Complex Locomotion Behavior Changes Are Induced in *C. elegans* by the Lack of the Regulatory Leak K⁺ Channel TWK-7

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Abstract

The change of locomotion activity in response to external cues is a considerable achievement of animals and is required for escape responses, foraging, and other complex behaviors. Little is known about the molecular regulators of such an adaptive locomotion. The conserved eukaryotic two-pore domain potassium channels (K_{2}P) channels have been recognized as regulatory K^{+} channels that modify the membrane potential of cells thereby affecting e.g. rhythmic muscle activity. By using the *Caenorhabditis elegans* system combined with cell type specific approaches and locomotion analyses in-depth, here, we found that the loss of K_{2}P channel TWK-7 increases the locomotor activity of worms during swimming and crawling in a coordinated mode. Moreover, loss of TWK-7 function results in a hyperactive state that (although less pronounced) resembles the fast, persistent and directed forward locomotion behavior of stimulated *C. elegans*. TWK-7 is expressed in several head neurons as well as in cholinergic excitatory and GABAergic inhibitory motor neurons. Remarkably, the abundance of TWK-7 in excitatory B-type and inhibitory D-type motor neurons affected five central aspects of adaptive locomotion behavior: velocity/frequency, wavelength/amplitude, direction, duration, and straightness. Hence, we suggest that TWK-7 activity might represent a mean to modulate a complex locomotion behavior at the level of certain types of motor neurons.
Introduction

Locomotion is a fundamental aspect of life, because it is required for escape responses, foraging, and other behaviors. In both invertebrates and vertebrates, rhythmic and patterned locomotion is usually controlled by specific motor circuits with autonomous rhythmic activities called central pattern generators (MARDER and CALABRESE 1996). The overall output of the motor network depends on the interplay of premotor interneurons, motor neurons, muscle cells, and the intrinsic membrane properties of the involved cells. For example, in insects, motor activity appears to increase during walking by tonic depolarisation of interneurons and/or motor neurons (LUDWAR et al. 2005). In response to environmental cues, animals often adjust their locomotion activity, which, in principle, can be regulated at the level of central pattern generators, interneurons, motor neurons, or muscle cells.

Two-pore domain potassium (K$_2$P) channels are evolutionarily conserved eukaryotic membrane proteins (ENYEDI and CZIRJAK 2010). They contain two pore domains per subunit and function as dimers building one conductance pore. K$_2$P channels operate as regulatory K$^+$ channels to stabilize the negative membrane potential and to counterbalance membrane depolarization. Closure of their potassium pore usually induces membrane depolarization and facilitates the excitability of cells. K$_2$P channels are specifically regulated by a variety of factors including temperature, pH, membrane stretch, fatty acids, and signaling pathway dependent phosphorylation. The physiological function of most K$_2$P channels remains to be elucidated. They have been implicated in the regulation of several processes, such as chemoreception, mechanical nociception, and excitation of motor neurons. Experiments on slice preparations of anaesthetized adult turtles revealed that the neurotransmitter serotonin increases the excitability of spinal motor neurons via inhibition of a K$_2$P-like K$^+$ current (PERRIER et al. 2003), for example.
In nature, the nematode *C. elegans* is mainly found on rotten fruits and vegetables (FELIX and BRAENDLE 2010; PETERSEN et al. 2015). These habitats are characterized by solid and liquid micro-niches suggesting that crawling and swimming are natural locomotory modes of the nematode. In the laboratory, the kinematic and biophysical parameters (e.g. mechanic forces) of *C. elegans* crawling and swimming have been described in detail (BOYLE et al. 2012; FANG-YEN et al. 2010; MAJMUDAR et al. 2012; PIERCE-SHIMOMURA et al. 2008). The frequency of spontaneous alternating C-shape conformations (swimming) is considerably higher compared to undulating S-shape conformations (crawling). Locomotion is controlled by excitatory cholinergic (A- and B-type) and inhibitory GABAergic (D-type) motor neurons that are localized in the ventral nerve cord of the worm. B-type motor neurons are responsible for forward, A-type motor neurons for backward locomotion (CHALFIE et al. 1985). A-, B- and D-type motor neurons are further divided into V and D subclasses that innervate the longitudinally aligned ventral and dorsal muscle cells, respectively (GIORGIJEVA J 2014; WEN et al. 2012; WHITE et al. 1976). A- and B-type motor neurons synapse not only onto their respective muscle cells but also onto corresponding inhibitory D-type motor neurons (VD or DD) leading to contralateral muscle inhibition (GIORGIJEVA J 2014; WEN et al. 2012; WHITE et al. 1976). Recently, it has been shown that the B-type motor neurons (responsible for forward locomotion) are coupled by proprioception thereby transducing the rhythmic movement, which may be initiated by a postulated central pattern generator near the head, into bending waves propagated driven along the body by a chain of reflexes (WEN et al. 2012). Moreover, studies by (KAWANO et al. 2011) revealed that an imbalanced neuronal activity between B-type to A-type motor neurons is responsible for directional movement. In response to mechanical, gustatory, olfactory, and thermal stimuli or food deprivation (BEN AROUS et al. 2009; CLARK et al. 2007; LUERSEN et al. 2014; LUO et al. 2008; SAWIN et al. 2000; SHTONDA and AVERY 2006), *C. elegans* shows adaptive locomotion behaviors. The stimulated backing escape response has been characterized in detail (CHALFIE et al. 1985;
DONELLY et al. 2013). However, the underlying mechanisms (channels, subgroup of neurons etc.) that are involved in distinct adaptive changes of forward locomotion behavior remain largely unknown.

*C. elegans* K$_2$P channels are designated as TWK channels (two-P (pore-forming) domain K+ channels). More than 40 TWK-encoding genes representing six subfamilies have been identified in the worm (BUCKINGHAM et al. 2005; SALKOFF et al. 2001). In *Drosophila* and mammals, only eleven and fifteen K$_2$P channels have been annotated, respectively. Most *C. elegans* TWK channels are expressed in a few cell types including body-wall muscle cells, chemosensory neurons, interneurons, and motor neurons (KRATSIOS et al. 2012; SALKOFF et al. 2001). Up to now, only two TWK channels, both expressed in body-wall muscle, have been functionally characterized in *C. elegans* (DE LA CRUZ et al. 2003; DE LA CRUZ et al. 2014; KUNKEL et al. 2000). TWK-18 has been implicated in locomotion activity in response to higher temperature. TWK-23 (SUP-9) is activated by a putative iodotyrosine deiodinase (SUP-18) and might be involved in the excitability of muscle membranes. Overall, our knowledge regarding the physiological functions of two-pore domain potassium channels, even in *C. elegans* and also in other experimental systems such as fly, zebrafish, or mouse is rather limited. Here, we found that TWK-7 is required in cholinergic B-type and GABAergic D-type motor neurons to maintain normal spontaneous locomotor activity and locomotion behavior. The coordinated hyperactive phenotype with fast forward and directed crawling caused by loss of TWK-7 function is consistent with the abovementioned impact of closure or inactivation of regulatory K$_2$P channels on the membrane potential of cells and the effect of hyperexcitability of motor neurons on *C. elegans* locomotion. Owing to the regulatory nature of K$_2$P channels, we suggest that TWK-7 may represent a prime candidate for the modulation of adaptive locomotion behavior that enables fast and directed forward locomotion.
Materials and Methods

Strains and culturing

All *C. elegans* strains were grown at 20°C on nematode growth media (NGM) agar plates seeded with OP50 *E. coli* as a food source (BRENNER 1974). The following strains were obtained from the *Caenorhabditis* Genetics Center (USA): Bristol N2 (used as wild-type), RB1239 *twk-30(ok1304)I* (1000 bp deletion), *twk-40(tm6834)III* (473 bp deletion), VC40352 *twk-43(gk590127)V* (substitution L232Ochre), VC40313 *twk-46(gk568572)V* (substitution G125E), *otE4803* (expressing DsRed under a 3000 bp *twk-7* promoter) (KRATSIOS et al. 2012), LY120 *twk-7(nf120)III*, VC40681 *twk-7(gk760044)III*, and RB1239 *twk-30(ok1304)I*. The strain MT1908 *nDf21/dpy-19(e1259),unc-32(e189)III* was used for genetic complementation studies. The strain used for cell specific expression pattern analyses was LX929 *unc-17p::GFP(vsIs48)*. For co-localization studies we used the thermosensitive strain GE24 *pha-1(e2123)III* transformed with the plasmid of interest and the rescuing plasmid pBX as co-marker (GRANATO et al. 1994). Double mutants were generated using standard genetic methods without additional marker mutations. Homozygosity of alleles in each double mutant was confirmed by PCR in case of deletion mutations, by restriction length polymorphism analysis in case of appropriate SNP mutations or in all other cases by sequencing of amplified genomic DNA.

