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In Vivo Imaging of *C. elegans* Mechanosensory Neurons Demonstrates a Specific Role for the MEC-4 Channel in the Process of Gentle Touch Sensation

Hiroshi Suzuki,[1,4] Rex Kerr,[1,4] Laura Bianchi,[2] Christian Frøkjær-Jensen,[1,3] Dan Slone,[2] Jian Xue,[2] Beate Gerstbrein,[2] Monica Driscoll,[2] and William R. Schafer[1,*]

[1]Division of Biology
University of California, San Diego
La Jolla, California 92093

[2]Department of Molecular Biology and Biochemistry
Nelson Biological Laboratories
Rutgers, The State University of New Jersey
Piscataway, New Jersey 08855

[3]Niels Bohr Institute
University of Copenhagen
Copenhagen
Denmark

Summary

In the nematode *C. elegans*, genes encoding components of a putative mechanotransducing channel complex have been identified in screens for light-touch-insensitive mutants. A long-standing question, however, is whether identified MEC proteins act directly in touch transduction or contribute indirectly by maintaining basic mechanoreceptor neuron physiology. In this study, we used the genetically encoded calcium indicator cameleon to record cellular responses of mechanosensory neurons to touch stimuli in intact, behaving nematodes. We defined a gentle touch sensory modality that adapts with a time course of approximately 500 ms and primarily senses motion rather than pressure. The DEG/ENaC channel subunit MEC-4 and channel-associated stomatin MEC-2 are specifically required for neural responses to gentle mechanical stimulation, but do not affect the basic physiology of touch neurons or their in vivo responses to harsh mechanical stimulation. These results distinguish a specific role for the MEC channel proteins in the process of gentle touch mechanosensation.

Introduction

Studies in organisms ranging from bacteria to mammals indicate that mechanotransduction can be mediated by specialized ion channels that open or close in response to mechanical stimuli such as stretch or pressure (Hamill and Martinac, 2001). In *C. elegans*, screens for mechanosensory defective (Mec) mutants (Chalfie and Sulston, 1981; Chalfie and Au, 1989) have identified several genes that encode candidate subunits of a mechanically gated ion channel responsible for sensing light touch in six body touch sensory neurons (Tavernarakis and Driscoll, 1997). In particular, *mec-4* and *mec-10* genes encode members of the DEG/ENaC sodium channel superfamily (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994; Welsh et al., 2002) that are coexpressed in the touch neurons (Huang and Chalfie, 1994; Mitani et al., 1993) and are required for sensation of gentle touch. The stomatin-related MEC-2 protein (Huang et al., 1995) and paraoxonase-related MEC-6, also essential for touch, associate with MEC-4 to enhance current conducted by MEC-4 channels expressed in *Xenopus* oocytes (Goodman et al., 2002; Chelur et al., 2002). In vivo, a MEC channel composed of these subunits has been hypothesized to make critical contacts to the extracellular matrix and to a specialized intracellular cytoskeleton that exerts the tension required for mechanical gating.

However, direct evidence for this model for MEC channel function has been difficult to obtain. The phenotypes of *mec-4* and *mec-2* loss-of-function mutants are identical to those caused by touch neuron ablation, leaving the nature of MEC channel's role in touch transduction an open question. Although the MEC channel might be specifically involved in mechanotransduction, an identical mutant phenotype would result if this MEC-4 channel were generally required for normal touch neuron physiology, as has been shown for a Mirp K^+ channel that maintains the touch neurons' transmembrane potential (Bianchi et al., 2003). Since human disorders involving stomatin defects are correlated with leaky plasma membrane channels (Stewart and Fricke, 2003), a role for the MEC proteins in general touch neuron physiology is not an unreasonable possibility. Although it has been possible to reconstitute sodium channels in heterologous expression systems using mutant forms of MEC-4 (Goodman et al., 2002), these heterologously expressed MEC channels are not mechanically gated, perhaps because the reconstituted system does not recapitulate specific forces required for mechanical gating. Since dissection protocols that expose tiny *C. elegans* neurons rupture the worm's cuticle and destroy the hydrostatic skeleton, in vivo electrophysiological recording from neurons of behaving *C. elegans* is not technically feasible. As a consequence, it has not yet been possible to directly assay the effects of the *mec* genes on the activity of touch neurons in response to natural touch stimuli.

To address this critical question, we established a protocol for measuring physiological neuronal responses to touch in living animals through in vivo optical imaging. Our strategy was to use the genetically encoded calcium indicator cameleon (Miyawaki et al., 1997) to monitor the activity of touch neurons in response to controlled mechanosensory stimuli. Cameleons are multidomain proteins that include YFP and CFP moieties linked by calmodulin and a calmodulin binding peptide; when Ca^{2+} binds the calmodulin domain, conformational changes allow fluorescence resonance energy transfer (FRET) between YFP and CFP such that ratios of fluorescence signals reflect intracellular Ca^{2+} changes (Miyawaki and Tsien, 2000). We had previously recorded calcium transients in muscles and electrically stimulated neurons (Kerr et al., 2000), suggesting that

*Correspondence: wschafer@biomail.ucsd.edu
[4]These authors contributed equally to this work.

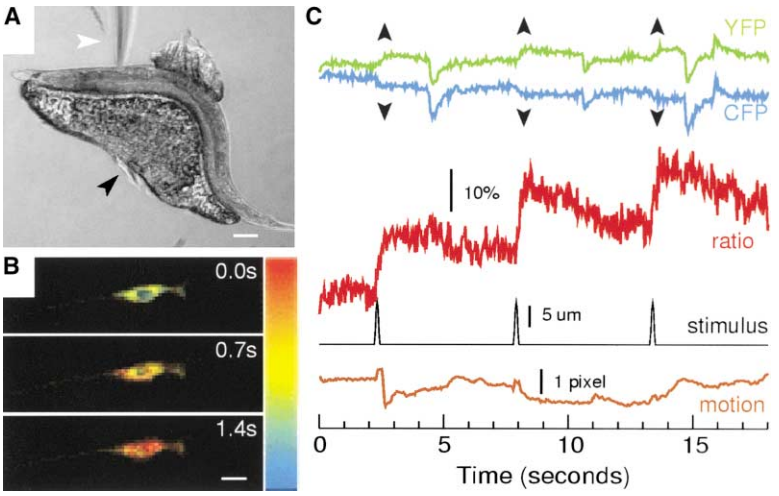

Figure 1. Detection of Touch-Activated Calcium Transients in Intact *C. elegans*

(A) An adult hermaphrodite *C. elegans* prepared for calcium imaging and mechanical stimulation. Worms are glued (black arrow) to a 2% agarose pad and immersed in extracellular saline. Mechanical stimulation is delivered by moving the probe (white arrow) against the worm by means of a computer-controlled motorized stage. Scale bar, 50 μm.

(B) Pseudocolor ratio image of mechanosensory neuron ALM before, during, and after a 1.4 s mechanical stimulation. Blue to red represents a ratio of 1.25–1.95. Scale bar, 5 μm.

(C) Response of ALM to mechanical stimulation. Mechanical stimulation (black line) consisting of a 10 μm deflection of the worm's body lasting 200 ms was delivered at 5 s intervals. The fluorescence ratio (red line) in the ALM touch neuron cell body responded with characteristic rises. CFP and YFP intensities (top two lines) changed reciprocally (black arrowheads), indicating that the ratio change was due to a change in fluorescence resonance energy transfer. Matched upwards or downwards spikes in YFP and CFP intensities reflect lamp instability and are effectively canceled out in the ratio. Motion of the cell was moderate (brown line) and had a different profile from ratio changes, indicating that motion was insufficient to cause substantial artifacts. Although the baseline increased, potentiation was not observed during closely repeated stimuli.

calcium transients could serve as a reliable indicator of neuronal depolarization in *C. elegans*.

Here we document the in vivo physiological responses to touch stimuli in *C. elegans* mechanoreceptor neurons. Using a cameleon-based reporter of transient calcium influx, we deduced the basic sensory/response capacities of the body touch neurons, distinguished a specific role for the MEC-4/MEC-2 channel in the sensation of gentle touch, and unexpectedly discovered that body touch receptor neurons also have the capacity to respond to harsh touch stimuli using molecular machinery distinct from the MEC-4/MEC-2 channel. These data significantly extend understanding of molecular mechanisms of touch transduction and establish feasibility of using cameleon reporters for analyses of nematode neuronal responses in a native context.

