Developmental regulation of whole-cell capacitance and membrane current in identified interneurons in *C. elegans*.

Serge Faumont¹, Thomas Boulin², Oliver Hobert², and Shawn R. Lockery¹

1. Institute of Neuroscience, 1254 University of Oregon, Eugene OR 97403
2. Howard Hughes Medical Institute, Columbia University Medical Center, New York, New York 10032

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Corresponding author: Shawn Lockery, 1254 University of Oregon, Eugene, OR 97403, email: shawn@lox.uoregon.edu, phone: 541-346-4590, fax: 541-346-4548
Abstract

Post-embryonic developmental changes in electrophysiological properties of the AIY interneuron class were investigated using whole-cell voltage-clamp. AIY interneurons displayed an increase in cell capacitance during larval development, whereas steady-state current amplitude did not increase. The time course of the outward membrane current, carried at least in part by K⁺ ions, matured, from a slowly activating, sustained current to a rapidly-activating, decaying current. We also investigated how the development of capacitance and outward current was altered by loss-of-function mutations in genes expressed in AIY. One such gene, the LIM homeobox gene \textit{ttx-3}, is known to be involved in the specification of the AIY neuronal subtype. In \textit{ttx-3} mutants, capacitance and outward current matured precociously. In mutants of the gene \textit{wrk-1}, an immunoglobulin superfamily (IgSF) member whose expression is regulated by \textit{ttx-3}, capacitance matured normally, whereas outward current matured precociously. We conclude that AIY interneurons contain distinct pathways for regulating capacitance and membrane current.

\textbf{Key Words}: mutant, \textit{ttx-3}, \textit{wrk-1}, patch clamp
Introduction

During development, neurons undergo dramatic changes in their shape and electrical properties, progressively acquiring their adult phenotype. Maturation of electrical properties includes changes in the density and kinetics of voltage-dependent ion channels (Carrascal et al. 2005; Lockery and Spitzer 1992; Vabnick and Shrager 1998). It is presumed that changes in ionic currents reflect the execution of specific genetic programs but little is known about which genes and regulatory mechanisms are involved. The nematode *Caenorhabditis elegans* is a good model organism with which to address this question, because it allows the combination of biophysical techniques with genetic manipulations to study the regulation of neuronal function during development in identified neurons (Francis et al. 2003; Hobert 2003).

A neuron class of particular interest in *C. elegans* is AIY, which consists of two left-right symmetrical neurons. AIY interneurons are involved in a number of different behaviors, including locomotion (Gray et al. 2005; Tsalik and Hobert 2003), chemotaxis (Hirotsu and Iino 2005; Remy and Hobert 2005; Tsalik and Hobert 2003), thermotaxis (Mori and Ohshima 1995; Ryu and Samuel 2002), odor adaptation (Hirotsu and Iino 2005) and associative learning (Gomez et al. 2001; Remy and Hobert 2005). In addition, certain aspects of its development have been studied at the genetic level (Altun-Gultekin et al. 2001; Wenick and Hobert 2004). The adult phenotype of AIY is reached progressively through a cascade of transcription factors. At the top of this cascade, the LIM homeobox gene *ttx-3* regulates many class-specific aspects of AIY’s identity, including its axonal morphology and the expression of neurotransmitter receptors (Altun-Gultekin et al. 2001; Wenick and Hobert 2004). In contrast, pan-neuronal markers are preserved in *ttx-3* mutants, suggesting that the product of this gene is mostly involved in
specifying the AIY neuronal subtype. Abnormal development in *ttx-3* mutants leads to abnormal AIY-mediated behavior (Mori and Ohshima 1995; Remy and Hobert 2005; Tsalik and Hobert 2003). Thus the *ttx-3*-mediated control of AIY development has important implications for adaptive behavior in *C. elegans*.