Molecular biology and transfection of *C. elegans*

Transgenic strains were generated by biolistic bombardment following the protocol of (WILM et al. 1999). For rescue experiments the *myo-2p::GFP::pPD118.33* plasmid was used as co-marker. Oligonucleotides used for amplification of the following constructs are listed in Tab. S1.
Genetic constructs

twk-7p::TWK-7::mCherry::let-858(3’UTR)

This rescue construct was generated by cloning a 12.1 kb genomic sequence including 3.0 kb upstream of the translational start site into the pPD118.33DD-mCherry vector.

twk-7p::TWK-7(G^{282}C)::mCherry::let-858(3’UTR) and twk-7p::TWK-7(T^{279}K)::mCherry::let-858(3’UTR)

A 3.5 kb fragment was cut off of the twk-7p::TWK-7::mCherry construct by using PstI. After gel purification the isolated fragment was further digested by BglII resulting in a 2.4 kb product of interest for subcloning into the vector pL4440. Site-directed mutagenesis was carried out by employing this construct and the complementary primer pairs corresponding to the sequences 5’-AACCGTCGTCACTACCATCGGAT ACGGTAATCCAGTTCCAG-3’ (underlined nucleotide sequence was changed to TGT) and 5’-CTTTGCCGTAACCGTC GTCACTACCATCGGATACGGTAATC-3’ (underlined triplet was changed into AAG) in order to obtain a G^{282}C and T^{279}K exchange in the TWK-7 protein sequence, respectively. The mutated fragments were verified by sequencing, before cloning back into the twk-7p::TWK-7::mCherry construct via SwaI and BglII.

unc-17p(4.2kb)::TWK-7::mCherry::let-858(3’UTR), unc-17(1kb)p::TWK-7::mCherry::let-858(3’UTR), and unc-17(1kb)p::TWK-7(G^{282}C)::mCherry::let-858(3’UTR)

The unc-17p(4.2kb) construct was generated by cloning the cholinergic neuron specific 4.2 kb promoter region of unc-17 (restriction sites used: ApaI and Ndel) in front of the twk-7 gene of 9142 bp (starting with translational start site) that is fused to mCherry reporter in the pBluescript vector. In addition, we replaced the 4.2 kb unc-17 promoter sequence by a 1 kb unc-17 promoter fragment that has been shown to drive gene expression solely in cholinergic motor neurons (Kratsios et al. 2012). For the dominant negative TWK-7 construct, a 2.4 kb
fragment containing the G<sup>282</sup>C exchange was excised from twk-7p::TWK-7(G<sup>282</sup>C)::mCherry vector by BglII and SacII and introduced into the *unc-17*(1kb)p::TWK-7::mCherry::let-858(3′UTR) construct.

unc-4p::TWK-7::mCherry::let-858(3′UTR), unc-4p::TWK-7(G<sup>282</sup>C)::mCherry::let-858(3′UTR), acr-5p::TWK-7::mCherry::let-858(3′UTR), acr-5p::TWK-7(G<sup>282</sup>C)::mCherry::let-858(3′UTR), unc47p::TWK-7::mCherry::let-858(3′UTR), and unc-47p::TWK-7(G<sup>282</sup>C)::mCherry::let-858(3′UTR)

These constructs were produced by ligation of the cholinergic neuron specific promoter regions of *unc-4* (4.2 kb genomic DNA, restriction sites: *Apa*I and *Nde*I) and *acr-5* (4.3 kb genomic DNA, restriction sites: *Apa*I and *Ase*I) and the GABAergic neuron specific promoter region of *unc-47* (2.9 kb genomic DNA, restriction sites: *Apa*I and *Nde*I) in front of the 9142 bp *twk-7* and *twk-7*(G<sup>282</sup>C) gene, respectively (starting with translational start site) that are fused to *mCherry* reporter in the ρBluescript vector.

**Short-term locomotion assays and analyses**

To quantify the number of body bends of swimming worms, 5 nematodes were transferred with a worm-picker (platinum wire) from standard NGM plates onto empty NGM plates to clean worms from bacteria. After approximately 1 min worms were placed in a 50 µl droplet of M9 buffer onto a diagnostic slide (3 wells, 10 mm diameter; Menzel). The worms were immediately filmed for 1 min with a VRmagic C-9+/BW PRO IR-CUT camera (VRmagic, Germany) attached to a Zeiss Stemi 2000-C microscope (Carl Zeiss AG, Germany). Mov-files were used to quantify the body bending swimming frequency by *ImageJ* wrMTrck wormtracker plugin (Pедерсен 2011) according to the protocol described in (http://www.phage.dk/plugins/download/wrMTrck.pdf). One body bend corresponds to the movement of the head region thrashing from one side to the other and back to the starting
position. For the locomotion analysis on NGM agar plates we followed the protocol of (MILLER et al. 1999) with minor modifications. The assay was set up by placing 500 late L3 or early L4 stage larva on locomotion assay plates spread with a thin lawn of OP50 bacteria (100 µl of an OP50 overnight culture per plate, incubated for 20 h at 37°C). Worm plates were incubated at 20°C for 24 h, before the locomotion of young adult animals was filmed three times for 1 min with the camera setup described above. Spontaneous body bending crawling frequency was assessed by visual inspection of worms that were captured for at least 5 seconds. One body bend corresponds to the movement of the tip of the tail from one side to the other. Velocity and straightness was analysed by using ImageJ wrMTrck wormtracker plugin. The forward locomotion efficiency (straightness) was calculated from the ratio of distance to track length, whereby the distance represents the straight line from start to end coordinates of each recorded animal. In addition, for more detailed analyses we split the locomotion behavior into forward, backward and dwelling time periods. The latter includes periods where worms move less than one body bend in forward or backward direction. Body bending crawling frequencies of single worms were separately assessed for forward locomotion periods. Furthermore, we determined the relative time the worms spent on dwelling and forward and backward locomotion.

Stimulated forward locomotion assays (picking transfer assays) were done as described in (GAGLIA and KENYON 2009). Staged young adult animals were transferred with a worm-picker from standard NGM plates to NGM assay plates spread with a thin lawn of OP50 food bacteria. Movies were taken immediately after transfer and every 30 min for 2 h. Body bending crawling frequencies were assessed by visual inspection of the movies. Speed and forward locomotion efficiency was analysed by using ImageJ wrMTrck wormtracker plugin. For all locomotion assays, mutants and transgenic strains were strictly compared with the respective control strains (wild-type, non-transgenic siblings) at the same day using the same batch of plates.
Long-term tracking assay and analysis

NGM plates were seeded with *E. coli* OP50 and incubated over night at 37°C. In order to determine the long term locomotion behavior, single well fed young adult animals were transferred into a 10 µl M9 drop on the freshly seeded NGM plates and incubated for 16 h at 20 °C. For track assessment, worm traces were visualized on the backside of each NGM plate using an pencil marker and then captured as .jpeg file with an VRmgic C-9+/BW PRO IR-CUT camera (VRmagic, Germany) attached to a Zeiss Stemi 2000-C microscope (Carl Zeiss AG, Germany). Recorded tracks were finally ranked by visual inspection counting the squares crossed by each worm.

Body curvature analyses

Single swimming or crawling worms were filmed for 10 s Subsequently, their bodies were skeletonized and the resulting spines were divided into ten segments by using MultiWormTracker (version 1.3.0-r1035) software (SWIERCZEK et al. 2011). The alteration of angles between adjacent segment pairs were calculated over time. Resulting values were transferred to MatLab and processed using the contour plot function.

Imaging of reporter strains

Transgenic animals were anesthetized and mounted in 5 mM levamisole, 5 mM aldicarb on a 2% agarose pad. Laser-scanning confocal fluorescence microscopy was carried out using a Zeiss Axio Imager Z2 upright microscope equipped with a Zeiss LSM 700 scanning confocal imaging system. Image acquisition and processing was performed by employing Zeiss ZEN software.
**Calculation of the slip values for locomotion efficiency**

Locomotion efficiency was introduced as the amount of slip (S) by (GRAY and LISSMANN 1964) for the locomotion of nematodes. Slip values were calculated from the ratios between velocity ($V_{\lambda}$) of the undulating wave propagated over the worm spine and the velocities ($V_x$) of the moving worm, where $f$ represents the bending frequency and $\lambda$ the wave length.

$$S = \left(1 - \frac{V_{\lambda}}{V_x}\right) \times 100; \text{ where } V_{\lambda} = f \times \lambda$$
Results

TWK-7 deficiency led to an enhanced activity of both swimming and spontaneous crawling in a coordinated mode

The *C. elegans* genome contains more than 40 genes encoding TWK channels. Of these TWK-7, -30, -40, -43, and -46 have been previously shown to be expressed in motor neurons (Kratsios et al. 2012; Salkoff et al. 2001). Because the evolutionary conserved K2P/TWK channels are known to set the membrane potential and, hence, influence the excitability of cells, we investigated whether these *C. elegans* channels have a physiological role in locomotion. When tested for their locomotor activity the *twk-7* mutant allele *nf120* showed an altered spontaneous crawling activity than wild-type worms, whereas mutant alleles of other TWK channels expressed in motor neurons did not induce a similar phenotype (Fig. 1A). Consistent with the general structure of K2P channels, *in silico* analyses predicted that the deduced TWK-7 protein contains four transmembrane domains (M1-M4) and the two pore domains P1 and P2 that specify ion selectivity (Fig. 1B; Fig. S1). In the *nf120* allele the *twk-7* gene contains a deletion of 303 bp which leads to the disruption of the P1 and M2 domains and a translational frameshift within the open reading frame.

On agar plates, the *twk-7(nf120)* worms moved with an elevated spontaneous crawling body bending frequency of $0.48 \pm 0.11$ Hz (wild-type: $0.16 \pm 0.05$ Hz) resulting in an increased crawling velocity of $0.15 \pm 0.01$ mm*s$^{-1}$ (wild-type: $0.10 \pm 0.02$ mm*s$^{-1}$) (Fig. 2A, B; File S1, S2). Of note, the 3-fold increase in body bending frequency of *twk-7(nf120)* worms led only to a 1.5-fold enhanced crawling velocity when compared to wild-type data. The *twk-7(nf120)* mutant worms also exhibited an enhanced swimming activity of $2.16 \pm 0.05$ Hz when compared to wild-type animals with a body bending swimming frequency of $1.68 \pm 0.09$ Hz (Fig. 2C; File S3, S4). We conclude that among the K2P mutants tested solely TWK-7
function is required to maintain spontaneous locomotor activity of *C. elegans*. Hence, for further analysis, we focused on TWK-7. Genetic analysis revealed that *nf120* is a recessive null allele of *twk-7* (Fig. 2D). Thus, *twk-7(nf120)* was specified as *twk-7(null)*. Accordingly, similar higher crawling and swimming activities were determined for a second loss-of-function allele of *twk-7*, *gk760044* that is characterized by a premature stop at position W325 (Fig. 1A, B; Fig. 2A-C; Fig. S1). Importantly, although the two *twk-7* mutants swam and crawled with higher frequencies than wild-types, they maintained both normal C-shape conformations during swimming and normal undulating S-shape conformations during crawling (Fig. 3A, B). These results suggest that loss of TWK-7 which most probably mimics a closure of the K\(^+\) channel affects swimming and crawling in a coordinated manner.