Results

In Vivo Detection of Touch-Evoked Neural Activity in *C. elegans* Mechanoreceptors

We generated transgenic lines that expressed chameleon YC2.12 (Nagai et al., 2002) in the touch neurons under the control of the *mec-4* promoter (Mitani et al., 1993). In the lines assayed, we found touch sensitivity to be normal (for example, for transgenic line *bzIs18* [$p_{mec\text{-}4}$YC2.12 + *lin-15(+)*], 82%, and for wild-type, 84% of animals respond to the first three successive touches), indicating that expression of cameleon in the touch receptors did not disrupt their function. To test whether we could detect calcium transients in response to mechanical stimulation, we glued individual adult hermaphrodites to 2% agarose pads and stimulated them with a round-tipped glass probe connected to a motorized stage (Figure 1A). Various types of gentle stimulation were given (Figure 2A): pokes, which represent brief stimuli in which the probe transiently pushes in and pulls out of the cuticle; presses, which give a longer stimulus of constant displacement; and buzzes, which are longer stimuli in which the probe is kept continually moving against the cuticle.

We first recorded from the ALM touch neuron. Images of CFP and YFP emission were captured simultaneously at 25 Hz, and custom software was used to compute the fluorescence ratio at each pixel and over the entire cell body. Long buzz stimuli produced clear and relatively uniform ratio changes across the entire cell body as shown in Figure 1B for a 1.4 s buzz. Short poke stimuli also generated reliable increases in the fluorescence ratio averaged over the ALM cell body (Figure 1C). The yellow and cyan intensities showed reciprocal changes (Figure 1C), as expected for a FRET change caused by an increase in calcium. All three classes of touch neurons, ALM, AVM, and PLM (Chalfie et al., 1985), exhibited reliable calcium responses when we delivered stimuli to the appropriate sensory region (anterior body for ALM and AVM, posterior body for PLM) and responded less reliably or not at all when the inappropriate body region was stimulated (data not shown; see also Figure 3F). Thus, we could robustly detect calcium transients in the touch neurons in response to mechanical stimulation.

To verify that our stimuli corresponded to gentle touch, we assayed the behavioral responses of partially immobilized wild-type and mutant nematodes under imaging conditions. Pokes and buzzes elicited vigorous thrashing from wild-type animals (7/8 responded to poke, 8/9 to buzz), but *mec* mutants failed to respond (0/9 *mec-4(u253)* animals responded to poke, 0/9 to buzz), indicating that our stimuli correspond to gentle touch typically delivered in behavioral tests by an eyelash stroke. We also noted an unexpectedly slow recovery of calcium levels to baseline, so we examined cell body responses with the lower affinity YC3.12 and observed a more rapid return to baseline (Figure 2B). This difference may be due to rapid reduction of calcium levels to below YC3.12 but not YC2.12 detection limits, or due to the higher affinity calcium buffering action of YC2.12 impeding the removal of calcium from the cell.

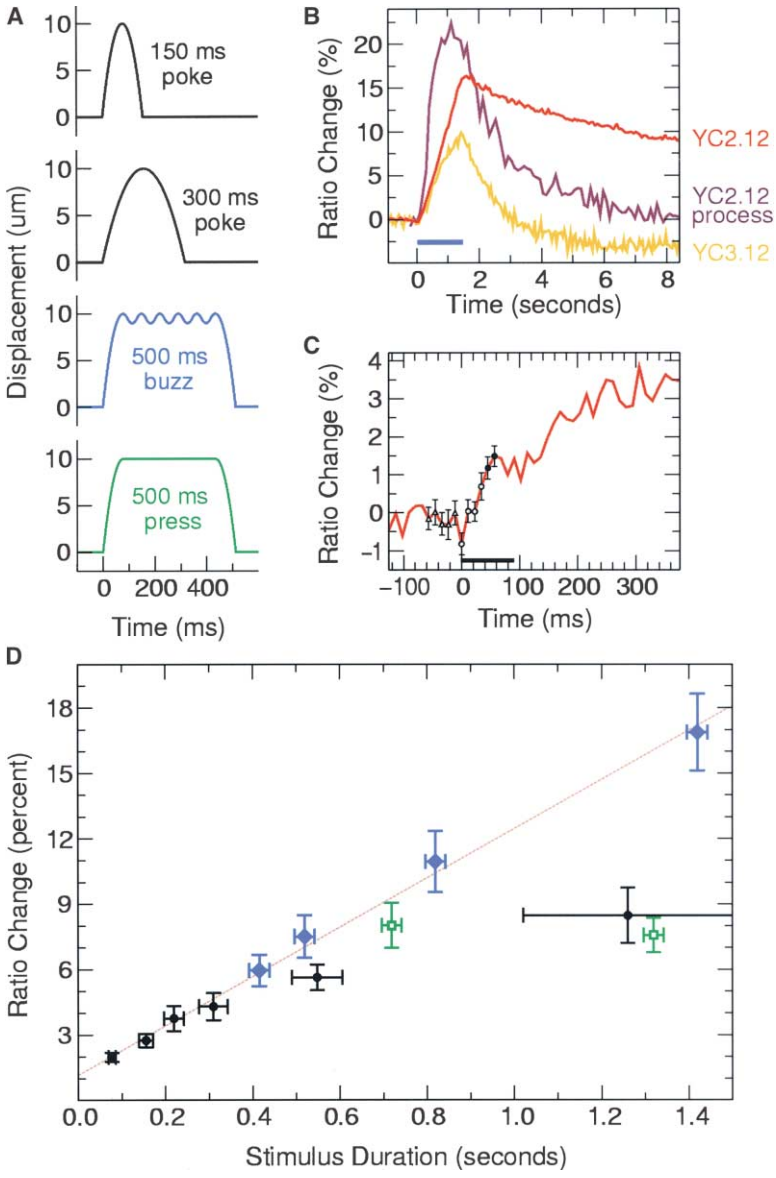

Figure 2. Magnitude and Latency of Wild-Type Responses to Gentle Touch

(A) Stimulation types. Idealized traces of the deflection of the worm cuticle are shown.

(B) Comparison of responses. Average responses to 1.4 s buzz (blue bar) are shown for the high-affinity YC2.12 (red line, n = 23 animals) and lower-affinity YC3.12 (orange line, n = 6 animals) in the ALM cell body. A single YC2.12 response from the process of ALM in the nerve ring is also shown (maroon line).

(C) Stimulus-response latency. Average response in the ALM cell body elicited by a 90 ms poke approximately 350 μm anterior to the cell body. Sample rate is 88 Hz. A statistically significant ($p < 0.001$) rise is observed 45 ms after the onset of stimulation (closed circles) as compared to the baseline 50 ms prior to stimulation (open triangles). 0–34 ms time points (open circles) are not significantly different from baseline. n = 60 (ten trials from six worms). Error bars indicate SEM.

(D) Response magnitudes under varying stimulus conditions. Shown is the mean magnitude of response in ALM to pokes (small black circles), buzzes (blue diamonds), and presses (green squares) of varying duration. Error bars indicate SEM of response (vertical) and expected deviation of stimulus duration (horizontal). Dashed line is fit of fastest four pokes and all four buzzes to a straight line, yielding $y = 11.3x + 1.2$. $n \geq 18$ animals for each data point shown.

Since YC2.12 produced larger responses, exhibited a linear rising phase over a wider range of calcium changes (up to about 30% ratio change), and did not disrupt touch behavior, we concluded that using YC2.12 would best allow us to accurately detect and quantify responses to mechanosensory stimuli in vivo.

To assess the effect of mechanical stimulation on calcium influx in regions other than the cell soma, we recorded from the ALM process in the nerve ring, where synaptic and gap-junction contacts are made. Calcium transients typically showed faster rises and decays than in the cell body (Figure 2B and data not shown), as might be expected from the greater surface-to-volume ratio in the process, but otherwise process responses seemed similar to cell body responses. Due to the technical difficulty and less favorable signal-to-noise ratio when recording from the process, we focused on the cell body for quantitative comparisons between different strains and stimulus conditions.

