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The origin of quantal size variation and high-frequency minis at the *C. elegans* neuromuscular junction

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Abstract

The neuromuscular junction (NMJ) of *C. elegans* has proved to be a very useful model synapse for investigating molecular mechanisms of synaptic transmission. Intriguingly, miniature postsynaptic currents (minis) at this synapse occur at an unusually high frequency (50–90 Hz in wild-type worms) and show large variation in quantal size (from <10 pA to >200 pA). It is important to understand the cellular and molecular bases for these properties of minis in order to interpret electrophysiological data from this synapse properly. Existing data suggest that several factors may contribute to the high frequency and quantal size variation, including (1) the establishment of multiple NMJs with each body-wall muscle cell, (2) diversity of postsynaptic receptors (two acetylcholine receptors and one GABA receptor), (3) association of one presynaptic site with several body-wall muscle cells, (4) effects of Ca^{2+} at the presynaptic site, and (5) a possibly elevated (less hyperpolarized) resting membrane potential in motoneurons. Neither the frequency nor quantal size of minis is affected by electrical coupling of body-wall muscle cells. Furthermore, quantal size variation is not due to synchronized multivesicular release. Analyses of the *C. elegans* NMJ may lead to better understanding of the mechanisms controlling the frequency and quantal size of minis of other synapses as well.

Keywords

C. elegans; neuromuscular junction; minis; quantal size; calcium

In the absence of action potentials or direct nerve stimulation, neurotransmitters may be released spontaneously and sporadically. Such synaptic events may be detected as changes in the membrane potential or current of the postsynaptic cell, which are known as miniature postsynaptic potentials (mPSPs) and miniature postsynaptic currents (mPSCs), respectively. Both mPSPs and mPSCs are commonly referred as minis. Minis were first discovered as membrane potential fluctuations at the end-plate of vertebrate neuromuscular junctions (NMJs), and were called miniature end-plate potentials (mEPPs). They were thought to represent elementary units of neurotransmitter release known as quanta (Del Castillo and Katz 1954; Fatt and Katz 1950). Until recently, minis were considered to have the following properties: (1) they reflect leakage of neurotransmitters under resting conditions and do not have specific physiological functions (Zucker 2003); (2) each mini represents the full-collapse fusion of one synaptic vesicle, and the release from the vesicle is all-or-none (Katz 1971); (3) the quantal size, as represented by the amplitude of minis, is constant (Del Castillo and Katz 1954); and (4) minis result from exocytosis of the same pool of synaptic vesicles as evoked responses (Del Castillo and Katz 1954; Groemer and Klingauf 2007). However, these earlier concepts are challenged by some recent data. For example, minis

have been implicated in several physiological functions, including postsynaptic receptor clustering (Saitoe et al. 2001), shift of NMDA receptor subunit compositions during development (Barria and Malinow 2002), regulation of dendritic protein synthesis (Sutton et al. 2006; Sutton et al. 2004), maintenance of dendritic spines (McKinney et al. 1999), and action potential firing (Carter and Regehr 2002; Sharma and Vijayaraghavan 2003). In addition, synaptic vesicles may be able to release neurotransmitters by establishing a transient fusion pore with the presynaptic plasma membrane without losing its vesicular identity, which is known as a kiss-and-run event (Aravanis et al. 2003; Gandhi and Stevens 2003; He et al. 2006), and minis and evoked responses appear to result from two distinct pools of synaptic vesicles (Chung et al. 2010; Fredj and Burrone 2009; Hablitz et al. 2009; Mathew et al. 2008; Sara et al. 2005). Moreover, quantal size appears to be variable at many synapses (Forti et al. 1997; Liu et al. 1999; Liu and Tsien 1995) due to a variety of pre- and postsynaptic factors, including variable neurotransmitter content among synaptic vesicles (Reimer et al. 1998; Wu et al. 2007), possibly incomplete discharge of synaptic vesicle content during kiss-and-run exocytosis (Harata et al. 2006), merging of synaptic vesicles prior to fusion with the presynaptic plasma membrane (He et al. 2009), synchronized release of multiple synaptic vesicles (Llano et al. 2000; Sharma and Vijayaraghavan 2003), and saturation of postsynaptic receptors by a single quantum of neurotransmitter (Edwards et al. 1990; Tang et al. 1994; Tong and Jahr 1994). Nevertheless, a great deal of controversy still exists with respect to some of these new findings. These uncertainties highlight the necessity and importance of determining the mechanisms regulating mini frequency and quantal size.