An age-dependent decline of locomotor activity has been reported for wild-type *C. elegans* (Schreiber et al. 2010). Both *twk-7* loss-of-function mutants also showed a constant decrease of swimming activity over 12 days starting with L4 larvae (Fig. S2). Nevertheless, during the whole aging period the *twk-7* mutants still had higher locomotor activities than age-matched control worms. Thus, TWK-7 function affects locomotor activity throughout *C. elegans* adulthood but does not influence the gradual reduction of activity caused by the aging process.

**An intact potassium pore is required for the function of TWK-7 in maintaining normal swimming and crawling activity**

To further investigate the function of TWK-7 in affecting locomotor activity, the *twk-7(nf120)* null mutant was transformed either with the *twk-7* containing cosmid F22B7 or with the construct *cauEx[twk-7p(3000)::TWK-7::mCherry::let-858(3’UTR)]* that consists of the full genomic region of *twk-7* including 3000 bp of the promoter. Analysis of the resulting transgenic worms showed that these constructs rescued both the accelerated crawling and
swimming activity of twk-7(null), even slightly below wild-type level (Fig. 4A-C; File S6). In contrast, the activity of swimming and crawling was not affected in the non-transgenic siblings.

In a functional K2P channel dimer, the pore domains P1 and P2 of both subunits contribute to the formation of one conductance pore specific for K⁺ (Enyedi and Czirjak 2010). Single amino acid substitutions within the ion selectivity filter of pore domain P1 inactivates channel function due to suppression of K⁺ conductance (Kollewe et al. 2009). Here, we introduced the mutations into our twk-7 construct cauEx[twk-7p(3000)::TWK-7::mCherry::let-858(3’UTR)] to analyze whether the potassium pore of TWK-7 is required for locomotor function. We found that neither mCherry tagged mutant channel TWK-7(G282C) nor TWK-7(T279K) is able to rescue twk-7(null) locomotion phenotypes (Fig. 4A-C; Fig. S3A, B) which is indicative for a disrupted TWK-7 channel function. Confocal microscopy analysis showed an expression pattern of the TWK-7(G282C)::mCherry mutant channel similar to that of wild-type TWK-7::mCherry (data not shown). Remarkably, expression of TWK-7(G282C)::mCherry in wild-type worms resulted in an elevation of both crawling and swimming activity when compared with non-transgenic siblings or non-transformed wild-type worms (Fig. 4A-C). Because the two-pore domain K⁺ channels are obligate dimers whose pore domains form a single ion pore, our data indicate a successful expression of a dominant-negative TWK-7 mutant protein that most probably suppresses potassium conductance owing to an impaired ion selectivity filter. We conclude that an intact K⁺ channel function of TWK-7 is necessary to maintain normal spontaneous locomotor activity.

TWK-7 is expressed in all types of motor neurons of the ventral nerve cord

To determine the expression pattern of TWK-7, we employed the ohEx[twk-7p(3000)::DsRed::let-858(3’UTR)] construct (Kratsios et al. 2012) expressing a cytosolic
DsRed reporter protein under the control of the twk-7 promoter. For co-localization studies, we used the vsIs48[unc-17p::GFP::let-858(3’UTR)] construct that expresses a GFP reporter protein in the entire set of cholinergic neurons with the exception of VC neurons (Kratsios et al. 2012). Confocal microscopy of transgenic worms revealed that the twk-7 promoter drives DsRed reporter expression in some head and tail neurons as well as in all A-, B-, and AS-type motor neurons of the ventral nerve cord (Fig. 5A-C; Fig. S4A-D). In addition, DsRed expression was seen in GFP-negative neurons. According to their number and stereotypic position, these TWK-7 expressing cells were identified as the 13 ventral and 6 dorsal GABAergic D-type motor neurons of the ventral nerve cord. The cell type specific expression pattern of TWK-7 was confirmed in worms that express a C-terminal tagged TWK-7::mCherry fusion protein under the control of the twk-7 promoter (Fig. 5D, E). TWK-7::mCherry was localized along the ventral nerve cord and in punctated structures in the soma of motor neurons (Fig. S4E-G) suggesting that TWK-7 is trafficked by the secretory Golgi-dependent pathway. Taken together, in accordance with the unraveled impact of TWK-7 on locomotor activity of C. elegans, TWK-7 is expressed in all types of motor neurons of the ventral nerve cord that control body wall muscle activity.

**TWK-7 is required in excitatory cholinergic motor neurons to maintain normal spontaneous crawling activity**

Cholinergic B-type and A-type motor neurons excite muscles on each side of the body during forward and backward crawling while indirectly inhibiting muscles through excitation of GABAergic D-type motor neurons on the complementary side (Chalfie et al. 1985; Sengupta and Samuel 2009). The B-type neurons act like a single command unit switching local segment oscillations on or off and modulate the speed and amplitude of the local segment waves (Bryden and Cohen 2008). Here we asked whether TWK-7 functions in a certain subset of neurons to maintain normal spontaneous locomotor activity. Expression of
TWK-7::mCherry exclusively in cholinergic excitatory motor neurons of the ventral nerve cord reduced both the crawling (Fig. 6 A, B) and swimming activity (Fig. 6C) of twk-7(null) comparable to the values obtained for transgenic worms expressing twk-7 under its own promoter. Ectopic expression of TWK-7::mCherry in all cholinergic neurons of twk-7(null) resulted in uncoordinated and almost immobile swimming animals (Fig. 6C) indicating that a correct spatial expression of TWK-7 is critical for its locomotor function. Complementary to the results of the rescue approach, expression of the dominant negative TWK-7(G282C)::mCherry mutant channel in cholinergic excitatory motor neurons of wild-type worms led to an increased crawling and swimming activity (Fig. 6A-C). Thus, functional TWK-7 is required in excitatory cholinergic motor neurons (A- and B-type) to maintain normal spontaneous locomotor activity.

**TWK-7 is required in cholinergic B-type motor neurons to maintain normal spontaneous crawling activity**

In order to examine the role of TWK-7 in single subtypes of the cholinergic and GABAergic neurons, we expressed the wild-type variant of TWK-7 in the twk-7(null) background, and the dominant negative TWK-7(G282C) variant in the N2 wild-type background under the control of the unc-4 (cholinergic A-type motor neurons), acr-5 (cholinergic B-type motor neurons) and unc-47 (GABAergic D-type motor neurons) promoter. Expression in A-type motor neurons did not induce any change in the spontaneous forward crawling behavior compared with corresponding non-transgenic animals (Fig. 7A, B). In contrast, worms with twk-7(null) background expressing acr-5p::TWK-7 (B-type) or the unc-47p::TWK-7 (D-type) construct exhibited markedly decreased locomotion rates (Fig. 7A, B). The spontaneous crawling activities of acr-5p::TWK-7 (0.18 ± 0.05 Hz; 0.08 ± 0.01 mm*s⁻¹) and unc-47p::TWK-7 (0.21 ± 0.01 Hz; 0.09 ± 0.01 mm*s⁻¹) transgenics were similar to those of wild-type animals (0.23 ± 0.02 Hz; 0.09 ± 0.01 mm*s⁻¹), but significantly reduced in comparison with the twk-7(null)
mutants (0.46 ± 0.07 Hz; 0.13 ± 0.02 mm*s⁻¹). Consistently, the dominant negative effect induced by the acr-5p::TWK-7(G²⁸²C) in wild-type background led to notably elevated spontaneous locomotion rates (0.47 ± 0.05 Hz; 0.13 ± 0.02 mm*s⁻¹) compared with the N2 control animals (0.23 ± 0.06 Hz; 0.09 ± 0.01 mm*s⁻¹) (Fig. 7A, B). Despite the fact that overexpression of TWK-7 in D-type motor neurons rescued hyperactive phenotype of twk-7(null), dominant negative TWK-7(G²⁸²C) did not accelerate locomotion of wild-type worms when expressed in D-type motor neurons. Thus, functional TWK-7 is required in cholinergic B-type but not in GABAergic D-type motor neurons of the ventral nerve cord to maintain the normal activity of spontaneous crawling.