Characterization of the Gentle Touch Mechanosensory Modality in ALM

We used the poke, buzz, and press stimulus paradigms to investigate the basic properties of the gentle touch sensory modality in ALM. Very fast (90 ms) pokes were used to examine the time course of the initial response (Figure 2C). We observed a significant increase in ratio within 45 ms, although there was no evidence of response for the first 22 ms. Since it was necessary to stimulate some distance from the site of recording to avoid motion artifacts, it is unclear what fraction of the 20–30 ms delay is due to propagation time down the process. Regardless, it is clear that the cell can respond within 50 ms of stimulus onset. To determine the duration and strength of this response, we applied a variety of stimuli for a range of durations. As shown in Figure 2D, the magnitude of response was roughly proportional to the stimulus duration over a wide range of stimuli. Responses induced by fast (<500 ms) pokes and by

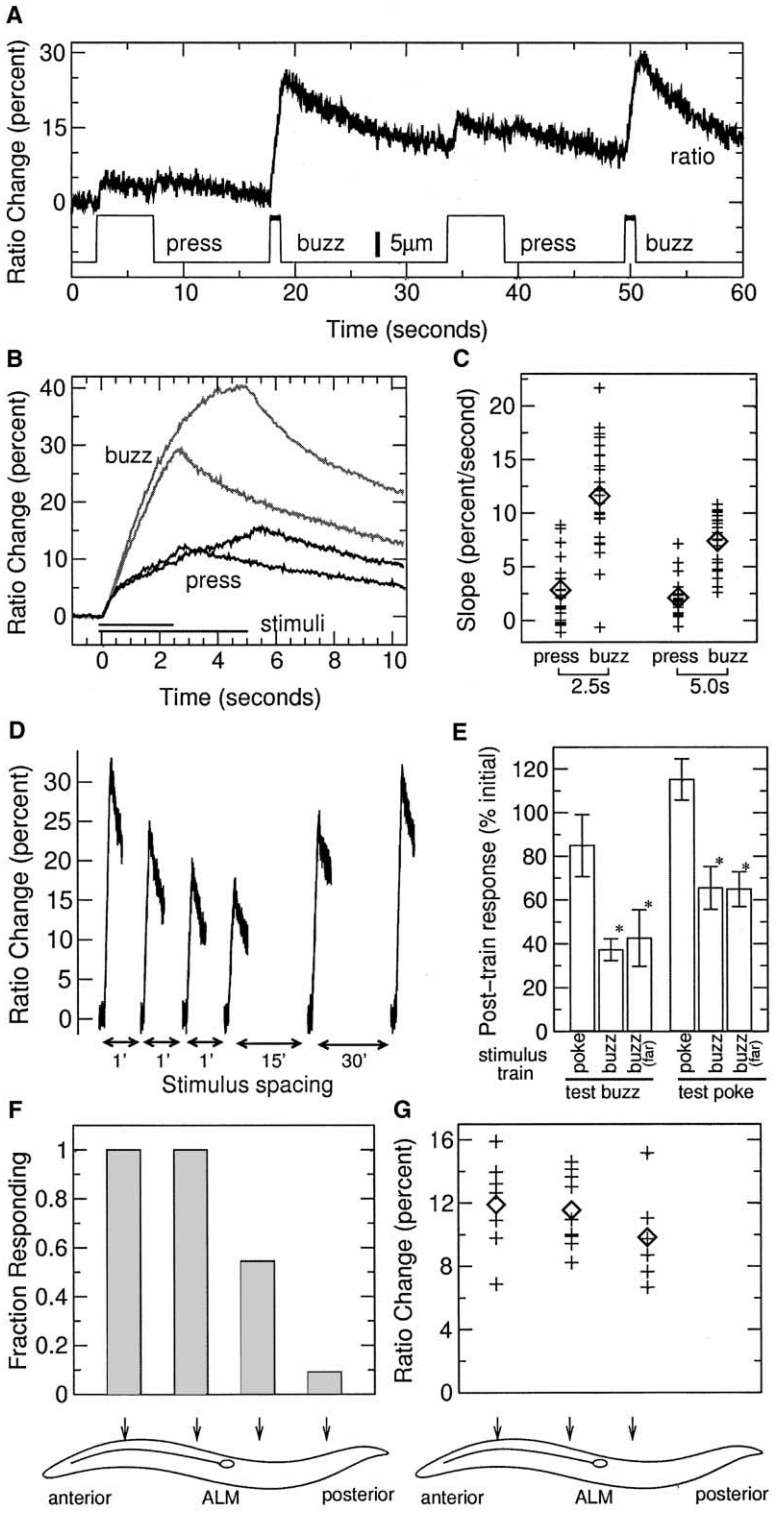

Figure 3. Characterization of the Wild-Type Gentle Touch Sensory Modality

(A) Typical response of ALM to motion and pressure. Two stimulation protocols were given (thin line), a 5 s press and a 1.5 s buzz. Responses in the ratio (thick line) were observed at the onset and removal of the press, and throughout the duration of the buzz, indicating that ALM is activated by motion but not by constant pressure.

(B) Average response of ALM to 2.5 and 5 s press (black line) and buzz (gray line). Note that the initial response is similar for ~500 ms. Average consists of responses from ≥19 animals aligned at stimulus onset.

(C) Quantification of late phase response. The slope of ratio change between 500 ms after stimulus onset until 300 ms before stimulus removal was calculated for 2.5 and 5 s press and buzz. Mean response (open diamonds) is lower for press in both cases ($p < 0.001$). Individual responses (pluses) show many presses with approximately zero slope (no stimulation) during the press, indicating complete adaptation to the motionless stimulus. Nonzero responses could reflect incomplete adaptation, or muscle contraction moving the worm against the probe.

(D) Reversible adaptation to multiple buzzes. A series of four 1.5 s buzzes were given at 60 s intervals, followed by 15 and 30 min rest periods.

(E) Adaptation to stimulus trains. Test stimuli were given 2 min before and after a training protocol, and adaptation was measured by comparing the magnitude of the two responses. The training protocol consisted of 30 stimuli at 10 s intervals. Test and training stimuli were either 150 ms pokes or 1 s buzzes. As shown, trains of buzzes but not pokes induced a significant decrease in response magnitude (*$p < 0.05$). This decrease was also observed when the 30 training buzzes were given far from the site where test stimuli were delivered, indicating a cell-wide reduction in response (bars labeled buzz [far]). n = 7 animals in each condition.

(F) Reliability of ALM responses across its receptive field. A 1.0 s buzz was given at four locations along the body (arrows, n = 10 worms each): two anterior locations that directly stimulate the specialized mechanosensory process of ALM, and two posterior locations that do not. Occasional responses from posterior stimulation may be due to mechanical stresses being transmitted through the worm's body.

(G) Magnitude of ALM responses across its receptive field. The magnitude of responses to stimulation in part (F) was quantified for each trial showing a response (pluses; open diamonds denote means). No stimulus location appeared significantly different from any other in response magnitude, despite the difference in response frequency.

buzzes fell along a line with a slope of 11.3%/s and intercept of 1.2%, while responses to other stimuli fell below this line. This line suggests an upper limit to the rate of response, which presumably corresponds to a maximally active cell for the duration of the stimulus. Longer, slower pokes and presses, during which the probe becomes essentially immobile, produced smaller stimuli. In particular, 0.7 and 1.3 s presses gave nearly identical responses, suggesting that the cell largely adapts to the constant pressure applied during the constant-deflection phase of the stimulus.

In vertebrates, mechanoreceptive neurons include

rapidly adapting (RA) motion sensors that initially respond but quickly stop responding even if the stimulus is maintained, as well as slowly adapting (SA) pressure sensor subtypes that continue to signal for extended durations of an applied stimulus (Koltzenburg et al., 1997). Vertebrate skin can be reproducibly stimulated by using von Frey hairs to deliver a constant force stimulus. The small size of *C. elegans* makes measuring force difficult, so we use deflections of constant size that presumably require a constant force to compress the body wall. Although the two methods are not directly comparable, presses should correspond to the constant force stimuli that SA mechanoreceptors respond to, while buzzes should provide varying force that would prevent adaptation to constant pressure. To address whether *C. elegans* touch mechanoreceptors were similar to either mammalian subtype, we compared neuronal responses to 5 s presses and 1.5 s buzzes (Figure 3A). In this trial, the press stimulus generated a response on application and removal of the pressure, but no response during the period of constant pressure, while the buzz caused a continual increase in calcium, suggesting that the gentle touch neurons are considerably more responsive to motion than to pressure per se. To further quantify this difference, we recorded the response to 2.5 and 5.0 s presses and buzzes (Figure 3B) and calculated the slope of the response from 500 ms after the onset of the stimulus to 300 ms before the removal of the stimulus (Figure 3C). These measurements confirmed that there is substantial adaptation to constant deflection, although in some cases residual responsiveness remained. This may reflect incomplete adaptation, or an artifact such as muscular contraction moving the worm's body against the stationary probe; in virtually all cases, there was detectable motion in the sample. In any case, ALM appears to function primarily as a motion sensor, consistent with the role of these cells in detecting vibrations caused by tapping a worm's plate.