Although many genes involved in AIY’s development have been characterized, almost nothing is known about the maturation of its electrical properties, and how these properties are affected by mutations in genes responsible for AIY’s identity. We therefore analyzed capacitance and whole-cell voltage-dependent current in AIY during larval development in wild-type (WT) animals, and also in animals in which specific AIY-expressed genes were genetically removed. We found that there is an age-dependent maturation in the electrical properties of AIY, and that this maturation is altered in animals that lack a LIM homeobox gene, *ttx-3*, and a specific target of this transcription factor, the IgSF member *wrk-1*. In particular, AIY interneurons undergo an increase in cell capacitance during larval development, without a concomitant increase in steady-state current amplitude. Furthermore, the time course of net outward currents changes from a slowly activating, sustained current to a rapidly-activating, decaying current as the animals age.

We found that in the case of the *ttx-3* and *wrk-1* mutants, AIY matured precociously, implying that the more mature state is a default state that AIY adopts in the absence of *ttx-3* and *wrk-1* function. The mutants also showed that developmental changes in capacitance can be genetically dissociated from changes in the time course of outward currents.
Materials and methods

Strains. The following C. elegans strains were used for the electrophysiological experiments: ttx-3(ks5) (Altun-Gultekin et al. 2001), ser-2(pk1357) (Tsalik and Hobert 2003), wrk-1(ok695), wrk-1(tm1099) (T.B., Pocock, R. and O.H., submitted). All animals contained the mgIs18 transgene (ttx-3prom::gfp) in the background to visualize the AIY interneurons with gfp (Altun-Gultekin et al. 2001); mgIs18 animals serve as WT control. We used the hypomorphic ks5 mutant allele of ttx-3, rather than available null alleles, because in null alleles gfp expression from the mgIs18 array is completely lost, therefore precluding the identification and recording from AIY. The two wrk-1 mutant alleles will be described in detail in T.B., Pocock, R. and O.H. (submitted). Briefly, both are deletion alleles with ok695 being a likely null allele.

Animals were grown in mixed-stage cultures at 20°C on 1.7% agar-filled plates containing nematode growth medium seeded with E. coli strain OP50 (Brenner 1974). We performed recordings at the 4 larval stages (L1 to L4) that characterize postembryonic C. elegans development (animals ranging in size from 200 to 628 µm). We did not record from adult animals because of the difficulty of exposing AIY for electrophysiological recordings at this stage. The following numbers of AIY recordings were obtained from these strains: wild-type, 14; ttx-3(ks5), 18; ser-2(pk1357), 12; wrk-1(ok695), 17; wrk-1(tm1099), 10.

Expression pattern. 4 kb of the upstream regulatory region of wrk-1 (F41D9.3) were fused to gfp using a PCR fusion technique and injected with pha-1(+) DNA into pha-1 mutants (Aurelio et al. 2002). Three independent arrays (otEx202-otEx204) showed similar expression patterns that will be described in more detail in T.B., Pocock, R. and O.H., submitted. Expression in AIY was assessed using the otIs133 transgene,
which labels the AIY interneurons with dsRed2 (Wenick and Hobert 2004). To assess \textit{wrk-1} regulation by \textit{ttx-3}, the \textit{otEx203} array was crossed into a \textit{ttx-3(ot22)} mutant background.

\textit{Electrophysiology.} Animals were prepared and dissected as previously described (Goodman et al. 1998; Lockery and Goodman 1998). Whole-cell patch clamp recordings were performed using electrodes manufactured from borosilicate glass (Sutter BF120-69-10, Novato, CA) and filled with (in mM): 125 K gluconate, 18 KCl, 4 NaCl, 1 MgCl$_2$, 0.6 CaCl$_2$, 10 HEPES, 10 EGTA (pH 7.2). External saline contained (in mM): 145 NaCl, 5 KCl, 1 CaCl$_2$, 5 MgCl$_2$, 10 HEPES (pH 7.2). Membrane currents were amplified with a modified Axopatch 200A (Axon Instruments, Foster City, CA), filtered at 10 kHz, and digitized at 25 kHz. Currents were further filtered at 2 kHz offline. Membrane currents were leak subtracted, and voltages were corrected for liquid junction potentials. AIY interneurons were identified by GFP expression (see strains). Steady-state current amplitude was measured as the average value during the last 5 ms of the voltage pulse. The early-to-late current ratio was defined as the ratio of the average current in the 5 ms time window beginning at $t = 5$ ms and the average current during the last 5ms of the voltage pulse. Recordings were performed randomly on either the left or right AIY neurons.

