitude in slo mutant animals and after pharmacological blockade with CTX could either be caused by elevated excitatory neurotransmitter release from CPG pre-motor neurones or by reduced MN $I_{\text{CF}}$ during synaptic excitation, or both. To distinguish between these possibilities, we next reduced $I_{\text{CF}}$ by targeted RNAi knockdown of slo in only aCC and RP2 MNs without affecting the rest of the network. The expression of two different slo-RNAi transgenes (VDRC 6723, 104421) under the control of the even-skipped promoter (RN2-GAL4; Fujioka et al. 2003) did not result in a significant knockdown of $I_{\text{CF}}$ in aCC (not shown). Therefore, we employed a Flippase strategy to express the UAS-RNAi constructs in a mosaic fashion under the control of the strong actin-GAL4 promoter (see Methods).

Targeted slo RNAi knockdown in aCC under the control of the actin promoter reduced $I_{\text{CF}}$ amplitude by ~50% (Fig. 6A) compared to non-expressing internal controls in genetic mosaics (see Methods). RNAi induced reduction in MN $I_{\text{CF}}$ resulted in decreased firing responses to ramp current injections (Fig. 6B), as was the case in slo mutants (Fig. 3E–H). However, even with the strong actin driver, knockdown efficacy remained variable. In some aCC MNs, $I_{\text{CF}}$ current was only slightly reduced, whereas it was absent in others (Fig. 6C). Combining current clamp with subsequent voltage clamp recordings from the same neurones revealed that aCC with clear reductions in $I_{\text{CF}}$ current also displayed lower firing frequencies upon somatic current injections (Fig. 6D). Therefore, $I_{\text{CF}}$ normally functions cell intrinsically to increase MN excitability. Generally, the expression of slo-RNAi under the control in actin-GAL4 in aCC MNs caused significant reductions of $I_{\text{CF}}$ and firing frequencies in response to ramp current injection, with values that were intermediate compared to wild-type and slo null mutants.

During ‘crawling-like’ locomotor patterns, RNAi knockdown restricted to a few MNs caused changes in aCC firing patterns that qualitatively resembled those observed in slo mutants. Representative bouts of aCC activity during locomotion for a wild-type control and a slo-RNAi knockdown are shown in Fig. 6E and F. Although overall cycle period was decreased in slo mutants, it was not affected by knockdown of $I_{\text{CF}}$ in a few MNs only (Fig. 6E, F and J). As in slo mutants, RNAi knockdown in aCC did not affect burst duration, although it increased drive potential amplitude and decreased aCC intraburst firing rates, as depicted in selective enlargements of representative single bursts of aCC activity for CS (Fig. 6G) and slo-RNAi (Fig. 6H). Statistical analysis revealed no significant differences in burst duration (Fig. 6K), drive potential duration at half-amplitude (Fig. 6L) or the time constant of membrane potential repolarization after a burst (Fig. 6M). These data indicated that $I_{\text{CF}}$ played no role in burst termination. By contrast, drive potential amplitude was significantly increased over controls, no matter whether all cells were slo mutant or whether $I_{\text{CF}}$ was selectively reduced in a few MNs only (Fig. 6N). Although RNAi knockdown of slo was not complete, it recapitulated the findings on MN drive potential amplitude and intraburst firing rates that were found in slo mutants. No statistical significant differences in drive potential amplitude were found between slo-RNAi restricted to postsynaptic MNs or slo mutants (Fig. 6N). This indicated that the increase in drive potential amplitude was caused by postsynaptic reduction of $I_{\text{CF}}$ in aCC and not by increased neurotransmitter release from CPG pre-motor neurones. Moreover, as in slo mutant animals, intraburst firing frequency was significantly reduced by reduced $I_{\text{CF}}$ in aCC, although this effect was significantly stronger in the null mutant (Fig. 6O).