Despite the lack of obvious adaptation during a single buzz stimulus, we wondered whether multiple stimuli would cause adaptation. Initially, we simply applied relatively long stimuli, 1.5 s buzzes, at 60 s intervals and observed a reduction in response magnitude that recovered after approximately 45 min (Figure 3D). Thus, we concluded that there is adaptation to repeated buzz stimuli. Behavioral habituation to repeated taps is well characterized (Jorgensen and Rankin, 1997). For instance, after 30 successive taps with a 10 s interstimulus interval, most animals fail to respond behaviorally. Since tap response is mediated by the touch neurons, we replicated this training protocol with both 150 ms poke and 1 s buzz stimuli and compared test responses before and after training. Both poke and buzz test stimuli were significantly reduced after training with 1 s buzzes (Figure 3E), as compared to pretraining levels and compared with mock training where no stimuli were given (data not shown). This reduction was also observed when test stimuli were given at a different location than the training stimulus, suggesting a cell-wide reduction in response rather than a local phenomenon of adaptation at a specific point along the sensory process. It is therefore likely that habituation protocols alter the response properties of the sensory cells. However, when pokes were used as the training stimulus, no significant change in touch neuron response was observed (Figure 3E). Furthermore, responses were reliable, if reduced in amplitude, after buzz training, in contrast to the near complete elimination of behavioral responses after habituation. Therefore, while adaptation of the sensory cell response may play a role in the habituation of behavior, other mechanisms are likely to also be involved.

To define the receptive field of ALM, we measured neural responses to poke and buzz stimuli applied at different locations along the body. We delivered both 150 ms poke and 1 s buzz stimuli in four locations: two locations anterior of ALM along its specialized process, and two posterior locations (Figure 3F). We observed reliable responses at the two anterior stimulus locations (19/19 buzz, 18/20 poke) and almost never saw a response at the far posterior location (1/11 buzz, 0/10 poke) (Figure 3F and data not shown); responses to the close posterior location were intermediate in occurrence (5/9 buzz, 2/11 poke). Thus, the receptive field for ALM is roughly coincident with its sensory process. Interestingly, in the successful trials, the magnitude and slope of transients caused by close and distant anterior stimulation were not significantly different (Figure 3G), suggesting either active propagation or very little loss during passive propagation down the process. Furthermore, when a response was elicited by posterior stimulation, it was also not significantly different in magnitude, despite the reduced probability for success (Figure 3G), suggesting a sharp threshold between submaximal and maximal stimulation.

Dependence of Touch Neuron Calcium Transients on L-Type Calcium Channels

Having defined stimulus conditions specific for the gentle touch response of ALM, we proceeded to investigate the molecular components required for induction of calcium transients. Neither reduction-of-function alleles of ER calcium release channels (the ryanodine receptor homolog *unc-68(e540)* and the IP3 receptor homolog *itr-1(sa73)*) (Maryon et al., 1996; Dal Santo et al., 1999) nor null alleles of the ER calcium storage molecule calreticulin *(crt-1(bz29))* (Xu et al., 2001) had significant effects on calcium transients in ALM induced by fast or slow poke or 3 s buzz (Figure 4B and data not shown). Likewise, putative null mutations *lj1* and *mu74* in the N-type voltage-gated calcium channel gene *unc-2* (Schafer and Kenyon, 1995) did not affect calcium transients evoked in ALM in response to touch stimuli. In contrast, the partial loss-of-function mutation *ad1006* in the L-type calcium channel gene *egl-19* significantly reduced the magnitude of calcium influx in response to poke and buzz stimuli. Thus, the calcium transients observed in response to touch appeared to be dependent on the influx of extracellular calcium through voltage-gated channels, supporting the hypothesis that the touch-induced calcium transients are an indicator of mechanosensory depolarization.

Effects of *mec-4* and *mec-2* on Touch Neuron Sensory Responses

We next assessed the effects of the *mec* genes on the process of mechanosensation in ALM. We first tested the effect of *mec-4*, which encodes a sodium channel subunit of the DEG/ENaC superfamily and has been hypothesized to be the core channel subunit of the

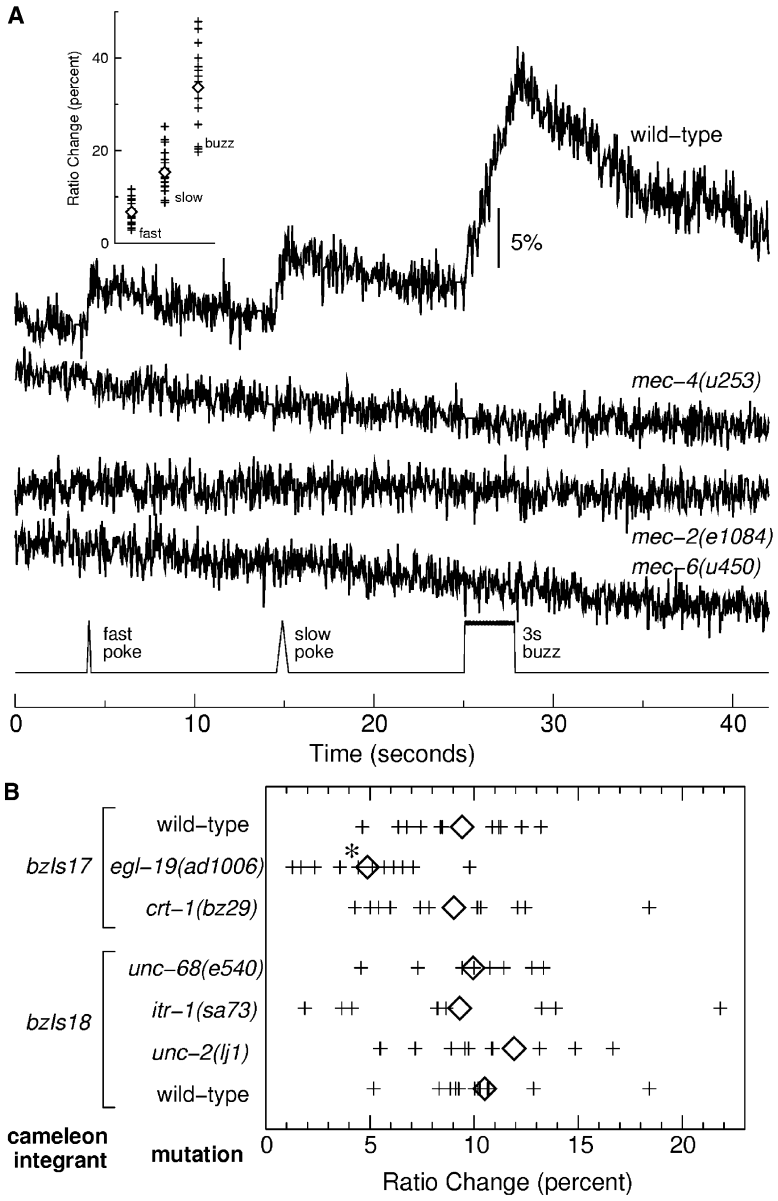

Figure 4. Effects of MEC Proteins and L-Type Calcium Channels on Gentle Touch-Evoked Calcium Transients

(A) Touch neurons in wild-type worms, but not *mec* mutants, respond to gentle-touch stimulation protocols. ALM was recorded during stimulation of worms with a fast (450 ms) poke, slow (1350 ms) poke, and a 3 s buzz. Typical traces are shown here; wild-type responses (top line) are observed for all three types of stimuli (thin line), while mutants in degenerin channel subunits *mec-4* or *mec-6*, or in the associated stomatin *mec-2* showed no response. Additionally, in lightly glued worms, all three stimuli induced a motor response consistent with touch-avoidance behavior in wild-type but not *mec-4* or *mec-2* worms ($n \geq 10$ animals for each assay). All three *mec* mutants also showed no response to prolonged (buzz-like) gentle stimuli administered by hand.