\textit{Statistics.} Data are expressed as mean ± SEM. Statistical analysis was performed with SPSS 11.0 software (SPSS Inc, Chicago, Ill). Statistical significance was assessed using a one-way ANOVA (Fig 1A, 1E, 2A, 2D, 4A, 5C, 6B, 7A, 7D), a one-way ANOVA for repeated measures (Fig. 1B, 2B, 4B, 6B), and a two-way ANOVA for repeated measures (comparison of WT and mutant IV curves). Statistical results are expressed in the form $F(a,b)=c$, where $a$ and $b$ are the degrees of freedom in the
ANOVA, and $c$ is the value of the F statistic.
Results

Development of wild-type electrical properties

To study the development of AIY’s electrical properties, we measured whole-cell capacitance and net membrane current as a function of developmental stage, using animal length as an approximate marker of stage. Animals were divided into two groups: L1 larvae and L2 to L4 larvae (L2-4), with an animal length of 350 microns being the cutoff between the two groups (Hirsh et al. 1976). We observed a 3-fold increase in whole-cell capacitance as the animal age increased (Fig. 1A; \( F(1,11) = 15.773, p < 0.01 \); see Material and Methods). However, the amplitude of steady-state membrane currents evoked by a family of voltage pulses remained constant (Fig. 1B; \( F(1,11) = 0.85, p > 0.05 \)). Thus there was an overall decrease in current density with age. This is surprising because other types of *C. elegans* neurons exhibit an increase in both capacitance and current amplitude as the animal grows (Goodman et al. 1998).

Although steady-state current amplitude remained constant over larval development, the time course of outward current changed. Fig. 1C shows average currents generated by voltage steps from –154 to +86 mV in L1 and L2-4 animals. In general, we observed a change from an outward current that activates slowly during depolarization (Fig 1C1, arrow) to an outward current that activates more rapidly and decays slightly over the time course of the depolarization (Fig 1C2, arrow). The change in time course of the outward current seems to result from the appearance of an early component in the outward current in L2-4 animals (Fig. 1D). We henceforth refer to this current as the fast-activating component of the outward current.

The change in outward current was quantified in two ways. First, the ratio of early-to-late current (see Materials and Methods) was displayed for the L1 and L2-4
larvae for the outward current generated by a voltage step from -74 to +86 mV (Fig. 1E). Note that a ratio above one indicates the appearance of the fast-activating component of the outward current. This ratio increased as the animals grew ($F(1,11) = 11.177, p < 0.01$). A similar increase was observed when ratios from a range of voltage steps were pooled (voltage steps to +26, +46, +66 and +86 mV; $F(1,11) = 7.601, p < 0.05$), demonstrating that this increase was not an artifact of using a particular voltage step.

Second, because there was considerable individual variability in early-to-late current ratios in WT and mutant animals (see below), we also compared the within-group frequency of cells displaying an early-to-late current ratio greater than one. A ratio greater than one was evident in over 80% of the cells from L2-4 animals, but evident only in 20% of cells from L1 animals (Fig. 1F). Thus the appearance of the fast-activating component of the outward current was a common feature of maturation in AIY neurons.

**Development of electrical properties is altered in ttx-3 mutants**

We next examined how the development of capacitance and voltage-dependent currents was affected in $ttx-3(ks5)$ mutants. In contrast to WT animals, AIY capacitance did not increase in $ttx-3$ mutants (Fig 2A; $F(1,16) = 0.894, p > 0.05$). Moreover, the capacitance observed in L1 $ttx-3$ mutants ($0.49 \pm 0.09$ pF) was similar to that of L2-4 WT animals ($0.68 \pm 0.11; F(2,21) = 1.09, p > 0.05$). This result suggests that capacitance matures precociously in $ttx-3$ and, further, that $ttx-3$ may function to reduce or retard maturation of AIY capacitance.