TWK-7 abundance in cholinergic B-type and GABAergic D-type motor neurons affects wave parameters during spontaneous crawling

The crawling pattern of C. elegans and other nematodes is determined by a sinusoidal wave propagated along the body from the head to the tail, where each segment of the worm’s body follows the preceding segment (GRAY and LISSMANN 1964; PARK et al. 2008) (Fig. 8A). In this context we were interested to know to what extent the elevated speed of progression of twk-7(null) worms affects parameters (i.e. amplitudes and wavelengths) which determine the shape of the wave (Fig. S6A, B). To compare worm strains with different body proportions (Fig. S6C), we calculated the respective wave parameters in percentage of body lengths. We found that twk-7(null) mutants moved with about 15 % lower amplitude to body length ratios than wild-types (Fig. 8B), whereas the wavelength to body length values of both strains were very similar (Fig. 8C). Therefore, since the propagation speed of the wave along the body (Fig. 8A) is determined by the frequency and the wave length, the higher bending frequency of twk-7(null) animals (Fig. 7A) was linearly translated into an increased wave velocity (Fig. S6D). Generally, increased wave velocities were associated with a decreased locomotion efficiency as indicated by the calculated slip values (Fig. S6E).
To further unravel the relationship between TWK-7 expression and wave shape modulation, we next analyzed the effects of an impaired TWK-7 function and of TWK-7 overexpression on amplitudes and wave lengths at the level of the cholinergic motor neurons. Similar to \textit{twk-7}(\textit{null}) mutants, transgenic wild-type worms expressing the dominant negative TWK-7(G\textsuperscript{282}C) allele in cholinergic motor neurons (driven by the \textit{unc-17}(1 kb) promoter) or solely in B-type motor neurons (driven by the \textit{acr-5} promoter) moved with about 13 % reduced amplitude to body length ratios when compared to wild-type (Fig. 8B). The amplitude to body length ratios were not affected by TWK-7(G\textsuperscript{282}C) expression in A-type (driven by the \textit{unc-4} promoter) or D-type motor neurons (driven by the \textit{unc-47} promoter). Moreover, for all transgenic wild-types expressing the dominant negative TWK-7(G\textsuperscript{282}C) the wave length to body length ratios were wild-type-like (Fig. 8C).

When the wild-type form of TWK-7 was overexpressed in cholinergic motor neurons (\textit{unc-17} (1 kb) promoter) or solely in the B-type motor neurons (\textit{acr-5} promoter) of \textit{twk-7}(\textit{null}) mutants, the amplitude to body length ratios were reduced by about 10 % and 20 % when compared to non-transgenic \textit{twk-7}(\textit{null}) and wild-type animals, respectively (Fig. 8B). Moreover, animals overexpressing TWK-7 under its own or both \textit{unc-17} promoter variants frequently initiated an additional wave along their bodies during spontaneous forward crawling (Fig. 8D, File S6) thereby showing reduced amplitude to body length- and wave length to body length ratios (Fig. 8D). Notably, the overexpression of the wild-type form of TWK-7 in D- type motor neurons of \textit{twk-7}(\textit{null}) worms (driven by the \textit{unc-47} promoter) induced drastic increases of the amplitude to body length ratios that were found to be about 35 % and 40 % higher than in wild-type and \textit{twk-7}(\textit{null}) animals, respectively (Fig. 8B, File S5). Moreover, the wave length to body length ratios were increased by about 10 % in these transgenics when compared to the corresponding non-transgenics (Fig. 8C). Remarkably, expression of the wild-type form of TWK-7 in cholinergic A-type motor neurons of \textit{twk-}
7(null) worms (driven by the unc-4 promoter) did not affect the wave parameters. These animals exhibited twk-7(null) phenotype. All transgenics that exhibited reduced crawling activity (Fig. 7A) also had decreased wave velocities (Fig. S6D).

Summarizing, the abundance of functional TWK-7 at the level of B- and D-type motor neurons affects the wave lengths and amplitudes during spontaneous crawling. An impaired TWK-7 in B-type neuron is sufficient to reduce the wave amplitude, whereas overexpression of wild-type TWK-7 in D-motor neurons is sufficient to elevate the wave amplitude. Increasing twk-7 expression specifically in B-type neurons was sufficient to frequently induce an additional wave during spontaneous crawling.

**TWK-7 abundance in cholinergic B-type and GABAergic D-type motor neurons affects the duration of spontaneous forward crawling**

Our findings on the impact of TWK-7 on locomotion activity prompted us to investigate whether the temporal distribution of crawling forwards, crawling backwards, and dwelling is affected in the twk-7(null) mutants. The inspection of tracks generated by twk-7(null) mutants over a longer time period (17 hours) revealed that the area explored by the mutant worms were noticeably larger than the area covered by wild-types animals (Fig. 9A). Consistently, compared to wild-type, these animals spent more time on crawling forwards in expense of reduced dwelling times, while the relative duration of crawling backwards was not altered (Fig. 9B). Wild-type worms crawled 41.9 ± 17.5 % of their time in the forward mode and 54.5 ± 15.8 % of their time they rested, whereas, the respective values of twk-7(nf120) were 63.7 ± 14.9 % and 31.0 ± 12.7 %. Mutants carrying the twk-7(gk760044) allele also allocated their time preferentially to crawling forwards (62.1 ± 9.7 % crawling forwards, 30.8 ± 9.1 % dwelling). Expression of TWK-7 under its own promoter as well as under the cholinergic motor neuron specific promoter completely rescued the crawling behavior phenotype of twk-
In accordance to this rescue approach, impairing TWK-7 channel function by expressing the dominant negative mutant variant TWK-7(G$^{282}$C) in cholinergic motor neurons of wild-type worms led to a temporal distribution of crawling behaviors similar to twk-7(null) (Fig. 9B). Regarding the subtype level of motor neurons, transgenic twk-7(nf120) animals expressing acr-5p::TWK-7 (B-type motor neurons) and unc-47p::TWK-7 (D-type) spent more time on dwelling and less time on forward crawling than the twk-7(null). Wild-type worms carrying the dominant negative TWK-7(G$^{282}$C) in the B-type motor neurons exhibited similar increased forward crawling activity like the twk-7(null) mutants. These transgenics spent more time in the state of forward crawling and exhibited shorter dwelling periods than the non-transgenic wild-types (Fig. 9C). The dominant negative TWK-7(G$^{282}$C) construct expressed in GABAergic D-type neurons had no influence on the duration of forward crawling and dwelling. Overall, the action of TWK-7 in cholinergic B-type and GABAergic D-type motor neurons of the ventral nerve cord influences the persistence of forward crawling.

**TWK-7 abundance in cholinergic B-type and GABAergic D-type motor neurons affects the straightness of forward crawling**

We noticed that the straightness of crawling forwards, which is defined by the ratio of distance to track length, was improved in twk-7(null) mutants (Fig. 10A). It was found to be $43.1 \pm 11.8\%$ for wild-type compared to $64.5 \pm 11.6\%$ for twk-7(null) and $67.5 \pm 16.8\%$ for twk-7(gk760044), respectively. This crawling behavior phenotype was rescued by TWK-7 expression driven by its own, by the cholinergic motor neuron specific, by the B-type motor neuron specific, and by the D-type motor neuron specific promoter, respectively (Fig. 10B, C). Overexpression of both TWK-7 and TWK-7(G$^{282}$C) in A-type motor neurons of twk-7(null) and wild-type, respectively, had no influence on the straightness of forward crawling. However, when expressed in the B-type motor neurons of wild-type worms, the dominant
negative TWK-7(G^{282}C) construct resulted in an improved straightness similar to twk-7(null), while its expression in GABAergic D-type neurons had no influence on the straightness of forward crawling. Taken together, these data indicate that functional TWK-7 is required in the cholinergic excitatory B-type and inhibitory GABAergic D-type motor neurons of the ventral nerve cord not only to maintain normal spontaneous locomotion activity but also normal spontaneous locomotion behavior.

Loss of TWK-7 function affects locomotion parameters in a manner characteristic for stimulated locomotion behavior

Compared to wild-type, crawling of twk-7 deficient mutants and of transgenics overexpressing a dominant negative from of TWK-7 is characterized by (i) an increased activity preferentially on crawling forwards, (ii) an extended time spent on crawling forwards, and (iii) an improvement of the straightness of crawling forwards. Remarkably, we found that these parameters were similarly affected in wild-type animals stimulated by the picking transfer assay (Fig. 11B; File S7, S8). The stimulated worms dramatically increased their forward crawling activities about 2.8-fold (Fig. S5A, B; Fig. 11B-E), straightness rates about 2-fold (Fig. 10B, C; Fig. 11F, G) and also reduced their amplitude to body length ratios by about 15% to the level of spontaneously crawling twk-7(null) mutants (Fig. 8B; Fig. 11H). Consequently, it is tempting to speculate that TWK-7 might be involved in a forward escape or foraging behavior. To further test our hypothesis, we have developed a forward escape score that is based on the distribution of time spent on spontaneous crawling forwards, the straightness of spontaneous forward movement, and the spontaneous velocity. This escape score is approximately four times higher in both twk-7(null) mutants compared to wild-type (Tab. 1). Expression of TWK-7::mCherry under its own promoter as well as under the cholinergic motor neuron specific promoter rescued the increased escape score of twk-7(null) mutants to the wild-type level (Tab. 1). In contrast to that and consistent with the results
presented above, the escape score of N2 wild-type worms that expressed the dominant negative TWK-7(G^{282}C) mutant protein in TWK-7-specific neurons or in cholinergic motor neurons was found to be similarly elevated as seen for twk-7(null) (Tab. 1). These results underline our hypothesis that TWK-7 may be specifically involved in escape-like locomotion behaviors by enhancing the activity, duration, and straightness and adjusting the wave parameters of moving forwards.

Next, we investigated how the constitutively hyperactive phenotype of twk-7(null) mutants is affected by picking transfer stimulation. Although starting from a hyperactive state under non-stimulated conditions, these animals further increased their forward crawling activity about 1.6-fold (Fig. S5A,B; Fig. 11B-E), and straightness rates about 1.5-fold (Fig. 10B, C; Fig. 11F, G), however, only up to the level of the stimulated wild-type worms. Therefore, the picking-transfer-process induced activating pathways that led to a further increase in these locomotion parameters. The amplitude to body length ratios of twk-7(null) worms remained unchanged after stimulation when compared to the spontaneous crawling conditions (Fig. 8B; Fig. 11H). Hence, the locomotion parameters of stimulated wild-type match those of stimulated twk-7(null) animals. Consistently, the expression of the dominant negative TWK-7(G^{282}C) in wild-types did not induce any significant effects on the stimulated wild-type crawling (Fig. 11B-I).