(B) Calcium transients rely primarily upon the *egl-19* L-type voltage-gated calcium channel (VGCC). Mean responses (diamonds) and individual responses (pluses) to 600 ms poke are shown for mutants in the L-type VGCC *egl-19*, non-L-type VGCC *unc-2*, ryanodine receptor *unc-68*, IP_3 receptor *itr-1*, and calreticulin *crt-1*, in either of two cameleon-expressing integrants *bzIs17* or *bzIs18*. Only the reduction-of-function mutant *egl-19(ad1006)* showed a significant reduction in response ($p < 0.01$), suggesting that the primary source of calcium is the *egl-19* L-type VGCC.

touch-transducing complex. In contrast to wild-type animals, we found that in all cases, null alleles *u253* and *e1497* of *mec-4* mutants showed no calcium response to poke, buzz, or press stimuli (Figure 4A and data not shown) as assessed either by eye or by our automated transient-detecting algorithm (see Experimental Procedures). Thus, the MEC-4 protein is required for touch neuron activity in response to two types of gentle touch stimuli. Similarly, the *mec-2* null mutant *e1084* and the *mec-6* null mutant *u450* showed no detectable calcium response in the touch neurons to either stimulus (Figure 4A). Thus, MEC-2 and MEC-6, like MEC-4, are required for touch neuron activity in response to gentle touch. In oocytes, MEC-2 potentiates the MEC-4 channel current as much as 40-fold, but is not critical for all MEC-4 channel activity (Goodman et al., 2002). The observation that *mec-2* mutants show in vivo defects as severe as those of *mec-4* may indicate the reduced conductance of the MEC-2-deficient channel is insufficient to generate a calcium transient in vivo. Alternatively, in the in vivo situation that we monitor, MEC-2 may be necessary to couple channel activity to the movement of intracellular structures such as microtubules.

MEC-4 and MEC-2 Do Not Alter the Basic Physiology of the Touch Neurons

Although our results established that the *mec* genes are required for transient calcium elevations in response to gentle touch, they did not rule out the possibility that *mec* mutations might disrupt generic properties of the touch neurons (e.g., by altering their general excitability or ability to generate a calcium transient in response to depolarization) rather than specifically affecting mechanotransduction. To address this question, we created primary neuronal cultures (Christensen et al., 2002) of cells derived from p_{mec-4}::YC2.12 expression strains. We

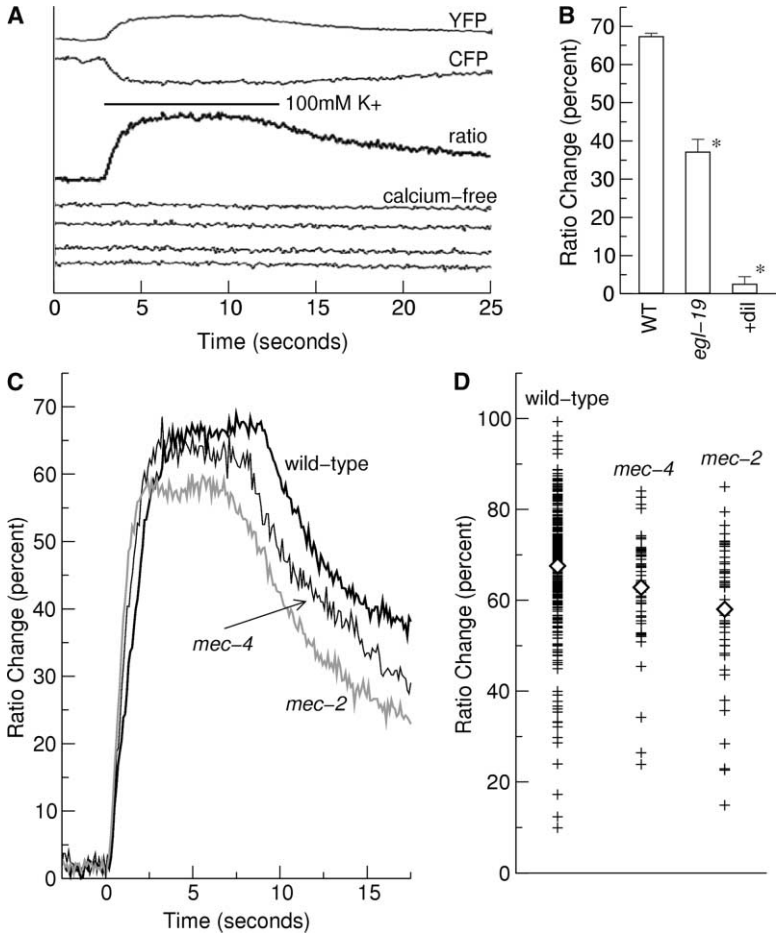

Figure 5. Effects of MEC Proteins and Calcium Channels on Depolarization-Evoked Calcium Transients

(A) Potassium depolarization in cultured neurons. The top traces show the individual YFP and CFP intensities and the bottom trace the ratio YFP/CFP. The ratio faithfully records the reciprocal ratio change in the individual intensities. Lower traces: no response to 40 mM K^+ is observed in calcium-free buffer with 2 mM EGTA.

(B) Dependence of depolarization-induced calcium transients on L-type calcium channels. The histogram shows mean ± SEM calcium transient amplitude in response to 100 mM K^+ for wild-type cells in normal buffer and in buffer with 1 mM diltiazem, an L-type channel blocker. Also shown is the calcium transient amplitude for *egl-19(ad1006)* cells in normal buffer.

(C) Cultured touch neurons expressing YC2.12 were exposed to 100 mM K^+ depolarization for approximately 8 s and resulting calcium transients were recorded. Responses were grossly similar in neurons cultured from wild-type, *mec-4(u253)*, and *mec-2(e1084)* animals, indicating that *mec-4* and *mec-2* are not essential for voltage-dependent calcium influx. Traces are aligned at the onset of the response.

(D) *mec-4* and *mec-2* do not alter the magnitude of calcium response to potassium depolarization. The magnitudes of depolarizations observed in cultured neurons of wild-type, *mec-4(u253)*, and *mec-2(e1084)* were recorded (plusses). Neither the mean (open diamonds) nor distribution of magnitudes was substantially affected. Conditions were as in (C). Sample sizes were n = 315 cells for wild-type, n = 56 for *mec-4*, and n = 50 for *mec-2*. Similar results were obtained for the null allele *mec-4(e1497)*.

depolarized the cultured touch neurons with extracellular potassium and assayed calcium changes using cameleon. All imaged neurons showed a robust ratio increase of homogenous size and similar time course coincident with the depolarization, and the ratio trace faithfully corresponded to reciprocal changes in yellow and cyan intensities as expected from an increase in cameleon FRET due to a rise in intracellular calcium. Calcium transients were completely abolished in nominally calcium-free depolarizing solutions (2 mM EGTA added to 40 mM K^+ solution without calcium; Figure 5A) and by the L-type calcium channel blocker diltiazem (Figure 5B). *unc-2*, *itr-1*, and *unc-68* strains all showed no significant difference from wild-type (Frøkjær-Jensen, 2002). These results indicated that the intracellular rise in calcium caused by potassium-induced depolarization was dependent on calcium influx through L-type voltage-gated channels as opposed to non-L-type channels, the IP3 receptor, or the ryanodine receptor.

To investigate whether the *mec* genes altered the excitability or general calcium dynamics of the touch neurons, we created cell cultures of cameleon-expressing *mec-4* and *mec-2* null touch neurons and compared their calcium responses to potassium-induced depolarization to those of wild-type cells (Figures 5C and 5D). Significantly, we observed responses in neurons from the *mec-4(u253)* and *mec-2(e1084)* null strains that were indistinguishable from those of wild-type neurons. In all cases, over 90% of cells gave a measurable response. It should be noted that while the *mec-4(u253)* and *mec-2(e1084)* mutations had no detectable effect on depolarization-induced calcium influx, they abolished in vivo calcium and behavioral responses to gentle touch. In contrast, the calcium influx in cells cultured from *egl-19* reduction of function mutants was significantly reduced in these assays relative to wild-type (Figure 5B). Thus, since *egl-19* hypomorphic mutants showed only a partial reduction in touch induced calcium influx and only slightly impaired touch avoidance behavior, the absence of touch-induced calcium transients in *mec-2* and *mec-4* animals could not be caused by a general defect in L-type calcium channel function in these mutants. Together, these results indicated that *mec-2* and *mec-4* do not affect the general excitability of the touch neurons or their ability to generate a calcium transient in response to depolarization.