As in WT animals, no change in steady-state current amplitude could be detected during larval development in $ttx-3$ mutants (Fig. 2B; $F(1,16) = 0.474; p > 0.05$). We therefore pooled the data from L1 and L2-4 $ttx-3$ animals, and compared the pooled data
with that of WT animals. No significant difference could be detected \((F(4,64) = 2.17, p > 0.05)\). Therefore \(ttx-3\) mutants do not differ from WT in terms of steady-state current amplitude and its maturation.

We next analyzed age-dependent changes in the time course of outward current in the mutants. Fig 2C shows average currents for both L1 and L2-4 animals. One important difference from WT is apparent in outward currents in \(ttx-3\): in L1 animals, early outward current is dominated by the fast-activating component, rather than by the slowly activating currents observed in WT L1 (Fig 2C1, compare with Fig. 1C1). Thus outward current time course matures precociously in \(ttx-3\) mutants. These differences are further quantified in Fig 2D and E for the current elicited by a voltage pulse to +86 mV. Fig. 2D shows the ratio of early-to-late current for L1 and L2-4 animals. Whereas WT animals displayed a progressive increase of the early-to-late current ratio as the animals grew (Fig. 1E), in \(ttx-3\) mutants this ratio remained constant (Fig. 2D; \(F(1,16) = 1.35; p > 0.05\)). Moreover, a ratio greater than one was evident in 88% of cells in L1 animals and 100% of cells in L2-4 animals (Fig. 2E). Therefore, AIY displayed the fast-activating component in outward currents irrespective of developmental stage in \(ttx-3\) mutants, suggesting \(ttx-3\) functions to prevent or retard the maturation of this current.

\textit{wrk-1} expression is regulated by \(ttx-3\) and alters the development of AIY properties.

We next sought to understand the mechanism of action of \(ttx-3\) by analyzing the effect of mutations in candidate downstream genes. We focused on the \(wrk-1\) gene, a GPI-anchored member of the immunoglobulin-superfamily with three Ig and one FnIII domain (Hutter et al. 2000), for two main reasons: \(1\) \(wrk-1\) is expressed in AIY (Aurelio et al. 2002), and therefore is a potential target of \(ttx-3\), and \(2\) members of the IgSF have
been shown to regulate potassium currents in other preparations (Rasband 2004; Traka et al. 2003).

In order to determine if \textit{wrk-1} is indeed a target of \textit{ttx-3}, we examined the expression of \textit{wrk-1} in WT and \textit{ttx-3} mutants. Expression of \textit{wrk-1} was detected using a \textit{wrk-1}\textit{prom.:gfp} reporter (Fig. 3A; see Material and Methods). In WT animals, \textit{wrk-1} was expressed in several neurons in the \textit{C. elegans} nerve ring (Fig 3A). In the same animals, AIY was specifically labeled using a \textit{ttx-3::RFP} reporter (Fig. 3B). The two reporters co-localized, confirming that \textit{wrk-1} is expressed in AIY (Fig. 3C). We found that \textit{wrk-1} was expressed in AIY in 100% of WT animals (Fig. 3D). In contrast, using the same reporter genes in a \textit{ttx-3} mutant background, we found that the frequency of expression of \textit{wrk-1} was greatly reduced (Fig 3D). We conclude that \textit{wrk-1} expression is regulated by \textit{ttx-3} in AIY.