In multiple types of neurones, firing frequency is in part regulated by BK channels via the time course and amplitude of the AHP (Sah & Faber, 2002). In general, the AHP can be subdivided into three phases: a fast, a medium and a slow AHP. Each phase is probably mediated by different ionic currents. BK channels are typically activated fast during the upstroke of the action potential and then close rapidly after repolarization of the membrane potential. Therefore, BK channels may influence spike shape and the amplitude of the fast AHP (Faber & Sah, 2003). To test whether $I_{\text{CF}}$ might allow for high firing rates of MNs specifically during Drosophila crawling, we analysed these parameters quantitatively (Fig. 7). First, we measured firing threshold, action potential amplitude, half-amplitude width, the time from action potential onset to maximum AHP, and the voltage difference between maximum AHP and firing threshold during tonic firing as evoked by somatic ramp current injections just above firing threshold (Fig. 7A, 1, spike threshold; 2, spike amplitude; 3, spike width; 4, AHP time to minimum; 5, AHP amplitude). Firing threshold was similar in control, slo mutant and slo RNAi knockdown in aCC MNs (not shown). By contrast, action potential amplitude and duration were significantly increased in slo mutants and after RNAi knockdown (Fig. 7B), indicating that $I_{\text{CF}}$ activated sufficiently fast to limit spike amplitude and duration in controls. By contrast, during tonic firing just above threshold, neither the amplitude, nor the duration of the AHP was affected in slo mutants or knockdowns compared to controls (Fig. 7C). Note that firing responses to somatic square pulse or ramp current injections just above threshold were also not affected by genetic manipulation of slo (Fig. 3). However, slo mutants showed significantly reduced firing frequencies in response to ramp current injection that resulted in firing frequencies above 30 Hz (Fig. 3).

During locomotion, aCC MNs fire in bursting patterns at frequencies well above 100 Hz (Fig. 6O).
Therefore, we tested whether $I_{CF}$ contributed to a fast AHP that was necessary to allow maximum intraburst firing rates, as occurring during locomotion. Figure 7D depicts representative action potentials, as occurring at maximum depolarization and maximum intraburst firing rates during crawling movements (control, black trace; slo mutant, red trace). Maximum intraburst firing frequencies were significantly higher in controls (up to 200 Hz) compared to slo mutant or RNAi knockdown (Fig. 7E). At these high firing rates and depolarized membrane potentials, the AHP was considerably smaller and ~5-fold faster (Fig. 7F) compared to low frequencies of tonic firing (Fig. 7C). Most importantly, in MNs without $I_{CF}$, the amplitude and the speed of the fast AHP were significantly reduced compared to controls (Fig. 7D and F). slo RNAi knockdown, which, on average, reduced

![Figure 6](image_url)