Rescued twk-7(null) mutant worms that overexpressed wild-type TWK-7 under the twk-7 promoter were not able to reach the stimulated forward crawling activity exhibited by wild-type as well as by twk-7(null) animals (Fig. 11B, D; File S9). This effect was also observed by overexpression of TWK-7 in the entire set of cholinergic, B-type or D-type motor neurons (Fig. 11C, E). The generation of the above mentioned additional wave seen during spontaneous crawling in rescued animals occurred more frequently after picking transfer stimulation (File S9).
Although our current data do not allow to draw the conclusion that TWK-7 contributes to the stimulated forward crawling behavior, we found that wild-type worms respond to the external stimulation by changing their crawling parameters reminiscent to the spontaneous hyperactive locomotion phenotype of twk-7(null).
Discussion

Here we have shown that the *C. elegans* two-pore domain potassium (K$_2$P) channel TWK-7 is required to maintain both normal spontaneous rhythmic locomotor activity and normal spontaneous locomotion behavior. In spite of predicted biochemical similarities within the *C. elegans* TWK family (Kratsios et al. 2012; Salkoff et al. 2001), we found that the locomotion phenotypes of two different *twk-7(null)* alleles were distinct from several other *twk* mutants. Our data indicate that these functions can be ascribed to a cholinergic B-type and GABAergic D-type neuron specific expression of TWK-7.

In the past two decades by means of molecular and electrophysiological methods it has been recognized that the eukaryotic K$_2$P channels are regulatory K$^+$ selective leak channels that modify the membrane potential of neurons and other cell types (Enyedi and Czirjak 2010). Nevertheless, data defining the physiological role of native K$_2$P channels are still limited. In principle, activated K$_2$P channels hyperpolarize the membrane potential by increasing potassium permeability. Diametrically opposed, closure or a genetic knock-out of K$_2$P channels depolarize the membrane potential resulting in an increased excitability of cells (Enyedi and Czirjak 2010). Accordingly, we suggest that the enhanced spontaneous locomotion activity of *twk-7(null)* animals is caused by an elevated excitability of neurons. This acceleration of *twk-7(null)* worms also implicates that in wild-type worms TWK-7 is activated during spontaneous crawling. An activated K$_2$P channel lowers the excitability of cells and, to that effect, overexpressing TWK-7 reduced the locomotor activity of *twk-7(null)* mutants even slightly below that of wild-type worms. Moreover, the additional wave that appears in worms with overexpressed TWK-7 channels can be explained by a reduced excitability of motor neurons that leads to decreased wave lengths and bending frequencies causing a slowed down forward propagation of the wave. The proprioception mechanism that is transduced by B-type cholinergic motor neurons (Wen et al. 2012) may be involved here.
given that a postulated rhythmic generator near the head initiates a certain wave frequency. The respective rhythmic wave initiation pattern appears to be periodically out of phase. Since the activity of $K_2P$ channels requires a dimeric structure (Enyedi and Czirjak 2010), the expression of TWK-7 mutant channels with disabled potassium selectivity filter should have an antimorphic effect by binding and thus inactivating the normal wild-type channels. As expected, the expression of the mutant channel TWK-7($G^{282}$C) solely in cholinergic excitatory B-type motor neurons did not influence the elevated locomotor activity of $twk$-$7$($loss$ of $function$) mutants but increases the activity of wild-type worms. Worthy of note, in contrast to the classical uncoordinated $C.$ $elegans$ mutants (Brenner 1974) which impair normal behavior and mainly define constitutive elements of neuromuscular functions, the $twk$-$7$($loss$ of $function$) mutants and the TWK-7($G^{282}$C) transgenics showed coordinated, directed and accelerated locomotion within a physiological range. Knock-out models and pharmacological approaches in the fly and in mammals suggested that $K_2P$ channels are involved in the regulation of rhythmic muscle activity (Aller et al. 2005; De la Cruz et al. 2003; De la Cruz et al. 2014; Decher et al. 2011; Donner et al. 2011; Kunkel et al. 2000; Lalevee et al. 2006). For example, the heart rate of $D.$ melanogaster was accelerated by the cardiac-specific inactivation of the $K_2P$ channel ORK1 via RNAi, while overexpression of ORK1 led to the stop of heart beating. Similarly, pharmacological activation of the $K_2P$ channel TASK is associated with decreased motor neuronal excitability, which most likely contributes to the immobilizing effect of anesthetics (Lazarenko et al. 2010). In good accordance with these reported findings, we suggest that our data may point to a regulatory function of $C.$ $elegans$ TWK-7 in cholinergic B-type motor neurons. Alternatively, a genuine regulator of locomotor activity may act upstream of TWK-7 within the same motor neurons or in upstream neurons (e.g. interneurons). However, up to now we cannot exclude that the activity of TWK-7 in motor neurons may represent a relevant background condition but not a mode of regulation in the context of spontaneous $C.$ $elegans$ locomotion.
According to the locomotion circuit diagram shown in Fig. 8A, activated cholinergic B-type motor neurons synapse on both the related body wall muscles and the corresponding GABAergic D-type motor neurons which then inhibit the contralateral body wall muscles. These inhibitory GABAergic motor neurons have been reported to be not essential for rhythmic activity during forward crawling (McIntire et al. 1993). Here, we found that adequate TWK-7 expression is required in D-type GABAergic motor neurons to maintain both activity and straightness of spontaneous movement suggesting that the excitability of these motor neurons may be important for specific locomotion behaviors. In particular, overexpression of TWK-7 in D-type motor neurons was found to counteract the changes in locomotor activity and behavior of twk-7(null) mutants. Given that B-type motor neurons activate the contralateral inhibitory D-type motor neurons, we assume that a specifically reduced excitability of D-type motor neurons due to TWK-7 overexpression may represent a limiting step in the otherwise elevated B-type motor neuron driven rhythmic muscle contraction of twk-7(null) mutants. Here, both muscle contraction and contralateral muscle relaxation are altered. Hyperexcitability of B-type motor neurons most probably elicits hypercontraction of body wall muscles and in accordance with the study of (Donnelly et al. 2013), a hypoexcited D-type motor neuron impairs relaxation of body wall muscle which leads to a more pronounced wave amplitude. However, these manipulations specifically affected dorsal or ventral GABAergic activity generating a directional bias. In our studies we observed that the twk-7(null) worms expressing native TWK-7 in GABAergic motor neurons moved slow and uncoordinated exhibiting larger S-shapes with higher amplitudes. A role of the GABAergic neurons in regulating shape and amplitude of the worms is in agreement with the literature (Bryden and Cohen 2008; McIntire et al. 1993; Yanik et al. 2004).

In contrast to the situation in the excitatory B-type motor neurons, an impaired TWK-7(G282C) channel was not able to induce increased velocity, straightness and duration of
forward movement or affect the wave form in wild-type animals via D-type motor neuron specific overexpression. Based on the neuronal locomotion circuit, the muscle contraction and D-type motor neuron dependent contralateral body wall muscle relaxation is B-type motor neuron dependent. Apparently, a potentially faster muscle relaxation caused by the hyperexcited D-type motor neuron is not able to affect the next contraction by the ipsilateral B-type motor neuron. Hence, an increased excitability of D-type motor neurons due to expression of an inactivated TWK-7 channel does not affect the rhythm and wave form of unstimulated forward crawling in wild-type worms. However, we can not exclude that cell-type specific differences in channel morphology or regulation might implicate distinct functions of TWK-7 in cholinergic and GABAergic motor neurons accounting for the lack of a dominant negative effect of TWK-7(G282C) in GABAergic motor neurons.

It is an interesting question how inactivation of TWK-7 in cholinergic motor neurons causes preferentially forward movement. It has been shown that distinct premotor interneurons (AVA, AVE, AVD, AVB, PVC) innervate the A- and B-type motor neurons to drive backward and forward movement (CHALFIE et al. 1985; ZHENG et al. 1999). Moreover, the cross-inhibition between the forward and backward circuit and the premotor interneuron-motor neuron coupling via gap junctions has been demonstrated to establish an imbalanced motor neuron output in favor of forward locomotion (KAWANO et al. 2011; ZHENG et al. 1999). The forward circuit is generally active and represents the default mode of locomotion direction, whereas the backward circuit is rather proactive regulated by touch and other stimuli (CHALFIE et al. 1985). Similar models of the neuronal control of locomotion direction have been unraveled in Drosophila (BIDAYE et al. 2014), crickets (SCHONEICH et al. 2011), and cockroaches (BURDOHAN and COMER 1996). All of these models predict that higher excitability of the motor neurons reinforce the activity of the forward circuit. In line with this,
we have shown that a closed TWK-7 channel solely in cholinergic B-type motor neurons is sufficient to enhance the duration of forward movement in expense of dwelling times.

Moreover, it is quite remarkable that loss of TWK-7 function solely in B-type motor neurons was sufficient to enhance the straightness of spontaneous crawling. We suggest that at least two factors are responsible for this locomotion phenotype. First, the lower backward crawling rate of twk-7(null) worms improves the straightness, because in C. elegans re-orientation is frequently associated with backing events (DONNELLY et al. 2013). Second, the lower amplitudes of twk-7(null) worms during forward crawling may favor a more directed movement.