We next analyzed the effects of two loss of function alleles of \textit{wrk-1}, \textit{ok695} and \textit{tm1099} (see Material and Methods), on the maturation of AIY electrical properties. The increase in AIY capacitance observed in WT worms was also evident in \textit{wrk-1} mutants (Fig 4A; $F(1,15) = 5.49, p < 0.05$ for the \textit{ok695} allele, and $F(1,7) = 16.31, p < 0.05$ for the \textit{tm1099} allele). These mutant strains resembled WT and \textit{ttx-3} in that they did not show any change in steady-state current amplitude during larval development (Fig. 4B; \textit{wrk-1(ok695)}: $F(1,15) = 0.870$; \textit{wrk-1(tm1099)}: $F(1,7) = 0.02; p > 0.05$), nor was steady-state current amplitude different from WT ($F(4,64) = 2.17, p > 0.05$). However, both alleles of \textit{wrk-1} resembled \textit{ttx-3} in that they exhibited precocious maturation of the time course of outward currents. Specifically, outward currents in \textit{wrk-1} exhibited the fast activating component throughout development (Fig. 5A, B). This trend is also reflected in the ratio of early-to-late current, which did not increase during development (Fig 5C; $F(1,15) =$
The fast-activating component was also evident in a high percentage of cells with an early-to-late current ratio greater than one in both L1 and L2-4 animals (Fig. 5D; wrk-1(ok695): 80 and 86% of cells, wrk-1(tm1099): 67 and 75%). We conclude that wrk-1 is necessary for suppressing the fast-activating component that is absent early in WT development of AIY interneurons, but not for suppressing the capacitance increase.

Using wrk-1(tm1099) as a representative case, we next asked whether we could convert the more mature form of the outward current into its immature form by pharmacological blockade. Given a positive result in such an experiment, the simplest model would be that maturation of the outward current involves expression of one additional type of ion channel. We therefore measured outward currents in AIY in the presence and absence of the potassium channel blocker 4-aminopyridine (4-AP). This drug was chosen because the fast activating component in AIY is also a transient current, and 4-AP is known to block transient potassium currents in other C. elegans cells (Jospin et al., 2002). We found that 3 mM 4-AP partially blocked both the fast and slow components of the outward current (Fig. 6A). Both components were blocked to the same extent (Fig. 6B; $F(1,16)=0.425; p>0.05$). Thus we could not convert the more mature form of the current into its immature form using 4-AP. This result supports the hypothesis that maturation of outward currents is more complex than expression of a single additional type of ion channel. However, the simple model could still be the case if 4-AP partially blocks both the new component and the original component of the outward current. Thus this experiment remains inconclusive.

In order to test whether the alteration of the developmental timing observed in wrk-1 mutants was a non-specific effect of mutating ttx-3 target genes, we analyzed the
effect of complete removal of ser-2, a putative tyramine receptor gene that is expressed in AIY and is regulated by ttx-3 (Tsalik et al. 2003). In ser-2 null mutants, AIY displayed an entirely WT sequence of maturation, that included: (1) an increase in capacitance with age (Fig 7A; $F(1,10) = 6.05, p < 0.05$), (2) a WT steady-state current amplitude that did not change during development (Fig 7B; $F(1,10) = 4.613; p > 0.05$), and that was similar to that of WT animals ($F(4,64) = 2.17, p > 0.05$), and (3) the presence of the fast-activating component in the outward current in L2-4 animals, but not L1 animals (Fig. 7C). This absence is further reflected in the ratio of early-to-late current (Fig 7D; $F(1,9) = 5.78; p < 0.05$), and the percentage of cells exhibiting an early-to-late current ratio greater than one (Fig 7E). Moreover, the values of these different parameters were similar to those of WT animals. Therefore, the developmental defects observed in wrk-1 mutants are not the result of a non-specific effect of mutating ttx-3 target genes.
Discussion

Development of AIY in wild-type worms

The electrical properties of wild-type AIY neurons exhibit a clear pattern of developmental maturation. The immature state is characterized by relatively low capacitance and slowly activating outward currents. The more mature state is characterized by relatively high capacitance and the addition of a fast activating component to the outward current.

The capacitance increase could be explained in two main ways: by an increase in membrane resistance, leading to better space-clamp, and thus a more accurate measurement of capacitance, or by growth of the cell. We favor the second explanation because AIY is likely to be isopotential at all developmental stages, given that larger C. elegans neurons are isopotential in adult worms (Goodman et al. 1998). However, our results do not rule out other potential factors, such as developmental increases in the conductance of gap junctions between AIY and other neurons (White et al. 1986). Premature appearance of such gap junctions could explain the increased capacitance observed at the first larval stage (L1) in \textit{ttx-3} mutants. The additional outward current component could be explained by modification of an existing current or addition of a new one. The changes in capacitance and membrane current that we observed could alter membrane excitability in ways that modify the integrative properties of AIY neurons.