**Figure 6.** Targeted RNAi knockdown reveals postsynaptic roles of $I_{CF}$ specifically in MNs

A–C, slo RNAi knockdown efficacy in aCC. A, current–voltage plots of isolated $I_{CF}$ reveal a mean 50% reduction in current amplitude in identified aCC MNs compared to internal controls (see Methods). B, RNAi knockdown of slo significantly decreases aCC firing responses to somatic ramp current injections compared to internal controls. C, variability of RNAi knockdown efficacy is reflected by variation of $I_{CF}$, current–voltage plots of isolated aCC neurones as shown in (O). Firing rates are markedly decreased (lower traces) in aCC without detectable $I_{CF}$ (lower traces). D, ramp current injections into the same aCC neurones as shown in (O). Firing rates are markedly decreased (lower traces) in aCC without detectable $I_{CF}$. E and F, representative bouts of crawling-like motor activity in control animals (E, CS) and after slo RNAi expression in aCC and RP2 MNs under the control of actin promoter (see Methods). Note that crawling speed appears to be similar. G–I, selective enlargements of typical aCC burst in a control animal (G) and after RNAi knockdown of slo in aCC (H). Dashed lines demark burst duration. J, crawling speed is increased in slo4 mutants but is not affected after $I_{CF}$ knockdown in MNs (control 1 is CS; control 2 is the GAL4 driver crossed with the genetic background of the UAS-RNAi fly strain). K–M, burst duration (K), drive potential duration at half-amplitude (L) or the time constant of post-burst repolarization (M) are not affected in slo4 mutants or after slo RNAi knockdown in MNs. N, drive potential amplitude is significantly increased in slo4 compared to controls, although it is not significantly different between slo mutants and slo RNAi knockdown in MNs. O, RNAi knockdown of slo in aCC causes intermediate intraburst firing frequencies compared to CS and slo mutants, similar to displaying intermediate levels of $I_{CF}$ (A). (Kruskal–Wallis ANOVA with posthoc U tests, *P < 0.05; **P < 0.01, ***P < 0.001).
$I_{\text{CF}}$ amplitude to $\sim50\%$ of controls but allowed MNs to express about twice the current of mutants, produced an intermediate but significant reduction in AHP amplitude and speed (Fig. 7F) and intermediate maximum intraburst firing rates (Fig. 7E). However, it remains unclear whether the significant reductions in maximum firing rates and fAHP amplitude in slo mutants were a sole consequence of the absence of $I_{\text{CF}}$. Note that, in slo mutants, aCC also displayed a higher amplitude drive potential (see above). This, in turn, may also have contributed to Na$^+$ channel inactivation or to a reduction in fAHP amplitude via increased inactivation of other transient K$^+$ currents. Although we did not attempt to separately quantify the individual contributions of the absence of $I_{\text{CF}}$ and the more depolarized drive potential on maximum firing rates and fAHP amplitude, both effects were large and highly significant in slo mutants, and both were also observed for drive potentials with minimally increased amplitude. Therefore, our data indicate that $I_{\text{CF}}$ functions in aCC crawling MNs to increase the speed and amplitude of the

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**Figure 7.** $I_{\text{CF}}$ facilitates the fast AHP in MNs during bursting but not during tonic firing

A, representative single action potential of aCC taken from a train of tonic firing at 1 Hz as evoked by somatic current injection. Numbers and dashed lines indicate measurements of (1) firing threshold, (2) action potential amplitude, (3) half-amplitude spike width, (4) the duration from action potential onset to maximum AHP (time to min) and (5) AHP amplitude as measured as the voltage difference between threshold and maximum AHP. B, mean action potential amplitude (white bars) and half-amplitude width (grey bars), as measured from eight animals per genotype, were significantly smaller in controls aCC compared to slo$^4$ or after targeted RNAi knockdown of slo (ANOVA with the Newman–Keuls posthoc test, *$P < 0.05$, error bars depict SD). C, neither the amplitude (white bars), nor the duration of the AHP (grey bars) is significantly affected in slo$^4$ or after slo RNAi expression in aCC (ANOVA, $P > 0.2$, error bars depict SD). D, overlay of action potentials taken from representative aCC bursts as recorded during crawling in a control (CS, black trace) and a slo$^4$ mutant (red trace). Note the different time scale compared to (A). Black and red dashed lines demark the different AHP amplitudes in both genotypes. E, maximum intraburst firing frequencies are significantly decreased in slo mutants and after $I_{\text{CF}}$ knockdown in aCC compared to controls (boxes show 25%, 50% and 75% quartiles; errors bars indicate the 10% and 90% values). Complete lack of $I_{\text{CF}}$ reduces maximum firing rates significantly stronger than reduction of $I_{\text{CF}}$ by RNAi knockdown (Kruskal–Wallis ANOVA with posthoc $U$ test, **$P < 0.01$, ***$P < 0.001$). F, lack or reduction of $I_{\text{CF}}$ causes significant reductions in the AHP amplitude (white bars) and speed (grey bars). Both effects are significantly stronger in null mutants compared to knockdowns (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ANOVA with Newman–Keuls posthoc test).
AHP specifically during the high frequency firing that occurs during bursting, thereby allowing for maximum MN firing rates during locomotion.