The regulation of velocity, duration, and direction of movement in response to external and internal cues is a hallmark of adaptive locomotion behavior. Escape behaviors and foraging are important examples for this. In agreement with a previous report (GAGLIA and KENYON 2009), we found that the transfer of worms to new assay plates by a platinum wire stimulates fast straightforward crawling when comparing to spontaneous movement. Since this effect - although declining - persists (Fig. S8), we suggest that it might reflect an escape-like behavior rather than an escape reflex. Spontaneously moving twk-7(null) mutants did not reach the activity level of stimulated wild-type worms indicating that, if TWK-7 is involved in this adaptive locomotion behavior, additional activating pathways are required. Although our current data do not allow to draw the conclusion that twk-7 contributes to this stimulated escape-like behavior, it is quite remarkable that five central aspects of locomotion - velocity/frequency, wave parameters, forward direction, duration, and straightness - that are constitutively affected by loss of TWK-7 function are also induced by external stimulation. Thus, we suggest that TWK-7 – together with other factors - may act as a putative player in the context of fast targeted forward locomotion. Since the K2P channels are regulated by a huge number of stimuli (ENYEDI and CZIRJAK 2010), preferentially the N- and C-terminal
region of TWK-7 might constitute a direct target for regulators such as protein kinases and/or fatty acids. It will be particularly important to test this hypothesis in order to get insight into the pathways that regulate TWK-7.

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Conflict of Interest

The authors declare that they have no conflict of interest.
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# Tables

## Table 1

<table>
<thead>
<tr>
<th>Allele/Transgenic</th>
<th>% Time forward ($T_{fw}$)</th>
<th>Straightness (St)</th>
<th>Velocity Rate ($V/v_s$)</th>
<th>Escape Score ($T_{fw}$ x St x V)</th>
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<td>N2</td>
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<td>twk-7(nf120)</td>
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<td>0.65 ± 0.12</td>
<td>0.64 ± 0.05</td>
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<td>twk-7(gk760044)</td>
<td>0.62 ± 0.10</td>
<td>0.68 ± 0.17</td>
<td>0.64 ± 0.03</td>
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<td>Ex[twk-7p:TWK-7]*</td>
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<tr>
<td>Ex[twk-7p:TWK-7(G282C)]*</td>
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<tr>
<td>Ex[twk-7p:TWK-7(G282C)]§</td>
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<td>0.67 ± 0.12</td>
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<td>0.22</td>
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\* twk-7(nf120) and § N2 background, respectively
Table legends

Table 1. Escape scores of TWK-7(null) animals compared to N2 wild-types and transgenic strains overexpressing the wild-type form of TWK-7 or the mutant channel TWK-7(G^{282}C). The velocity rate V reflects the ratio between stimulated (v) and spontaneous velocity (v_s). The values for percentage of time crawling forwards (T_{fw}), straightness (St) and the velocity rate (V) represent the means ± SD.

Figure legends

Figure 1. twk-7 mutant alleles exhibit enhanced spontaneous crawling activity. (A) A small scale locomotion screen revealed that twk-7(nf120) and twk-7(gk760044) animals exhibited notably enhanced velocities during spontaneous crawling when compared with wild-type and mutant strains of other TWK-family members that are known to be expressed in motor neurons. The dotted line indicates the wild-type level. The values represent the means (± SEM) of N ≥ 3 independent experiments involving n ≥40 animals. *p < 0.05; **p < 0.01; ***p < 0.001 (Student’s t-test). (B) The schematic diagram of the TWK-7 protein indicates the predicted position of the pore domains P1 and P2 (dark grey) and the transmembrane domains M1-M4 (light grey). The polypeptide region that is not encoded by the deletion mutant allele nf120 and the localization of the premature stop of the allele gk760044 are depicted.

Figure 2. The hyperactive allele nf120 is a recessive null allele of twk-7. (A) The spontaneous body bending crawling frequencies (BBCF) and (B) the corresponding spontaneous crawling velocities of twk-7 mutant alleles. (C) The body bending swimming frequencies (BBSF) of the twk-7 mutant alleles. (D) Genetic complementation analyses
revealed that only worms homozygote for the twk-7 deletion allele (nf120/nf120) exhibited increased BBSF. Heterozygote worms (+/nf120) swam like wild-type (+/+). Bringing the nf120 allele in trans to the chromosomal deletion nDf21 of the balanced deficiency mutant nDf21/dpy-19(e1259),unc-32(e189)III resulted in worms (nf120/nDf21) with elevated BBSF similar to twk-7(nf120) homozygotes. Symbols represent single worms. Genotypes of the tested worms were determined by PCR and/or phenotype analyses of progeny n ≥ 20 animals. Dotted lines indicate the respective wild-type level. The values represent the means (± SEM) of (A-C) N ≥ 4 and (D) N = 3 independent experiments involving n ≥ 40 animals. *p < 0.05; **p < 0.01; ***p < 0.001 (Student’s t-test).

**Figure 3.** twk-7(null) animals swim and crawl in a coordinated manner. (A) The S- and (B) C-shape conformations of crawling and swimming worms were visualized using the curvature matrices. Sequence continuities indicate that twk-7(nf120) worms crawled (A) and swam (B) in a coordinated manner similar to wild-types although with higher body bending frequencies. The color code illustrates the dorsal (D, red) and ventral (V, blue) changes of body shape angles (up to 40° for swimming and 60° for crawling, respectively) over time during locomotion (for details see Material and Methods). The sinusoidal curves below depict the corresponding rhythmic curvature of the neck (for swimming) and midbody segment (for crawling) over time (segments are marked in yellow).

**Figure 4.** TWK-7 functions in motor neurons to affect locomotor activity. (A) The body bending crawling frequencies and (B) the respective crawling velocities of transgenics (+) expressing TWK-7 and the selectivity filter mutant TWK-7(G282C) under its own promoter. The values for the corresponding non-transgenic siblings (-) are shown. (C) The body bending swimming frequencies (BBSF) of transgenic twk-7(nf120) and transgenic N2 wild-type worms are depicted. Transgenics (+) are directly compared with non-transgenic (-) siblings.
Overexpression of TWK-7 in a twk-7(null) background restores the BBSF even below wild-type level. In contrast, the selectivity filter mutant TWK-7(G282C) did not affect the swimming rate of twk-7(nf120), but has a dominant negative effect on the swimming activity in a N2 genetic background.

**Figure 5. TWK-7 is expressed in ventral nerve cord, head and tail neurons.** (A) Cytosolic DsRed expression driven by the twk-7 promoter is overlaid with the cholinergic neuron specific cytosolic GFP expression mediated by the unc-17 promoter. Co-expression is indicated by yellow color. (B) Maximum projection of a z-series through the head region revealed that DsRed fluorescence is seen in cholinergic and non-cholinergic head neurons (C) Maximum projection of a z-series through the cholinergic region of the ventral nerve cord. Detailed co-expression analyses of TWK-7 and UNC-17 expressing cells in the ventral nerve cord indicates that TWK-7 is present in the cholinergic A-, AS- and B-type motor neurons as well as in UNC-17 negative cells. According to their stereotypic positions, these were identified as GABAergic D-type motor neurons. In addition, TWK-7 expression is seen in unidentified tail neurons. (D) Maximum projection of a z-series through the cholinergic region of the ventral nerve cord. Expression of a TWK-7::mCherry fusion protein, which includes the entire TWK-7 channel protein, leads to a punctate subcellular and membrane associated localization. (E) Co-expression of cytosolic green fluorescence protein is driven by the cholinergic neuron specific unc-17 promoter.

**Figure 6. TWK-7 function in cholinergic motor neurons is sufficient to affect the spontaneous locomotor activity of C. elegans.** (A) Crawling and (B) swimming activity analyses of transgenics expressing TWK-7 cell specifically. By employing two different unc-17 promoter constructs, TWK-7 and the dominant negative selectivity filter mutant channel TWK-7(G282C) were cell-specifically expressed in all cholinergic neurons (unc-17(4.2kb)p)
or solely in the subset of cholinergic motor neurons ($unc-17(1 \ kb)p$). For each transgene (+) the corresponding locomotor activity of non-transgenic sibling (-) is shown. The values represent the means (± SEM) of N ≥3 independent experiments involving n ≥ 30 animals. *p < 0.05; **p < 0.01; ***p < 0.001 (Student’s t-test). Dotted lines indicate the respective wild-type level.

**Figure 7. Cholinergic B-type and GABAergic D-type motor neuron specific expression of TWK-7 affects the spontaneous crawling activity of *C. elegans*.** (A) BBCF and (B) crawling velocity were analysed in $twk-7(null)$ and wild-type animals overexpressing the wild-type and the dominant negative G$^{282}$C mutant form of TWK-7 in A-type, B-type or D-type motor neurons, respectively. The transgenic worms (+) are directly compared with the non-transgenic (-) siblings. Dotted lines indicate the respective wild-type level. The values represent the means (± SEM) of N ≥3 independent experiments involving n ≥30 animals. *p < 0.05; **p < 0.01; ***p < 0.001 (Student’s t-test).

**Figure 8. TWK-7 acts in cholinergic B-type and GABAergic D-type motor neurons to affect the wave parameters during spontaneous crawling.** (A) Schematic of muscle control during forward crawling driven by B- and D-type motor neurons is depicted, where A, P, V and D indicate the anterior, posterior, ventral and dorsal direction, respectively. Excitatory cholinergic B-type motor neurons (VB, DB) innervate both body wall muscle cells and GABAergic motor neurons (VD, DD), the latter of which inhibit the activity of contralateral body wall muscles by GABA release. The premotor interneurons AVA and PVC which regulate the forward locomotor programme are shown. Figure is adapted from and wormatlas.org. (B) The amplitude to body length and (C) wave length to body length ratios during spontaneous crawling were analysed in $twk-7(null)$ and wild-type worms, respectively, expressing the wild-type and the dominant negative G$^{282}$C form of TWK-7 at the level of A-
type, B-type and D-type motor neurons. Dotted lines indicate the respective wild-type level.
The values represent the means (± SEM) of N ≥3 independent experiments involving n ≥ 30
animals. *p < 0.05; **p < 0.01; ***p < 0.001 (Student’s t-test). (D) Examples of S-shape
body curvatures of N2 wild-type, twk-7(nf120) mutant and transgenic animals during
spontaneous crawling are depicted. Worms overexpressing TWK-7 under the control of the
twk-7 promoter (Ex[twk-7p::TWK-7]), the cholinergic neuron specific (Ex[unc-
17p(4.2kb)::TWK-7]), or the cholinergic motor neuron specific unc-17 promoter (Ex[unc-
17(1kb)p::TWK-7]) frequently exhibited two overlapping S-shape curvatures during
spontaneous crawling thereby generating an additional phase (green dot). The colored dots
mark the phases (yellow: 0, red: π, blue: 2π, green: 3π) of wave propagation. The

corresponding body lengths, wave lengths and amplitudes of each worm determined by
ImageJ are presented below.