Interestingly, not all aspects of AIY electrophysiology exhibit a process of maturation, in that steady-state outward current amplitude remained constant over the developmental period studied. Under the assumption that increased capacitance reflects cell growth, the constancy of current amplitude implies that the number of channels carrying this current remains approximately constant as the cell grows, leading to a
decrease in current density. This stands in contrast to the development of the ASE chemosensory neurons in *C. elegans*, in which steady-state outward current amplitude increases but current density remains constant (Goodman et al. 1998). It remains to be seen which trend is more common in *C. elegans*.

**Mutant analysis of the electrophysiological maturation of AIY**

Mutations in the genes *ttx-3* and *wrk-1* result in precocious maturation of AIY electrical properties. In *ttx-3* mutants, capacitance and outward current already display a more mature phenotype by the L1 stage. In *wrk-1* mutants, capacitance develops normally, but outward current display a more mature phenotype by the L1 stage, as in *ttx-3* mutants. The fact that these effects were produced by loss of function alleles indicates that both genes normally act to retard the electrophysiological maturation of AIY, implying that the more mature state is the default state.

Our genetic analysis indicates that AIY contains distinct pathways for regulating cell growth and ionic currents. This follows from the fact that *wrk-1* mutants exhibit normal development of capacitance, but precocious development of outward current. Thus, *wrk-1* appears to be part of the pathway for regulating outward current, but not part of the pathway for regulating capacitance. In contrast, *ttx-3* appears to be part of both pathways because development of capacitance and outward current are precocious in *ttx-3* mutants. In addition, we found that *ttx-3* is required for normal levels of *wrk-1* expression. Taken together, our results are consistent with a model in which *ttx-3* acts in the outward current pathway by positively regulating *wrk-1*.

The mechanism by which *wrk-1* retards the development of outward currents remains to be elucidated. IgSF proteins have been shown to alter the localization of K+...
channels in the node of Ranvier (Rasband 2004; Traka et al. 2003), and are able to trigger second-messenger cascades in neurons (Walsh and Doherty 1997). Both mechanisms could be related to alteration of the outward current we observed.

**Limitations**

The number of the developmental changes in AIY electrical properties, as well the extent to which their maturation is altered in the mutants, may have been underestimated in this study in three main ways. First, *ttx-3*(ks5) is a hypomorphic rather than a null allele (Altun-Gultekin et al. 2001) and, although hypomorphic and null alleles of *ttx-3* mutants are indistinguishable from one another by almost all known criteria, we note that in theory, a null allele could have produced a stronger electrophysiological phenotype. Although null alleles of *ttx-3* are available, AIY neurons can not be identified in these strains after dissection because these animals do not express the *ttx-3prom::gfp* reporter construct (Altun-Gultekin et al. 2001). Second, because we analyzed net whole cell-currents, which were dominated by outward currents, we might have missed changes in the amplitude or time course of inward currents. For example, Ca$^{2+}$ currents, which were not resolved under the conditions of this study, might also be developmentally regulated. Third, it is possible that we missed changes in currents that activate on a slower time scale than the one studied here (Weinshenker et al. 1999). Nevertheless, this report establishes the AIY interneuron as an effective system in which to investigate the genetic control of the development of electrical properties of neurons.

Whereas we could implicate *wrk-1* in the electrophysiological maturation of the AIY interneurons, using two independently isolated *wrk-1* alleles, we note that we have not formally demonstrated that *wrk-1* indeed acts in the AIY interneurons, e.g. by
rescuing the gene cell-specifically. There is, however, no indication that \textit{wrk-1} is expressed in neurons that are either synaptically connected to AIY or whose axons are in close physical proximity to AIY. Thus the most parsimonious explanation of our findings is that \textit{wrk-1} acts cell-autonomously in AIY.