To confirm that deletion of the *mec-4* gene does not affect the general functionality of touch neurons, we assayed ionic currents in cultured touch neurons prepared from wild-type and *mec-4(u253)* nematodes. We

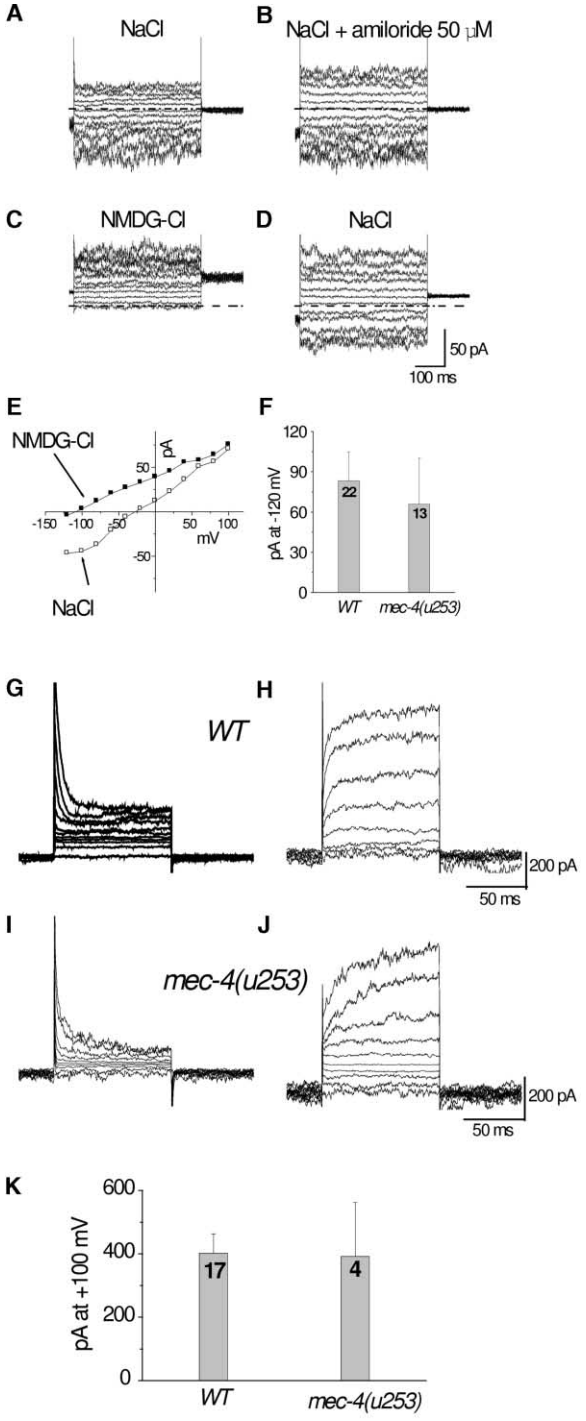

Figure 6. Effects of MEC Proteins on Resting Currents in Touch Neurons

(A) Example of whole-cell currents recorded from a WT touch neuron. Voltage steps were from −120mV to +100mV, from a holding potential of −50mV. The cell was perfused with a NaCl solution (see Experimental Procedures).

(B) The same cell was perfused with NaCl plus 50 mM amiloride.

(C) Perfusion of the same cell with a solution in which NaCl had been substituted with NMDG-Cl.

(D) Upon washout and reperfusion with NaCl, the inward component of the current has reappeared.

(E) Current-voltage relationships of the currents shown in (A) and (C). Notice the disappearance of the inward component of the current during perfusion with NMDG-Cl.

(F) Average current at −120mV recorded from WT and *mec-4(u253)* touch neurons. Data are expressed as mean ± SE. The number of assayed touch neurons is indicated in the graph.

(G) Family of outward currents likely carried by K+ recorded from a WT touch neuron. Voltage steps were from −80mV to +100mV from a holding potential of −80mV.

(H) Only example of slowly activating outward current found in a WT touch neuron.

(I and J) Same as (G) and (H) for *mec-4 (u253)* touch neurons.

(K) Average outward current at +100mV measured at the beginning of the depolarizing voltage step. Data are expressed as mean ± SE; the number of assayed touch neurons is indicated in the graph.

applied the patch-clamp technique in the whole-cell configuration, which allows the stimulation of the cell membrane with polarizations more resembling those seen in vivo. We used intracellular and extracellular perfusing solutions that allow the detection of sodium currents so that we could determine if a *mec-4*-dependent current was constantly present in touch neurons from WT nematodes and ablated in *mec-4(u253)* touch neurons. In both wild-type and *mec-4*, we could detect three types of ionic currents: a voltage-insensitive cationic current and two types of voltage-sensitive outward currents likely carried by potassium ions. The cationic current conducted sodium as well as potassium (data not shown), but did not conduct the much larger cation NMDG and it was not sensitive to amiloride (Figures 6A–6E). Of the two voltage-gated outward currents, one type activated and inactivated very quickly (inactivation τ at +100mV = 5.5 ± 0.9 ms, n = 6, Figures 6G and 6I), and the other displayed much slower activation kinetics and did not appear to inactivate during pulses as long as 400 ms (Figures 6H and 6J and data not shown). The slowly activating outward current was rare and was found in only one wild-type and one *mec-4(u253)* touch neuron. When we measured current amplitudes at −120mV and +100mV to determine if deletion of *mec-4* affected current density, we did not find difference between neurons cultured from the WT and *mec-4(u253)* (Figures 6F and 6K). Thus, we were again unable to detect any differences between the basic physiology of wild-type and *mec-4* mutant touch neurons.

MEC-4-Independent Sensory Responses in the ALM Touch Neurons

To further test the specificity of the *mec* mutants' touch neuron defects, we assayed the in vivo responses of the *mec* mutants to a distinct sensory stimulus, harsh touch. Avoidance of harsh touch (typically delivered by hand by prodding with a platinum wire) does not require the activity of the *mec* genes (Chalfie and Sulston, 1981), although cell ablation experiments suggest that the touch neurons might contribute to sensing these stimuli (Way and Chalfie, 1989). We observed reliable behavioral responses to harsh touch in *mec-4, mec-2*, and *mec*-6 animals, as anticipated. To determine the effect of harsh touch on calcium influx in ALM, we developed a protocol to deliver an analog of harsh touch to immobilized animals: a high-speed (2.8 mm/s), 30 μm deflection to the worm's body wall using a flat metal probe (here

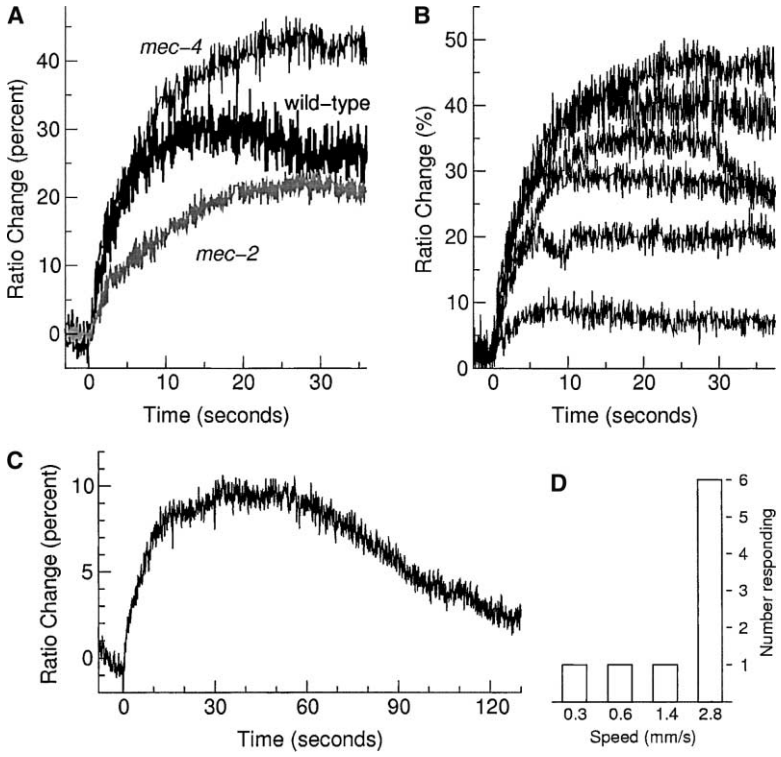

Figure 7. Effects of MEC Proteins on In Vivo Calcium Responses to Harsh Touch

(A) *mec-4*-independent response to harsh stimulation. Individual worms were stimulated at 10 s intervals with a jab consisting of a very rapid 30 μm displacement into the body of the worm; example traces are shown. Responses were observed in a subset of wild-type (thick line, 4 of 7), *mec-4(u253)* (thin line, 12 of 20), and *mec-2(e1084)* (gray line, 3 of 8), indicating that response to jab does not depend on the gentle touch receptors. After a brief (approx 90 s) refractory period, jabbed animals also responded to subsequent jabs (data not shown).