Figure 9. Loss of TWK-7 function in cholinergic motor neurons increases the duration of
spontaneous forward crawling. (A) For long-term analyses of spontaneous C. elegans
locomotion activity, the crawling tracks of single worms incubated for 17 h on standard NGM
plates are depicted. (B) The persistence of forward and backward crawling and dwelling
periods was analyzed in animals overexpressing the wild-type and the dominant negative
form G282C of TWK-7 in the twk-7-specific neurons, all cholinergic motor neurons (unc-
17(4.2kb)p), the cholinergic motor neurons of the ventral nerve cord (unc-17(1.0kb)p) and (C)
in the A-, B- and D-type motor neuron subtypes. The dotted lines indicate the respective wild-
type level. The values represent the means (± SEM) of at least N ≥ 3 independent experiments
involving n ≥ 30 animals. *p < 0.05; **p < 0.01; ***p < 0.001 (Student’s t-test).

Figure 10. Loss of TWK-7 function in cholinergic motor neurons increases the
straightness of spontaneous forward crawling. (A) Representative 1 min locomotion tracks
of twk-7(null) and N2 wild-types. (B) The straightness of forward crawling was analyzed in twk-7(null) and wild-type worms overexpressing the wild-type and the dominant negative form G^{282}C of TWK-7 in the twk-7-specific neurons, all cholinergic motor neurons (unc-17(4.2kb)p), the cholinergic motor neurons of the ventral nerve cord (unc-17(1.0kb)p) and (C) in the cholinergic and GABAergic motor neuron subtypes. The transgenics (+) are directly compared with non-transgenic (-) siblings. Dotted lines indicate the respective wild-type level. The values represent the means (± SEM) of N ≥ 3 independent experiments involving n ≥ 30 animals. *p < 0.05; **p < 0.01; ***p < 0.001 (Student’s t-test).

Figure 11. Stimulation of C. elegans forward crawling affects five locomotion parameters that are similarly altered in twk-7(null) mutants and TWK-7(G^{282}C) transgenics during spontaneous crawling. (A) To assay the stimulated crawling activity, groups of 10 young adult worms (age 72 h) were transferred to a new NGM agar plate and recorded immediately by camera. As shown here for wild-type worms, the animals leave the transfer point “T” by crawling straight forward (indicated by dotted arrows). Two pictures of the same movie are overlaid showing the position of worms at time point 0 s (pale worms) and 10 s (dark worms). (B, C) The stimulated BBCF and (D, E) the respective velocities of stimulated forward crawling were immediately determined for wild-type, twk-7(nf120) and transgenic animals. (F, G) The straightness rates, (H) amplitude to body length and (I) wave length to body length ratios of stimulated non-transgenics and transgenics are depicted. Dotted lines indicate the respective wild-type level. The values in (B-I) represent the means (± SEM) of N ≥ 3 independent experiments involving n ≥ 30 animals. *p < 0.05; **p < 0.01; ***p < 0.001 (Student’s t-test).
**Supplemental files**

**Figure legends**

**Figure S1. Amino acid sequence analysis of *C. elegans* TWK-7.** The gene *twk-7* encodes a predicted protein of 557 amino acid residues with only moderate sequence similarity (20-25%) to other K$_2$P channels, such as *C. elegans* SUP-9/TWK-38, mammalian TASK3 and the predicted *Drosophila melanogaster* K$_2$P channel CG43155. Similarity is restricted mainly to the two pore domains P1 and P2 including the K$^+$ channel specific ion selectivity filter and the transmembrane domains M1, M2, and M4. The crucial amino acid residues T$^{279}$ and G$^{282}$ within the selectivity filter are indicated by asterisks. Note that the TWK-7 sequence in the alignment starts at position 121. Owing to an unusually extended N-terminus of approximately 160 amino acid residues, the deduced TWK-7 polypeptide with a predicted molecular mass of 64.2 kDa is considerably larger than mammalian K$_2$P channels or *C. elegans* SUP-9/TWK-38 with deduced molecular masses of about 42 and 37 kDa, respectively.

**Figure S2. Analysis of age-dependent swimming activity.** The swimming activity (BBSF) of wild-type, *twk-7(nf120)* and *twk-7(gk760044)* animals were determined at different ages. The bars represent the means (± SD) of N ≥ 3 independent experiments involving n ≥ 40 animals.

**Figure S3. Expression of TWK-7 selectivity filter mutations G$^{282}$C and T$^{279}$K did not affect the swimming activity of *twk-7(nf120)* mutant worms.** The body bending swimming frequencies (BBSF) of three independent transgenic lines (+) expressing (A) the TWK-7(G$^{282}$C) and (B) the TWK-7(T$^{279}$K) channel mutant were compared with those of their non-transgenic siblings, N2 and *twk-7(nf120)* worms. Each bar represents the mean (± SD) of three independent experiments involving n = 30 animals.
Figure S4. Expression pattern of TWK-7. (A-D) Confocal microscopy analysis of worms expressing the reporter proteins GFP and DsRed under the control of the cholinergic neuron specific unc-17(4.2kb) and the twk-7(3.0kb) promoter, respectively. The box in (A) marks the posterior part of the body shown in more detail in (B-D), where A-, B-, AS- and D-type motor neurons of the posterior ventral nerve cord are labeled according to their numbering on wormatlas.org. (E-G) Laser scanning confocal microscopy analysis of worms expressing GFP and a TWK-7::mCherry fusion protein under the control of the unc-17(4.2kb) and twk-7(3.0kb) promoter, respectively. Small white arrows exemplarily indicate motor neurons that contain cytosolic GFP and punctated expression of mCherry in the soma. Gray arrows point to D-type neurons that express solely mCherry.

Figure S5. Body bending frequencies during periods of spontaneous forward crawling. L4 worms were transferred to fresh NGM assay plates and after 24 h at 20°C their body bending frequencies were determined only during periods of spontaneous forward crawling. Dwelling and periods of backward crawling were not considered. Data for (A) wild-type, twk-7(null) alleles and worms expressing wild-type TWK-7 or dominant negative TWK-7(G282C) in the TWK-7 specific neurons, cholinergic motor neurons and in (B) the motor neuron subtypes A, B, and D are shown. Columns represent the means ± SEM of at least three independent experiments with n ≥ 30 animals (Students t-test, * p < 0.05, ** p < 0.01 and *** p < 0.001). Dotted lines indicate the wild-type levels.

Figure S6. Wave parameters (absolute values), worm lengths and locomotor efficiencies during spontaneous crawling. (A) The absolute values of the wave amplitudes, (B) wave lengths and (C) worms lengths measured with ImageJ are depicted. (D) The wave velocity is given by the product of the body bending crawling frequency and the wave length. (E) The amount of slip reflects the locomotor inefficiency as described by (GRAY and LISSMANN 1964). Columns represent the means ± SEM of at least three independent experiments with n
≥ 30 animals (Students t-test, * p < 0.05, ** p < 0.01 and *** p < 0.001). Dotted lines indicate the wild-type levels.

Figure S7. The wave parameters (absolute values), wormlengths and locomotor efficiencies during stimulated crawling. (A) The absolute values of the wave amplitudes, (B) wave lengths and (C) worms lengths measured with ImageJ are depicted. (D) The wave velocity is given by the product of the bending frequency and the wave length. (E) The amount of slip reflects the locomotor inefficiency as described by (GRAY and LISSMANN 1964). Columns represent the means ± SEM of at least three independent experiments with n ≥ 30 animals (Students t-test, * p < 0.05, ** p < 0.01 and *** p < 0.001). Dotted lines indicate the wild-type levels.

Figure S8. Persistence of crawling velocities of N2 and twk-7(nf120) after stimulation by picking transfer. The crawling velocities of young adult C. elegans worms (age 72 h) were monitored over time after stimulation by transfer to fresh NGM agar plates. Each data point represents the mean ± SD of four independent determinations with n = 40 animals. The velocities of stimulated N2 and twk-7(nf120) worms declined significantly within the first 30 min (p = 0.0006 and p = 0.0074, respectively) and reached a plateau after 60 min with values similar to the spontaneous crawling velocities. Starting with similar stimulated crawling velocities, significant differences between the two strains became apparent and are indicated (Students t-test, * p < 0.05, ** p < 0.01 and *** p < 0.001).

Table legend

Table S1. Oligonucleotides and restriction sites used for the identification of mutant strains and the generation of genetic constructs.
**Movie legends**

**File S1.** Spontaneously crawling N2 worms on NGM agar under *ad libitum* feeding at 20°C.

**File S1.** Spontaneously crawling *twk-7(nf120)* worms on NGM agar exhibit elevated locomotion rates under *ad libitum* feeding at 20°C.

**File S3.** N2 wild-type worms swimming in M9 buffer at 20°C.

**File S4.** *twk-7(nf120)* mutants show increased locomotor activity during swimming in M9 buffer at 20°C.

**File S5.** Spontaneously crawling *twk-7(nf120)* worms overexpressing TWK-7 under the control of the GABAergic neuron specific *unc-47* promoter exhibit elevated amplitudes on NGM agar under *ad libitum* feeding at 20°C.