For technical reasons (see Materials and Methods), no adult animals were included in this study. Therefore, we cannot rule out further maturation of membrane currents in AIY past the L4 stage, nor can we rule out a role for \textit{ttx-3} and \textit{wrk-1} past the L4 stage.

\textbf{Future work}

Adult \textit{ttx-3} mutants exhibit several behavioral deficits (Mori and Ohshima 1995; Remy and Hobert 2005; Tsalik and Hobert 2003). The fact that \textit{ttx-3} acts in the AIY neurons to cause these behavioral defects strongly implicates this neuron class in these deficits. Such deficits could arise from alterations in intrinsic electrical properties or from alterations in synaptic function, including synaptic connectivity. The fact that the changes in intrinsic properties in \textit{ttx-3} mutants are minor seems to argue for changes in synaptic function as the main cause of behavioral deficits. However, as noted, we may have missed certain alterations in intrinsic properties.

The functional consequences of the maturation of outward currents remain unclear as little is known about the behavior of \textit{C. elegans} larvae and how it changes as the animals grow (Chiba and Rankin 1990). Further work is therefore required to characterize the emergence of AIY-mediated behaviors during larval development of \textit{C. elegans}. This would establish AIY as a model to study how genetic alterations in electrophysiological properties translate into behavioral defects.
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References


Figures legends
Figure 1. Development of electrical properties of AIY interneurons in wild-type animals.

A. Whole-cell capacitance vs. developmental stage. B. Steady-state current vs. voltage.
Currents were obtained in response to a family of voltage pulses from -154 to +86 mV in
20mV increments from a holding potential of -74 mV. C. Time course of average
membrane current in response to the voltage commands in B. Currents were averaged
across animals (one series of voltage pulses per animal). Capacitive transients were_clipped.
D. Overlay of the currents at +86 mV in C1 and C2. Bars above traces indicate
the time windows used in computing the ratios in Fig. 2E. E. Early-to-late current ratio at
+86 mV vs. developmental stage. F. Percentage of cells displaying a ratio greater than
unity vs. developmental stage. In A-F, n = 7 L1 animals and n = 6 L2-L4 animals, with
one neuron having been recorded per animal; asterisks represent p < 0.05.

Figure 2. Development of electrical properties of AIY interneurons in \( ttx-3 \) mutants. A.
Whole-cell capacitance vs. developmental stage. B. Steady-state current vs. voltage.
Currents were obtained in response to a family of voltage pulses from -154 to +86 mV in
20mV increments from a holding potential of -74 mV. The grey zone represents pooled
WT data from Fig. 1B. C. Time course of average membrane current in response to the
voltage commands in B. Currents were averaged across animals (one series of voltage
pulses per animal). Capacitive transients were clipped. D. Early-to-late current ratio at
+86 mV vs. developmental stage. E. Early-to-late current ratio at +86 mV vs.
developmental stage. F. Percentage of cells displaying a ratio greater than unity vs.
developmental stage. In A-F, n = 8 L1 animals and n = 10 L2-L4 animals, with one
neuron having been recorded per animal; asterisks represent p < 0.05. Dashed lines
indicate corresponding values of WT animals in Fig. 1.

Figure 3. Expression pattern of \textit{wrk-1} in WT animals and \textit{ttx-3} mutants. A. Expression of a \textit{wrk-1}$_{\text{prom}}$:gfp reporter gene in a WT animal. B. AIY-specific expression of a \textit{ttx-3}$_{\text{prom}}$:DsRed2 reporter gene in the animal shown in A. C. Merged image of A and B, showing that \textit{wrk-1} is expressed in AIY. D. Percentage of animals expressing the \textit{wrk-1} construct in a WT and \textit{ttx-3(ot22)} background in the AIY neurons.