Discussion

We show that slo specifically underlies $I_{CF}$ in Drosophila larval aCC MNs. Targeted genetic manipulation combined with in situ patch clamping during locomotion revealed critical roles of $I_{CF}$ in shaping MN excitability and firing output in response to CPG activity. $I_{CF}$ functions postsynaptically to limit the amplitude of MN synaptic drive potentials and to increase intraburst firing rates during crawling. The underlying cellular mechanisms and resulting behavioural relevance are discussed below.

slo specifically underlies fast activating/fast inactivating Ca$^{2+}$-dependent potassium current in larval MNs

Electrical and pharmacological separation of K$^+$ outward components in larval aCC MNs reveal a transient (peak current after 2.1 ± 0.4 ms; 90% inactivation after 5 ms) Ca$^{2+}$-dependent K$^+$ current ($I_{CF}$) that is selectively eliminated in slo mutants and CTX-sensitive. This is consistent with the reported properties of slo currents in muscle (Elkins et al. 1986; Singh & Wu, 1990; Komatsu et al. 1990). However, activation and inactivation in MNs is faster than in muscle. Functional diversity of slo channels is mediated by numerous post-transcriptional and post-translational modifications (Salkoff et al. 2006; Contreras et al. 2013) that occur in a tissue specific manner (Yu et al. 2006). Moreover, BK channel activation and inactivation kinetics are profoundly affected by auxiliary β-subunits (e.g. β2 mediates fast inactivation in CA1 hippocampal neurones) (Hicks & Marrion, 1998). Although the mechanisms underlying transient properties of $I_{CF}$ in Drosophila MNs remain unknown, the current is sufficiently fast to modulate Na$^+$-dependent action potentials and the fast AHP (see below). Therefore, $I_{CF}$ probably contributes to Na$^+$ channel de-inactivation during high frequency firing. Consistent with this, slo mutant MNs show significantly decreased maximum firing frequencies in response to ramp current injections.

slo mutant animals are hyperexcitable and show decreased cycle periods

slo mutant larvae are able to produce co-ordinated peristaltic waves of segmental contractions as is characteristic for larval crawling (Fox et al. 2006; Heckscher et al. 2012), indicating that $I_{CF}$ is not mandatory for co-ordinated CPG output. Although compensatory up-regulation of Shaker K$^+$ channels in slo mutants occurs at the Drosophila larval MN synaptic terminals (Lee et al. 2008; 2014), we have no indications for any compensatory mechanisms in the somatodendritic domain of MNs. In slo mutants, total transient outward current as recorded from the soma is smaller than in wild-type controls, and sole voltage-dependent transient K$^+$ current amplitude is identical to controls. This indicated that permanent loss of $I_{CF}$ function is not compensated for by up-regulation of other transient K$^+$ currents, although we cannot exclude homeostatic compensation by voltage-dependent transient K$^+$ channels in axonal compartments that are too distant to be resolved in somatic voltage clamp recordings. However, intrinsic MN excitability is altered in slo mutant MNs. Additionally, acute blockade of $I_{CF}$ by CTX recapitulates specific aspects of altered MN firing during crawling as observed in permanent genetic manipulation.

In slo mutants, overall activity in the semi-intact preparation is increased and cycle period is significantly shorter. This may be a result of increased neurotransmitter release from synaptic terminals throughout the CNS. In general, BK channels localized to presynaptic terminals negatively regulate synaptic transmission by limiting the influx of Ca$^{2+}$ through voltage-gated channels (Robitaille et al. 1993; Hu et al. 2001; Wang et al. 2001; Raffaelli et al. 2004; Sun et al. 2004; Lee et al. 2014). At the Drosophila neuromuscular junction, slo and Shaker operate together, although slo function dominates during repetitive firing by compensating for Shaker channel inactivation (Ford, Davis, 2014). Therefore, hyperactivity as a result of CNS-wide loss of BK channel function appears to be logical. Accordingly, seizures can be evoked by conditional slo knockout in Drosophila (Guan et al. 2005) and BK channel mutations have been linked to human epilepsy (Du et al. 2005; Lorenz et al. 2007; Yang et al. 2010).