The nematode *Caenorhabditis elegans* (*C. elegans*) is a very useful model system for studying cellular and molecular mechanisms of synaptic transmission due to several major advantages. First, most cellular and molecular mechanisms of synaptic transmission are conserved between *C. elegans* and mammals. Second, *C. elegans* has a simple nervous system with only 302 neurons. The entire neural circuit, including all the chemical synapses and gap junctions, has been described through analyses of serial section electron microscopy (Chen et al. 2006; White et al. 1986). Third, viable mutant alleles of many genes related to synaptic transmission already exist in *C. elegans*; and mutants of new genes may be obtained with relative ease through either forward or reverse genetics. Fourth, *C. elegans* is transparent, which allows convenient analyses of expression and subcellular localization patterns of genes/proteins using fluorescent proteins as reporters. Fifth, many public resources exist to support research with *C. elegans*, such as the *Caenorhabditis* Genetics Center where existing *C. elegans* mutants may be obtained, gene knockout facilities where targeted gene knockout may be requested at no charge, and extensive web resources on *C. elegans* genome (www.wormbase.org), anatomy (www.wormatlas.org) and biology (www.wormbook.org).

In *C. elegans*, analyses of minis and evoked postsynaptic currents are performed almost exclusively with the NMJs for technical reasons. In this assay, one electrode is used to voltage-clamp a body-wall muscle cell (typically at -60 mV) to monitor postsynaptic currents while a second electrode is placed near the ventral nerve cord to stimulate axons innervating the muscle cell. mPSCs are recorded in the absence of nerve stimulation whereas evoked postsynaptic currents (ePSCs) are caused by applying a square-wave current pulse through the stimulation electrode (Richmond et al. 1999). Compared with many other synapses, the frequency of minis at the *C. elegans* NMJ is high (typically 50–90 Hz) (Gracheva et al. 2006; Liu et al. 2005; Richmond and Jorgensen 1999). Furthermore, minis at the *C. elegans* NMJ show great variation in amplitude (Figure 1). While the mean amplitude is approximately 25 pA, many events are smaller than 10 pA or larger than 50 pA, and some are as large as 100–200 pA (Liu et al. 2005). The presence of many large-amplitude minis causes a severe skew of the amplitude distribution histogram (Figure 1, B), which is in sharp contrast to the normal distribution of vertebrate mEPPs (Del Castillo and

Katz 1954). What are the mechanisms for the high frequency and great quantal size variation of minis at the *C. elegans* NMJ? Answers to these two questions are potentially valuable for the interpretation of synaptic data from *C. elegans* and the understanding of fundamental mechanisms of synaptic transmission. In this review, I discuss several factors implicated in controlling the frequency and quantal size of minis at the *C. elegans* NMJ.

1. Each body-wall muscle cell forms synapses with multiple presynaptic sites

C. elegans has 95 body-wall muscle cells, which are organized into four longitudinal groups known as quadrants. Two quadrants are located on the left and right sides of the ventral nerve cord while the other two quadrants are located on the left and right sides of the dorsal nerve cord. Each quadrant consists of 24 muscle cells except for the ventral left quadrant, which contains 23 (Dixon and Roy 2005; Waterston 1988). Axons of motoneurons establish en passant synapses with muscle arms (thin processes) from body-wall muscle cells in the ventral and dorsal nerve cords. There are three major classes of motoneurons innervating body-wall muscle cells of *C. elegans*: A, B and D. The A and B classes are cholinergic motoneurons whereas the D class consists of GABAergic motoneurons. Depending on the muscle cells that they innervate in the adult, either the letter “V” (ventral) or “D” (dorsal) is used as a prefix of the class names. Thus, VA, VB and VD motoneurons innervate the two ventral quadrants whereas DA, DB and DD motoneurons innervate the two dorsal quadrants. In addition to the A, B and D classes, AS motoneurons innervate only dorsal muscle cells and VC motoneurons innervate vulval and ventral body-wall muscle cells (Chalfie and White 1988; White et al. 1976). Motoneurons in the AS class use acetylcholine (ACh) as their neurotransmitter whereas those in the VC class use either ACh or serotonin as their neurotransmitter (Rand and Nonet 1997). Cell bodies of all the motoneurons are located in a row on the ventral side. Axons of motoneurons innervating the ventral quadrants run in the ventral nerve cord whereas those of motoneurons innervating the dorsal quadrants first project laterally as commissures and then run in the dorsal nerve cord. Within the ventral and dorsal nerve cords, axons of the A and B classes project in the anterior and posterior directions, respectively; axons of the D class motoneurons often project in both directions (White et al. 1976). The number and positions of NMJ regions (active zones) along the motoneuron axons have been mapped through analyses of serial electron microscopy sections. Excluding those with pharyngeal muscle cells, 1410 NMJs have been counted in adult *C. elegans* (Chen et al. 2006). Given that there are 95 body-wall muscle cells (Dixon and Roy 2005; Waterston 1988), each body-wall muscle cell might on average receive 15 NMJs. However, as described in a later section, this number is greatly underestimated because several muscle cells are often contacted by a single active zone. Thus, one important cause for the high frequency of minis is that each muscle cell forms many NMJs with motoneurons.