(B) Six example traces from *mec-4(u253)* illustrating that the response is variable and long lasting.

(C) Average wild-type response to jab stimulation. Individual wild-type worms expressing the reduced-affinity cameleon YC3.12 were stimulated with 50 μm jabs where the probe was moved at 0.3, 0.6, 1.4, or 2.8 mm/s. The averaged response of the six least noisy traces is shown.

(D) Distribution of first responses to jabs of increasing speed. While occasional responses were observed at lower speeds, the highest speed gave the most reliable stimulation.

designated a "jab" stimulus). In contrast to pokes and buzzes, jabs elicited normal avoidance behavior in *mec-4* mutants (10/12 worms responded). Thus, the jab stimulus appeared to be analogous by genetic and behavioral criteria to harsh touch.

When we monitored calcium influx in response to jab stimuli in ALM neurons, we observed a large and prolonged calcium influx of variable amplitude in wild-type as well as *mec-2* and *mec-4* mutant animals (Figures 7A and 7B). To examine the sensitivity and response profile of this response, we applied 50 μm jabs at varying speeds to wild-type worms (Figure 7C) and found that only the fastest 2.8 mm/s stimulus induced the characteristic response (Figure 7D). Responses appeared to last approximately 90 s in worms expressing YC3.12, which was used in order to reduce the possibility of extending the apparent response via calcium buffering. These results demonstrate that the *mec* genes are not generally required for touch neuron function or health in vivo, but rather, are specifically required for sensory responses to gentle touch. Further, these results suggest that the touch neurons may contain a molecularly distinct, *mec-4*-independent mechanosensory modality that is activated by harsh touch (i.e., jabbing) but not by gentle touch (i.e., poking and buzzing).

Discussion

Assessing the Specificity of *mec-4*/*mec-2* Mechanotransduction Defects at the Cellular Level

The genetically based model for touch transduction in *C. elegans* (Ernstrom and Chalfie, 2002) has served as an elegant working hypothesis for how mechanosensation occurs. However, the fundamental premise of this model, that the *mec* genes encode components of a complex specifically involved in mechanosensation, has long been difficult to test at the cellular level. Since *mec* mutations eliminate the behavioral response to gentle touch, the proteins encoded by these genes are plausible candidates for components of a mechanotransducing complex. On the other hand, an equally plausible hypothesis is that a given MEC protein might simply be needed for the basic neuronal function of the touch receptor neuron, and that the touch-insensitive phenotype would result because the touch receptor would be impaired for signaling. In humans, the correlation of leaky red blood cell membrane permeability with stomatin deficiency (Stewart and Fricke, 2003) gives credence to this alternative hypothesis. Since *C. elegans* neurons are small (2 μm in diameter at the cell body) and touch neurons are particularly inaccessible (embedded tightly within the worm cuticle), it has not been possible to assess the specificity of the MEC-4/MEC-2 channel's effect on mechanosensation at the cellular level using standard electrophysiological approaches.

Using in vivo optical imaging, we have shown that the MEC-4 Na^+ channel subunit and its associated accessory subunit MEC-2 are specifically required for cellular responses by *C. elegans* mechanoreceptor neurons to a defined set of gentle mechanical stimuli. Since *mec-2* and *mec-4* are required for generating calcium transients in response to touch but are not required for maintained resting currents, the capacity for potassium-induced depolarization, or for harsh touch responses in vivo, the MEC-4/MEC-2 channel must act at some step between the sensation of mechanical force and the generation of the mechanosensory depolarization. This is

consistent with the hypothesis that MEC-2 and MEC-4 comprise subunits of a mechanically gated ion channel, and validates a central and specific role for the MEC-4/MEC-2 complex in the process of mechanosensation.

Key questions regarding the MEC complex and its role in touch transduction nonetheless remain unresolved. For example, it is not clear whether the MEC proteins alone are sufficient for mechanotransduction in the touch neurons, or whether they regulate or act in concert with other channel proteins. Nor is it established that all of the genetically defined MEC proteins act in the same aspect of the mechanosensory process as MEC-2 and MEC-4. Future in vivo imaging studies of additional *mec* mutants and their effects on touch neuron responses to mechanical stimuli will provide important insight into these questions.

What a Touch Neuron Feels

Our results indicate that the *mec-4*-dependent gentle touch mechanoreceptors respond primarily to motion rather than continuous pressure. More specifically, after 0.5 s, responses elicited by a constantly moving touch stimulus are larger in amplitude than those elicited by constant pressure. The decreased response to constant pressure is unlikely to result from fatigue or general adaptation of the cellular response, because the neuron can sustain responsiveness to a continuously moving stimulus. Rather, it appears that the touch receptor neuron adapts rapidly to constant pressure in the stimulus range we delivered, but is tuned toward sensing and responding to light but sustained motion across the receptor field. The in vivo sensitivity we document is consistent with the behavioral assay in which an eyelash is typically gently dragged across the body.

Our data also reveal that the neurons defined as critical for responses to gentle body touch mount a distinct physiological response to harsh touch stimuli. It is known that in the absence of functional touch receptor neurons, *C. elegans* can respond to harsh touch stimuli such as the prod of a wire (Chalfie and Sulston, 1981), and the capacity to respond to harsh mechanical stimuli has been proposed to reside in FLP and PVD neurons (Way and Chalfie, 1989). Our data establish that the gentle touch receptor neurons are also activated by harsh mechanical stimuli and thus may normally contribute, directly or indirectly, to this mechanosensory behavior. Importantly, the response to at least some harsh mechanical stimuli in touch neurons occurs independently of the MEC-4 and MEC-2 channel subunits. This suggests the possibility that specialized mechanosensory neurons may have the capacity to respond to a range of stimuli and may employ different molecular machinery to do so. The harsh touch-transducing channel might involve different homo- or heteromeric combinations of non-MEC-4 degenerin channels and distinct stomatins (nine stomatins are encoded in the *C. elegans* genome), or may involve a second class of mechanotransduction channels.

Calcium Channels in Mechanotransductory Neurons

We have implicated one specific L-type channel subunit, EGL-19, in the calcium responses of touch receptor neurons to gentle touch stimuli. Our findings in vivo and in culture together support the straightforward model that MEC-4 channel activity depolarizes touch neurons and activates voltage-gated calcium channels. It is not yet clear how critical EGL-19 function is to touch perception. Null alleles of *egl-19* are lethal and the partial loss-of-function allele we worked with (*ad1006*) (Lee et al., 1997) does not markedly disrupt touch sensitivity (B.G. and M.D., unpublished data). The residual cameleon-reported responses we find in the *egl-19(1006)* mutant may suffice for touch sensory nerve conduction, or another channel type may contribute to the response. Recently, a T-type channel has been implicated in a specific D-type mechanoreceptor class in mouse (Shin et al., 2003). The sole T-type calcium channel in the *C. elegans* genome is encoded by the *cca-1* gene (Jeziorski and Greenberg, 2000). We have not found any major touch abnormality in touch sensitivity in a null *cca-1* background, and thus, *cca-1* does not play a mandatory role in gentle touch sensation. Still, it remains possible that combinations of specific calcium channels may act to modulate touch sensitivity, a hypothesis that awaits further evaluation of combinations of null mutations in the touch neurons.

Our results also have interesting implications regarding the role in calcium channels in basic nematode neuronal physiology. Nematode genomes appear to lack homologs of voltage-gated sodium channels (Bargmann 1998), raising the question of how neuronal depolarization is propagated down the neural process. It has been suggested that the high input resistance of *C. elegans* neurons might allow propagation to occur exclusively through passive electrical conductance (Goodman et al., 1998). However, several of our results suggest that active propagation may occur in *C. elegans* touch neurons. In particular, we observed that stimulation of the sensory process in a region proximal to the cell body induced somatic calcium transients that were neither larger nor more reliably evoked than those induced by more distal stimulation. Moreover, although stimuli applied outside the core receptive field of the touch neurons evoked calcium transients much less reliably than stimuli applied over the sensory process, in the instances in which calcium transients were evoked, transients were approximately equal in magnitude (relative to stimulus duration). This all-or-none neural response pattern suggests the existence of active currents in *C. elegans* touch neurons, possibly mediated by voltage-gated calcium channels.