Figure 4. Development of whole-cell capacitance and steady-state current amplitude in two loss of function alleles of \textit{wrk-1}, \textit{ok695} and \textit{tm1099}. A. Whole-cell capacitance vs. developmental stage. B. Steady-state current vs. voltage. Currents were obtained in response to a family of voltage pulses from -154 to +86 mV in 20mV increments from a holding potential of -74 mV. The grey zone represents pooled WT data from Fig. 1B. In A and B, $n = 10$ \textit{ok695} and $n = 5$ \textit{tm1099} L1 animals; $n = 7$ \textit{ok695} and $n = 4$ \textit{tm1099} L2-L4 animals. One neuron was recorded per animal; asterisks represent p < 0.05. Dashed lines indicate corresponding values of WT animals in Fig. 1.

Figure 5. Development of the time course of outward current in two loss of function alleles of \textit{wrk-1}, \textit{ok695} and \textit{tm1099}. A and B. Time course of average membrane current in response to the voltage commands in Fig. 4B. Currents were averaged across animals (one series of voltage pulses per animal). Capacitive transients were clipped. C. Early-to-late current ratio at +86 mV vs. developmental stage. D. Percentage of cells displaying a ratio greater than unity vs. developmental stage. In A-D, $n = 10$ \textit{ok695} and $n = 6$ \textit{tm1099} L1 animals; $n = 7$ \textit{ok695} and $n = 4$ \textit{tm1099} L2-L4 animals. One neuron was recorded per
animal; asterisks represent p < 0.05. Dashed lines indicate corresponding values of WT animals in Fig. 1.

Figure 6. Pharmacological block of the outward current. A. Time course of membrane current in response to voltage pulses (from -154 to +8.6 mV in 20 mV increments from a holding potential of -74 mV) in a L1 wrk-1(tm1099) animal, before (Control) and after addition of 3 mM 4-aminopyridine (4-AP). B. Percent reduction in the outward current generated by a voltage pulse at +86 mV, for the fast-activating component and the steady-state component (n=9 animals).

Figure 7. Development of electrical properties of AIY interneurons in ser-2(pk1357) null mutants. A. Whole-cell capacitance vs. developmental stage. B. Steady-state current vs. voltage. Currents were obtained in response to a family of voltage pulses from -154 to +86 mV in 20 mV increments from a holding potential of -74 mV. The grey zone represents pooled WT data from Fig. 1B. C. Time course of average membrane current in response to the voltage commands in B. Currents were averaged across animals (one series of voltage pulses per animal). Capacitive transients were clipped. D. Early-to-late current ratio at +86 mV vs. developmental stage. E. Early-to-late current ratio at +86 mV vs. developmental stage. F. Percentage of cells displaying a ratio greater than unity vs. developmental stage. In A-F, n = 3 L1 animals and n = 9 L2-L4 animals, with one neuron having been recorded per animal; asterisks represent p < 0.05. Dashed lines indicate corresponding values of WT animals in Fig. 1.
figure 1
Figure 2

(A) Comparison of capacitance in WT L1 and L2-4 with ttx-3(ks5) knockout. (B) Graph showing steady-state current amplitude (pA) vs. voltage (mV) for L1 and L2-4. (C1) Recording of early current in L1. (C2) Recording of early current in L2-4. (D) Graph showing early/late current ratio for L1 and L2-4. (E) Graph showing percentage of cells with ratio > 1 for WT L2-4 and L1.
Figure 3

A) wrk-1prom::gfp

B) ttx-3prom::dsRed2

C) merge

D)

<table>
<thead>
<tr>
<th>wrk-1prom::gfp expression in AIY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
</tr>
<tr>
<td>ttx-3(ot22)</td>
</tr>
</tbody>
</table>

Figure 3
Figure 4
Figure 5
Figure 6

A

Control

4-AP

B

% reduction

fast-activating component

steady-state current

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Figure 7

(A) ser-2(pk1357)

 capacitor (pF)

 WT L2-4

 WT L1

 (C1) steady-state current amplitude (pA)

 voltage (mV)

 L1

 L2-4

 (C2)

 100 pA

 10 ms

 (D) early/late current ratio

 WT L2-4

 WT L1

 (E) % cells with ratio > 1

 WT L2-4

 WT L1

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