2. Three types of postsynaptic receptors exist in body-wall muscle cells

At the *C. elegans* NMJ, there are three types of postsynaptic receptors, including two types of acetylcholine receptor (AChR) and one type of GABA receptor (GABAR). The two types of AChRs are nicotine-sensitive (N-AChR) and levamisole-sensitive (L-AChR). The physiological agonist for both AChRs is ACh. In addition, N-AChRs and L-AChRs are selectively activated by the pharmacological agonists nicotine and levamisole, respectively (Richmond and Jorgensen 1999). The N-AChR appears to be a homomeric complex of ACR-16, which is closely related to the $\alpha 7$ -subunit of vertebrate nicotinic receptors (Touroutine et al. 2005), whereas the L-AChR appears to be a pentamer of five different subunits, including three α -subunits (LEV-8, UNC-38 and UNC-63) and two non- α -subunits (LEV-1 and UNC-29) (Boulin et al. 2008). The GABAR at the *C. elegans* NMJ is an

ionotropic receptor and thus belongs to the GABA_A type. It is formed by UNC-49B and UNC-49C, which are proteins translated from alternatively spliced *unc-49* mRNA (Bamber et al. 2005; Jorgensen 2005).

AChRs are typically non-selective cation channels whereas GABARs are chloride channels. Under physiological conditions, AChRs and GABARs conduct inward and outward currents, respectively, in body-wall muscle cells. However, postsynaptic currents are generally recorded from body-wall muscle cells at a negative holding potential (typically -60 mV) using extracellular and pipette solutions that give a chloride equilibrium potential of approximately 0 mV. Under such experimental conditions, GABARs conduct inward currents because the chloride equilibrium potential is more positive than the holding potential. Thus, minis recorded under these experimental conditions are caused by two different neurotransmitters acting on three different postsynaptic receptors (Richmond and Jorgensen 1999). Minis mediated by the three different postsynaptic receptors show different mean amplitudes (GABAR > N-AChR > L-AChR) (Liu et al. 2005). Even when only one of the receptors is functional, amplitudes of minis are still variable and do not show a normal distribution (Figure 2) (Liu et al. 2005). These observations suggest that the existence of three different postsynaptic receptors contributes to both the high frequency and large quantal size variability of minis at the *C. elegans* NMJ.