**File S6.** The elevated locomotion rate of spontaneously crawling *twk-7(nf120)* mutants is slowed slightly below the normal level of wild-types by the expression of the rescue construct *cauEx[twk-7p::TWK-7]*.

**File S7.** Stimulated N2 animals exhibit a fast and directed forward locomotion at 20°C on NGM agar plates seeded with *E. coli* OP50. Worms were recorded immediately after transfer to a new NGM agar plate using a platinum wire.

**File S8.** Stimulated *twk-7(nf120)* mutant worms show a similar fast and directed forward locomotion than wild-types at 20°C on NGM agar plates seeded with *E. coli* OP50. Worms were recorded immediately after transfer to a new NGM agar plate using a platinum wire.

**File S9.** Stimulated *twk-7(nf120)* mutants overexpressing the rescue construct *cauEx[twk-7p::TWK-7]* exhibit decreased locomotion rates at 20°C on NGM agar plates seeded with *E. coli* OP50. Worms were recorded immediately after transfer to a new NGM agar plate using a platinum wire.
Figures

Figure 1

A

Spontaneous crawling velocity [mm*s⁻¹]

B

1

258-315 (nf120) W120Stop (gk76044)
Figure 2

A

Spontaneous BBCF [Hz]

N2
twk-7(nf120)
twk-7(gk760044)

0.00
0.10
0.20
0.30
0.40
0.50
0.60
*** ***

B

Spontaneous crawling velocity [mm*s⁻¹]

N2
twk-7(nf120)
twk-7(gk760044)

0.00
0.03
0.05
0.08
0.10
0.13
0.15
0.18
0.20
*** ***

C

BBSF [Hz]

N2
twk-7(nf120)
twk-7(gk760044)

0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
*** ***

D

BBSF [Hz]

+/+
+/nf120
nf120/nf120+/unc,dpy
nf120/unc,dpy
+/nDf21
nf120/nDf21
0.0
0.5
1.0
1.5
2.0
2.5
3.0
*** ***
Figure 3

A

B

-60° -30° 0° 30° 60°

-60° -30° 0° 30° 60°

-60° -30° 0° 30° 60°

-60° -30° 0° 30° 60°

Dorsal

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Figure 4

A

Transgene: - twk-7p ::
TWK-7
-
(+) (-) (+) (-) (+) (-)
twk-7p ::
TWK-7
(G282C)
***
twk-7(null)
N2
Background:
Spontaneous BBCF [Hz]

B

Transgene: - twk-7p::
TWK-7
-
(+)(-) ... [Hz]

C

Transgene: - Cosmid
twk-7p::
TWK-7
(G282C)
Figure 5
Figure 6

A

B

C

Transgene: - - twk-7p::TWK-7 unc-17::TWK-7 unc-17 (1kb)p::TWK-7 unc-17 (1kb)p::TWK-7 (G282C) -

chol. chol. chol. chol.
neurons MN MN MN MN

Background:

***

***

***

**

***

**

Spontaneous BBCF [Hz]

Spontaneous crawling velocity [mm*s⁻¹]

BBSF [Hz]
Figure 7

A

Motor neurons: A B D
Transgene: - TWK-7 TWK-7(G282C)

Spontaneous BECF [Hz]

B

Motor neurons: A B D
Transgene: - TWK-7 TWK-7(G282C)

Spontaneous crawling velocity [mm/s]

Background:
- twk-7(null)
- N2

Spontaneous BBCF [Hz]

Spontaneous crawling velocity [mm/s]
Figure 8

**A**

- Direction of wave propagation
- Direction of forward crawling

**B**

- Ratio of amplitude to body length [%]
- Motor neurons: chol. MN A B D
- Transgene: TWK-7 TWK-7(G282C)

**C**

- Ratio of wave length to body length [%]
- Motor neurons: chol. MN A B D
- Transgene: TWK-7 TWK-7(G282C)

**D**

- Strain	| N2 | twk-7(null)| Ex[twk-7p::TWK-7] | Ex[unc-17p::TWK-7] | Ex[unc-17(1kb)p::TWK-7] |
- Body length [mm] | 1.24 | 1.22 | 1.18 | 1.15 | 1.06 |
- Wave length [mm]  | 0.62 | 0.60 | 0.43 | 0.39 | 0.38 |
- Amplitude [mm]    | 0.15 | 0.11 | 0.08 | 0.09 | 0.08 |
Figure 9

A

N2 10 mm

twk-7(nf120)

B

Crawling activity:
f = forward
d = dwelling
b = backward

C

Motor neurons:
A B D

Transgene: TWK-7	TWK-7(G282C)

Background:
twk-7(null)
N2

Crawling activity:
f = forward
d = dwelling
b = backward

Transgene: TWK-7

Temporal distribution of spontaneous crawling [%]

0 10 20 30 40 50 60 70 80 90 100

** * * * * * *

N2 twk-7(nf120) twk-7(gk760044)
Figure 10

A

N2

twk-7(null)

B

Motor neurons:

Transgene:

A

D

- TWK-7

(+)

(-)

N2

Background:

Spontaneous crawling straightness [%]

C

Motor neurons:

Transgene:

A

B

D

- TWK-7

(+)

(-)

N2

Background:

Spontaneous crawling straightness [%]

twk-7(nf120)
twk-7(gk760044)
N2

Background:

Spontaneous crawling straightness [%]

Chol. MN

**

***

0
10
20
30
40
50
60
70
80
90
100

0
10
20
30
40
50
60
70
80
90
100

(-)

(+)
Figure 11

A

B

C

D

E

Background:


twk-7(nf120)

twk-7(gk760044)

N2

Motor neurons:

Transgene:

A

D

- TWK-7

TWK-7(G282C)

B

A

B

D

- TWK-7

TWK-7(G282C)

- - -

chol. MN

Stimulated BBCF [Hz]

0.00

0.25

0.50

0.75

1.00

1.25

1.50

Transgene:

- - -

twk-7(nf120)

twk-7(gk760044)

N2

Background:

- - -

Stimulated BBCF [Hz]

0.00

0.25

0.50

0.75

1.00

1.25

1.50

Motor neurons:

Transgene:

A

D

- TWK-7

TWK-7(G282C)

B

A

B

D

- TWK-7

TWK-7(G282C)

chol. MN

Stimulated crawling velocity [mm*s^{-1}]

0.00

0.05

0.10

0.15

0.20

0.25

0.30

Transgene:

- - -

twk-7(nf120)

twk-7(gk760044)

N2

Background:

- - -

Stimulated crawling velocity [mm*s^{-1}]

0.00

0.05

0.10

0.15

0.20

0.25

0.30

Motor neurons:

Transgene:

A

D

- TWK-7

TWK-7(G282C)

B

A

B

D

- TWK-7

TWK-7(G282C)

chol. MN

Stimulated BBCF [Hz]

0.00

0.25

0.50

0.75

1.00

1.25

1.50

Transgene:

- - -

twk-7(nf120)

twk-7(gk760044)

N2

Background:

- - -

Stimulated BBCF [Hz]

0.00

0.25

0.50

0.75

1.00

1.25

1.50

Motor neurons:

Transgene:

A

D

- TWK-7

TWK-7(G282C)

B

A

B

D

- TWK-7

TWK-7(G282C)

- - -

chol. MN

Stimulated crawling velocity [mm*s^{-1}]

0.00

0.05

0.10

0.15

0.20

0.25

0.30

Transgene:

- - -

twk-7(nf120)

twk-7(gk760044)

N2

Background:

- - -

Stimulated crawling velocity [mm*s^{-1}]

0.00

0.05

0.10

0.15

0.20

0.25

0.30

Motor neurons:

Transgene:

A

D

- TWK-7

TWK-7(G282C)

B

A

B

D

- TWK-7

TWK-7(G282C)

- - -

chol. MN

Stimulated BBCF [Hz]

0.00

0.25

0.50

0.75

1.00

1.25

1.50

Transgene:

- - -

twk-7(nf120)

twk-7(gk760044)

N2

Background:

- - -

Stimulated BBCF [Hz]

0.00

0.25

0.50

0.75

1.00

1.25

1.50

Motor neurons:

Transgene:

A

D

- TWK-7

TWK-7(G282C)

B

A

B

D

- TWK-7

TWK-7(G282C)

- - -

chol. MN

Stimulated crawling velocity [mm*s^{-1}]

0.00

0.05

0.10

0.15

0.20

0.25

0.30

Transgene:

- - -

twk-7(nf120)

twk-7(gk760044)

N2

Background:

- - -

Stimulated crawling velocity [mm*s^{-1}]

0.00

0.05

0.10

0.15

0.20

0.25

0.30

Motor neurons:

Transgene:
Figure 11

**Legend:**
- **A** Motor neurons:
  - **D** Transgene:
  - TWK-7
  - TWK-7 (G282C)

**Background:**
- N2
- twk-7(null)
- twk-7(nf120)
- twk-7(gk760044)

**Graphs:**
- **F** Stimulated crawling straightness [%]
  - Transgene:
    - - TWK-7
    - TWK-7 (G282C)

- **G** Stimulated crawling straightness [%]
  - Motor neurons:
    - A
    - B
    - D
  - Transgene:
    - - TWK-7
    - TWK-7 (G282C)

- **H** Ratio of amplitude to body length [%]
  - Motor neurons:
    - chol.
    - MN
    - A
    - B
    - D
  - Transgene:
    - - TWK-7
    - TWK-7 (G282C)

- **I** Ratio of wave length to body length [%]
  - Motor neurons:
    - chol.
    - MN
    - A
    - B
    - D
  - Transgene:
    - - TWK-7
    - TWK-7 (G282C)