However, even with strongly reduced cycle period, MN burst durations and synaptic drive potential durations remain unaltered in slo mutants and after acute pharmacological blockade of $I_{CF}$. This indicates that $I_{CF}$ is not required to regulate the duration of each segmental excitation. Burst termination could either be mediated by other intrinsic MN conductances or by inhibitory synaptic input to MNs, or by both. Synaptic connections clearly play a role in burst termination in Drosophila larvae because it was recently shown that acute silencing of inhibitory glutamatergic interneurones prolongs segmental motor bursts (Kohsaka et al. 2014). However, the present study focuses on intrinsic mechanisms in MNs. The specific advantage of the Drosophila system is that targeted genetic manipulation allows to specifically address the function of $I_{CF}$ in MNs without affecting the rest of the network.

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The role of slo in shaping MN firing patterns during larval locomotion

Contrasting our findings in MNs, in hippocampal pyramidal neurones (Golding et al. 1999) and Purkinje neurones (Womack & Khodakhah, 2004), BK currents modulate burst duration. However, a direct role in burst termination is difficult to reconcile with the rapid inactivation of $I_{\text{CF}}$ in Drosophila MNs. Similarly, burst termination in trigeminal MNs is not mediated by transient BK currents but, instead, by slowly inactivating, apamin-sensitive $\text{Ca}^{2+}$ activated K$^+$ currents (del Negro et al. 1999), and sustained $I_{\text{CS}}$ underlies the slow AHP in guinea pig vagal MNs (Yarom et al. 1985). This suggests that burst termination with SK but not BK channels might be a common principle in MNs but not necessarily other types of central neurones.

Despite normal burst durations, MNs without $I_{\text{CF}}$ display increased postsynaptic drive potential amplitudes but decreased intraburst firing frequencies. These effects are also observed when restricting RNAi knockdown of $I_{\text{CF}}$ to MNs without affecting the rest of the network, thus indicating that both effects are caused solely by changes in MN membrane excitability. The question remains as to how rapidly inactivating $I_{\text{CF}}$ affects the amplitude of a broad synaptic drive potential (∼700 ms) and also facilitates high frequency firing.

$I_{\text{CF}}$ keeps MN postsynaptic depolarizations in response to CPG input in check

In many central neurones, sustained $I_{\text{CS}}$, mostly mediated by SK channels, mediates an outward conductance that is well suited to suppress long lasting postsynaptic responses (Faber et al. 2005). However, in amacrine cells, rapidly inactivating BK channels locally counter fast AMPAR-mediated depolarizations at single postsynaptic sites (Grimes et al. 2009). Notably, this suppression persists over a broad time scale because BK channels also limit postsynaptic activation of dendritic L-type voltage-gated $\text{Ca}^{2+}$ channels. L-type $\text{Ca}^{2+}$ channels are also localized to Drosophila MN dendrites (Worrell & Levine, 2008; D. Kadas, A. Klein and C. Duch, personal observations). Therefore, $I_{\text{CF}}$ may limit dendritic L-type $\text{Ca}^{2+}$ channel activation during synaptic excitation. Similarly, somatodendritic BK channels of layer 5 pyramidal neurones in rat somatosensory cortex inhibit the occurrence of dendritic $\text{Ca}^{2+}$ spikes (Benhassine & Berger, 2009). Alternatively, $I_{\text{CF}}$ may dampen MN postsynaptic responses by locally counteracting excitatory synaptic inputs throughout the duration of the synaptic drive potential (∼700 ms). Either way, in multiple different types of vertebrate and invertebrate central neurones, transient BK channels limit the magnitude of the postsynaptic response to a given synaptic drive.