3. A single quantum of neurotransmitter causes minis in multiple muscle cells

Ultrastructural analyses of *C. elegans* synapses show that a single presynaptic site, as defined by one active zone, often has several postsynaptic partners. This is true for both NMJs and neuron-neuron synapses (White et al. 1986). For example, one active zone of a cholinergic or GABAergic motoneuron often associates with muscle arms from several body-wall muscle cells (Liu et al. 2007; White et al. 1986). Depending on the number of postsynaptic partners, synapses of this kind are referred as dyadic synapses, triadic synapses, or simply as polyadic synapses. However, it cannot be determined from the morphological data whether a single presynaptic site forms functional synapses with the different postsynaptic partners, especially because postsynaptic densities are not obvious in *C. elegans*. If one presynaptic site forms functional synapses with several muscle cells, exocytosis of a single synaptic vesicle could potentially cause minis in all the muscle cells associated with this presynaptic site but amplitudes of the minis might vary due to different diffusion distances and different durations of exposure to acetylcholinesterase in the synaptic cleft. To address this possibility, we determined whether an unusually high frequency of concurrent mPSCs may be observed in different pairs of neighboring body-wall muscle cells. We found that adjoining muscle cells in parallel along the longitudinal body axis (e. g. R1-R2 or L1-L2 in Figure 3) show high frequencies of concurrent minis that cannot be explained by random coincidence whereas adjoining muscle cells in series (e. g. R1-R1 or R2-R2 in Figure 3) show low frequencies of concurrent minis that are consistent with random coincidence (Liu et al. 2007). Although body-wall muscle cells are electrically coupled (Liu et al. 2006), electrical coupling does not contribute to either the high frequency or quantal size variation of minis at the *C. elegans* NMJ (Liu et al. 2007), as discussed in a later section. Because there are four rows of muscle cells in the two dorsal or ventral quadrants (Figure 3) (Dixon and Roy 2005; Waterston 1988), a single quantum of neurotransmitter could cause minis in up to four ventral or dorsal muscle cells. Thus, the polyadic structure of *C. elegans* NMJs contributes to both the high frequency and large quantal size variation of minis.

4. Ca^{2+} is important to the occurrence and quantal size variation of minis at the *C. elegans* NMJ

Minis at the *C. elegans* NMJ are dependent on Ca^{2+} , which comes from both influx from extracellular space and release from the endoplasmic reticulum (ER) (Liu et al. 2005). Ca^{2+} channels containing the UNC-2 subunit, a homologue of mammalian N-type and P/Q-type α_1 -subunits (Bargmann 1998), are important to Ca^{2+} influx (Richmond et al. 2001) while ryanodine receptors (RyRs) allow Ca^{2+} release from the ER (Liu et al. 2005). Without extracellular Ca^{2+} and presynaptic RyR function, minis are essentially absent at the *C. elegans* NMJ, suggesting that Ca^{2+} is required for spontaneous neurotransmitter release (Liu et al. 2005). Ca^{2+} influx is important to the occurrence but not quantal size of minis because elimination of extracellular Ca^{2+} decreases the frequency greatly but does not alter the amplitude distribution of minis (Liu et al. 2005). In contrast, RyR-mediated Ca^{2+} release controls both the frequency and quantal size of minis. In null mutants of *unc-68*, which is the only RyR gene of *C. elegans*, minis at the NMJ show ~70% decrease in frequency and ~40% decrease in mean amplitude. Large-amplitude minis (arbitrarily defined as ≥ 50 pA), which are common in wild-type worms, are essentially absent in *unc-68* mutants (Liu et al. 2005). Thus, Ca^{2+} influx and RyR-mediated Ca^{2+} release appear to play distinct roles with respect to minis at the *C. elegans* NMJ. While both of them promote the occurrence of minis, only RyR-mediated Ca^{2+} release controls the quantal size (Liu et al. 2005).

How do RYRs control the quantal size? There are several potential mechanisms. First, RYRs may increase vesicle size. Larger vesicles may contain more neurotransmitter than do smaller vesicles. However, this does not appear to be the case since the size of synaptic vesicles is similar between the wild-type and *unc-68* mutant (Liu et al. 2005). Second, RYRs may increase the number of docked vesicles, as suggested by Ca^{2+} -dependent translocation of synaptic vesicles at *Drosophila* NMJs (Koenig et al. 1993), and these newly docked vesicles might contain more transmitter than other docked vesicles. However, the number of morphologically docked vesicles is not changed in *unc-68* mutant (Liu et al. 2005). Third, RYRs may promote vesicle loading with neurotransmitters, resulting in higher concentrations in the wild-type compared with *unc-68* mutants. Fourth, RYR may stimulate inter-vesicular fusion to form larger vesicles prior to exocytosis. The larger vesicles may contain neurotransmitter that was originally packed into several smaller vesicles (He et al. 2009). Fifth, RYRs may increase either the size or duration of the fusion pore during kiss-and-run synaptic release. Ca^{2+} entry-dependent regulation of quantal size has been shown to occur for catecholamine release from chromaffin cells, probably through modulation of kiss-and-run exocytosis (Elhamdani et al. 2001). Conceivably, quantal size in neurons might be regulated by RYR-mediated Ca^{2+} release through a similar mechanism. These possibilities need to be examined in future studies.

5. The resting membrane potential of motoneurons might favor spontaneous exocytosis

Voltage-gated Ca^{2+} channels (VGCCs) at the presynaptic site of *C. elegans* motoneurons appear to be important to the occurrence of minis, as suggested by the great decrease of mini frequency following elimination of extracellular Ca^{2+} (Liu et al. 2005), hyperpolarization of motoneurons (Liu et al. 2009), or mutation of the *unc-2* gene (Richmond et al. 2001). However, mammalian homologues of UNC-2 constitute high-voltage-activated Ca^{2+} channels with an activation threshold of approximately -20 mV (Bargmann 1998; Wang et al. 2008). Therefore, it is intriguing why VGCCs appear to be active at rest at the presynaptic site of *C. elegans* motoneurons. There are two potential reasons for VGCCs being important in mediating minis at the *C. elegans* NMJ: (1) the resting membrane

potential of *C. elegans* motoneurons might be more depolarized compared with mammalian motoneurons (approximately -65 mV) (Hall 1982), and (2) VGCCs at the presynaptic site of *C. elegans* motoneurons might function at a more hyperpolarized voltage range compared with high-voltage-activated Ca^{2+} channels of mammals. At present, functional properties of UNC-2 Ca^{2+} channels are largely unknown; and there is no report on the resting membrane potential of *C. elegans* motoneurons. The resting membrane potentials of only two different *C. elegans* neurons (AVA and RMD interneurons) have been reported. The resting membrane potential of AVA neurons is typically -20 and -30 mV whereas that of RMD neurons is approximately -70 mV (Mellem et al. 2008). However, RMD neurons are unusual in that they resembled Schmitt triggers with bistable membrane potentials (approximately -70 mV and -20 mV) (Mellem et al. 2008). The resting membrane potential of *C. elegans* body-wall muscle cells is -27 mV (Liu et al. 2006). If the resting membrane potential of *C. elegans* motoneurons was similar to those of the AVA interneurons and body-wall muscle cells, it would be favorable to the activation of VGCCs.

6. Quantal size variation at the *C. elegans* NMJ is not due to synchronized multivesicular release

Although the mean amplitude of minis at the *C. elegans* NMJ is approximately 25 pA under experimental conditions, a significant portion of minis is ≥ 50 pA. These large-amplitude events are essentially absent in mutants of the *unc-68* RyR gene. At least two previous studies have suggested that presynaptic RyRs cause large-amplitude minis at mammalian central synapses by promoting synchronized release of multiple synaptic vesicles (Llano et al. 2000; Sharma and Vijayaraghavan 2003). However, large-amplitude minis at the *C. elegans* NMJ are not due to synchronized multivesicular release based on several lines of evidence (Liu et al. 2005). First, the fraction of large-amplitude minis remained the same after eliminating extracellular Ca^{2+} that is required for synchronized synaptic exocytosis. Second, the proportion of large-amplitude minis does not decrease in syntaxin or SNAP25 mutants, which are defective in synchronized synaptic exocytosis. Third, the rise time of minis is constant regardless of amplitude. Thus, the large quantal size variability of minis at the *C. elegans* does not appear to be due to synchronized multivesicular release (Liu et al. 2005).

7. Electrical coupling of body-wall muscle cells does not contribute to the high frequency and quantal size variation of minis

Gap junctions exist between body-wall muscle cells, as shown by ultrastructural and electrophysiological analyses (Chen et al. 2007; Liu et al. 2006; White et al. 1986). There are two important features for electrical coupling of body-wall muscle cells (Liu et al. 2006). First, muscle cells are electrically coupled in a highly organized and specific pattern, and coupling is only detected between muscle cells at the same position along the longitudinal body axis. Second, junctional conductance is very small. These features are potentially beneficial to the sinusoidal locomotion of worms because they may synchronize activities of muscle cells at the same position along the longitudinal body axis without causing simultaneous contraction or relaxation of all muscle cells.

Can minis go through gap junctions? If so, minis recorded from one muscle cell would represent spontaneous synaptic activities involving multiple muscle cells. We performed two analyses to answer this question. First, we compared the mean rise times of small-amplitude minis (< 50 pA), large-amplitude minis (≥ 50 pA), and junctional currents induced by applying a voltage step to a neighboring muscle cell. We reasoned that if some of the minis were due to electrical coupling, they would likely be of the smaller amplitudes, and would

have a similar rise time as junctional currents. However, we found that the rise times of large- and small-amplitude minis were identical but much shorter than that of junctional currents. Second, we compared properties of minis between the wild-type and an *unc-9* innexin mutant, which is deficient in electrical coupling of body-wall muscle cells. Both the frequency and mean amplitude of minis are indistinguishable between the wild-type and *unc-9* mutant. These analyses suggest that gap junctions do not contribute to the high frequency and large quantal size variation of minis at the *C. elegans* NMJ (Liu et al. 2007).