$I_{\text{CF}}$ is required for maximum intraburst firing rates of MNs

Information transfer to muscle is encoded by MN firing rates. In many neurones, firing rates are regulated by action potential AHPs, which can be categorized into fast, medium and slow AHPs. The fast AHP typically lasts between 1–10 ms and is a result of the activation of A-type and/or BK channels (Lancaster & Nicoll, 1987; Sah & Faber, 2002). The medium AHP often underlies spike frequency adaptation and is mostly mediated by SK channels (Faber & Sah, 2003). Accordingly, we demonstrate that Drosophila BK current has no detectable effect on the median AHP during slow tonic MN firing. However, $I_{\text{CF}}$ significantly increases the amplitude and speed of the fast AHP. This in turn allows for maximum MN firing rates in rhythmical bursting, as occurs during crawling. Similarly, in CA1 pyramidal neurones, BK current does not affect firing rates below 40 Hz but is required for maximum initial firing rates during bursting (Gu et al. 2007). The fast AHP probably minimizes the refractory period by promoting $\text{Na}^+$ channel de-inactivation and limiting the activation of slow voltage-gated K$^+$ currents. Moreover, in Drosophila MNs (present study) and in cerebellar Purkinje neurones (Haghdoot-Yazdi et al. 2008), BK current is required for spike shape and spike frequency control only in bursting mode but not during tonic firing. Here, BK channel properties appear to be tuned so that (i) full activation occurs only during strong and prolonged depolarizations with coincident $\text{Ca}^{2+}$ influx such as in burst mode; (ii) activation is sufficiently fast to occur within the duration of a single $\text{Na}^+$ spike within bursts; and (iii) inactivation is sufficiently fast so that BK outward current does not oppose the next spike of the burst.

Narrow tuning of BK channel inactivation kinetics also has clinical relevance. For example, a mutation in the BK $\alpha$-subunit resulting in increased $\text{Ca}^{2+}$ sensitivity has been proposed to cause a familial form of generalized epilepsy and paroxysmal movement disorder by increasing neuronal excitability via an enhanced fast AHP (Du et al. 2005). Furthermore, a $\beta$4-subunit that slows BK current kinetics protects from temporal lobe seizures (Brenner et al. 2005). The proposed mechanism is that slower BK channel kinetics preclude them from sharpening the action potential to support higher firing rates. Our data on $I_{\text{CF}}$ function in Drosophila MNs are in accordance with these reports and provide clear evidence that $I_{\text{CF}}$ is required for maximal firing rates and thus optimal muscle force production during normal motor behaviour.

However, multiple solutions exist to increase maximum firing rates through outward K$^+$ currents that limit Na$^+$ channel inactivation or aid Na$^+$ channel de-inactivation (Baranauskas, 2007). In many fast spiking mammalian neurones, fast delayed rectifier currents mediated by Kv3
family proteins assist action potential repolarization and, consequently, speed up the recovery of Na⁺ channels from inactivation (Baranauskas et al. 2003; Erisir et al. 1999). In hippocampal interneurons, for example, Kv3 channels are kinetically optimized for high frequency action potential generation (i.e. activation is slower than Na⁺ channel inactivation, so that Kv3 channels do not prevent Na⁺ channel inactivation but, instead, activate sufficiently fast to speed up the recovery of Na⁺ channels) (Lien & Jonas, 2003). Such kinetics also cause minimal overlap of Na⁺ inward current and K⁺ outward current during the action potential, which in turn decreases the energy demand for action potentials (Alle et al. 2009).

Although our whole cell patch clamp recordings from the large larval Drosophila MNs do not allow a highly accurate analysis of BK channel kinetics, our combined voltage and current clamp data indicate that I CF in aCC is well tuned to facilitate maximum MN firing responses to CPG activity during locomotion, thus providing direct confirmation of the functional relevance of precisely regulated BK properties in motor control.

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### Additional information

#### Competing interests

The authors declare that they have no competing interests.

#### Author contributions

All authors approved the final version of the manuscript submitted for publication. DK: conducted most of the experiments and contributed to research design, data analysis and manuscript writing. SR: contributed to some experiments, research design and manuscript writing. CD: contributed to research design, data analysis and manuscript writing.

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