Concluding remarks

The frequency of minis is subject to influence by experimental conditions, including the solutions used for electrophysiological recordings. Compositions of *C. elegans* intracellular and extracellular solutions are unknown. When minis were first recorded from the *C. elegans* NMJ (Richmond and Jorgensen 1999; Richmond et al. 2001), extracellular and pipette solutions were prepared based on those used for electrophysiological recordings in *Ascaris lumbricoides*. Identical or essentially identical solutions have been used for recording minis in the majority of subsequent studies (e. g. (Gracheva et al. 2006; Liu et al. 2005; Schuske et al. 2003; Wang et al. 2001; Yeh et al. 2008)). However, one group of investigators has used very different solutions, and observed lower frequencies of minis (10–15 Hz in the wild-type) at the *C. elegans* NMJ (McEwen and Kaplan 2008; Simon et al. 2008). An important cause for the much lower frequencies of minis observed in these two studies was that GABAergic minis were eliminated using pipette and extracellular solutions with the chloride equilibrium potential near the holding potential. Besides solutions, other differences in experimental conditions may also affect the frequency of minis. For example, the mean frequency of minis at the NMJ of wild-type worms ranged from 30 to 90 Hz in different studies performed by the same group of investigators using identical or essentially identical solutions (Gracheva et al. 2006; Richmond and Jorgensen 1999; Schuske et al. 2003). Thus, it is important to keep experimental conditions strictly identical when comparing the frequencies of minis among different groups.

Mechanisms regulating mini frequency and quantal size at the *C. elegans* NMJ are complex. The mechanisms discussed above are mostly related to specific anatomical or functional properties of *C. elegans*. However, there must be a set of conserved mechanisms regulating mini frequency and quantal size across many synapses of many species. As described at the beginning of this article, a great deal of controversy still exists regarding mechanisms controlling the frequency and quantal size of minis. *C. elegans* is potentially very useful for advancing research in this field due to its powerful molecular genetics. As the number of labs performing electrophysiological analyses with *C. elegans* increases, and as more techniques are adapted to the NMJ of *C. elegans*, the worm will likely emerge as an unusually powerful tool for understanding spontaneous synaptic exocytosis.

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Figure 1.

Miniature postsynaptic currents (minis) at the *C. elegans* neuromuscular junction occur at a high frequency and show great variation in amplitude. **A:** A representative trace of minis shown at a compressed (**a**) or expanded (**b**) time scale. The trace in **b** is a fragment of the trace in **a**. **B:** Amplitude histogram of minis from wild-type worms with the number of events shown either in the linear (**a**) or logarithmic (**b**) scale. The amplitude distribution is skewed due to the presence of many large-amplitude events. The logarithmic scale allows a better view of events with larger amplitudes, which occur at a lower frequency. This figure is based on data of a previous study (Liu et al. 2005)

Figure 2.

Properties of miniature postsynaptic currents (minis) mediated by different postsynaptic receptors at the *C. elegans* neuromuscular junction. Body-wall muscle cells have three different postsynaptic receptors, including two acetylcholine receptors (nicotine-sensitive receptor, N-AChR; and levamisole-sensitive receptor, L-AChR) and one GABA receptor (GABAR). **A:** Representative traces of minis mediated by each of the three receptors. **B:** Amplitude histograms of minis mediated by the three different receptors. This figure is based on data of a previous study (Liu et al. 2005).

Figure 3.

Diagram of ventral body-wall muscle. A section of ventral body-wall muscle with the ventral nerve cord is shown. The ventral muscle includes the right (R) and left (L) quadrants with each quadrant consisting of two rows of muscle cells (R1 and R2 or L1 and L2). Axons of motoneurons establish en passant synapses with muscle arms, which are fine projections from body-wall muscle cells. Gap junctions exist at intercellular junctions of muscle cells within the same quadrant as well as near the ventral cord, where muscle arms from the left and right quadrants interdigitate.