DEG/ENaCs in Mechanosensation from Nematodes to Mammals

The mammalian MEC-4-related ASIC channels BNC1, ASIC2, and ASIC3 are expressed in rodent cutaneous mechanoreceptor neurons and can modulate their responses to specific mechanosensory stimuli (see Bianchi and Driscoll, 2002). Thus, a basic role in mechanotransduction appears conserved from nematodes to humans. Interestingly, BNC1 and ASIC3 deficiency disrupts the function of rapidly adapting mechanoreceptor neurons in mouse skin (Price et al., 2000), and thus, these channel types might be predicted to act most similarly to the MEC-4/MEC-2 channel. However, al-

though knockouts of BNC1 and ASIC3 alter the stimulus response properties of RA (and some other) mechanoreceptors, they have not been found to eliminate responses to any specific stimuli as we observed for *mec-4*. Why might gentle touch be eliminated in nematodes but only modestly modified in mammalian mechanoreceptors when DEG/ENaCs are lacking? One possible explanation for the BNC1/ASIC3 phenotypes is that mammalian mechanoreceptors may contain multiple functionally redundant ENaC or ASIC channels with overlapping specificities for different touch stimuli. Alternatively, DEG/ENaC channels may play a more modulatory role in the process of mechanosensation that is more stringently required for gentle touch sensation in nematodes than in mammals.

In Vivo Probes of Neuronal Activity

Our studies establish the feasibility of using in vivo optical imaging with a genetically encoded sensor to assess the roles of the MEC proteins in a measurable cellular response to touch. Future application of these methods in touch receptor neurons should enable us to significantly extend hypotheses about the properties and sensitivity of the touch-transducing complex, contributions of individual molecules to mechanosensation, the role of Ca^{2+} on adaptation properties, and the consequences of specific mutations within an in vivo context. Our methods should be readily extensible to analyses of other neuronal classes in *C. elegans* and other organisms for in vivo physiological studies of neuronal function that are critical for understanding biological function in a native context.

Experimental Procedures

Construction of Strains

The $p_{mec\text{-}4}$*::YC2.12* expression plasmid was generated as follows. A 1078 bp *mec-4* promoter fragment was obtained by PCR on *C. elegans* genomic DNA using primers CCTTGTGGCTCAGTGC GAAACTGTC and GACAGTTTCGCACTGAGCCACAAGG followed by SalI and HindIII digestion. A recent version of the cDNA for YC2.12 was kindly supplied by Atsushi Miyawaki, and a SalI site was introduced 18 bp upstream of the initiation Met by PCR using primers GCGTCGACGCGGCCGCCACCATGGTGAGCA and GGAATTCTTA CTTGTACAGCTCGTC. The two fragments were incorporated into a Fire Lab '97 Vector kit plasmid L3613 backbone cleaved with HindIII and EcoRI. The resulting plasmid was injected into *lin-15(n765)* worms along with a rescuing *lin-15* marker (Huang et al., 1994) to isolate stable extrachromosomal lines (Mello and Fire, 1995); these were then integrated using standard γ-irradiation techniques (Mello and Fire, 1995) to obtain lines *bzIs17* and *bzIs18* which were used for imaging. Mutant lines expressing cameleon were obtained by crossing *bzIs17* or *bzIs18* with existing mutants and isolating progeny homozygous for both the mutation of interest and the cameleon construct. $p_{mec\text{-}4}$*::YC3.12* strains were constructed similarly, but not integrated.

Calcium Imaging

Optical recordings were performed on a Zeiss Axioskop 2 upright compound microscope fitted with a Hamamatsu Orca ER CCD camera, a Hamamatsu W-View emission image splitter, and a Uniblitz Shutter (Vincent Associates). Fluorescence images were acquired and saved using MetaVue 4.6 (Universal Imaging). Samples were typically taken at 20–30 Hz (33–50 ms exposure time) with 4×4 or 8×8 binning, using a $63\times$ Zeiss Achroplan water immersion objective. Filter/dichroic pairs were: excitation, 420/40; excitation dichroic, 455; CFP emission, 480/30; emission dichroic, 505; and YFP emission, 535/30 (Chroma). To limit YFP photobleaching, neutral density filters were used to control illumination intensity; in some animals with particularly dim cameleon expression (Figures 2B and 4A), the more intense illumination used consequently resulted in greater photobleaching. Photobleaching in Figure 2B was corrected for presentation purposes by fitting inactive portions of the trace to a single exponential.

Individual worms (adult hermaphrodites, typically $\sim$24 hr past L4) were glued with Nexaband S/C cyanoacrylate glue to pads made from 2% agarose (Figures 1–3) or 5% agarose (Figures 4 and 7) dissolved in extracellular saline (Lockery and Goodman, 1998): 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM HEPES, 20 mM d-glucose, pH 7.2, or as described for culture experiments. Various salt solutions gave similar results, but worms seemed to survive consistently for longer in some type of extracellular saline. No difference was observed between worms mounted on pads made from different agarose concentrations, so whichever concentration was most convenient for handling was used. The same saline, without agarose, was used as the immersion medium.

Mechanical Stimulation

Gentle stimuli were delivered with a M-111.1DG microtranslation stage (Polytec PI) mounted at a 45° angle on a three-axis micromanipulator. The stage was used to drive a probe (pulled glass microcapillary with a rounded tip 15 μm in diameter for Figures 1–3, or a platinum wire with similar tip size for Figures 4 and 7) against the side of a glued worm. The tip was positioned near to the body wall and driven forward to cause a 10 μm (Figures 1–3) or 15 μm (Figures 4 and 7) deflection of the body wall. Optical and mechanical stimuli were synchronized by flashing a white LED on the sample when triggered by the stage.

Harsh stimuli were delivered with a Burleigh Inchworm micropositioning device, which can create very abrupt motions. To deliver the jab stimulus, a platinum wire probe was placed against the worm and driven 30 μm into the worm at a velocity of approximately 2.8 mm/s, and then retracted after 50 ms. Behavioral responses to jab and other stimuli were evaluated by recording the immobilized animals' behavior under transmitted light and determining whether they initiated tail thrashing within 0.5 s of the stimulus application.

C. elegans Primary Cultures

C. elegans primary cultures were prepared from synchronized adult worms as described by Christensen et al. (2002). Briefly, young gravid adults were treated with a 0.5 M NaOH and 1% NaOCl solution for 5–10 min to release eggs. After three washes with egg buffer (118 mM NaCl, 48 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 25 mM HEPES, pH 7.3, 340 mOsm), eggs were isolated from adult carcasses by centrifugation in a 30% sucrose gradient. The isolated eggs were resuspended in egg buffer containing 1 U/ml chitinase (Sigma Chemical, St. Louise, MO) and digested for 15–20 min on a turning rack. Eggs were dissociated with a 27 gauge needle or an Eppendorf 1 ml pipettor and resuspended in fresh L-15 (Life Technologies, Grand Island, NY) containing 10% fetal bovine serum (Life Technologies), 50 U/ml penicillin, 50 μg/ml streptomycin, and osmolarity of 340 mOsm. Single cells were isolated from undissociated eggs and larvae by filtration through a 5 μm Durapore filter (Millipore, Bedford, MA). Dissociated cells plated on cover glass (Fisher Scientific, Pittsburgh, PA) coated with 1 mg/ml peanut lectin (Sigma). Cells were cultured in 24-well Costar culture plates (Corning Inc., Corning, NY) and left for 2 hr on cover glass to adhere before substituting the L-15 growth medium. Cells were grown in plastic boxes containing wet tissues in a 25°C incubator.

Potassium Depolarization in Cell Culture

Primary cell cultures from wild-type, *mec-4(u253)*, *mec-4(e1497)*, and *mec-2(e1084)* worms expressing cameleon in the touch neurons from the *bzIs18* array were prepared from synchronized worms as described in Christensen et al. (2002). Coverslips with adherent 2- to 4-day-old cells were placed in the perfusion chamber and attached to the bottom of the well with vacuum grease. Cells were perfused with an extracellular saline solution (145 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM d-Glucose, pH 7.2, and brought to 340 mOsm with sucrose) to remove culture medium and nonadherent cells. Prior to recording, the cells were

perfused with extracellular solution for 5 s to stabilize the solution level in the recording chamber. The recording protocol consisted of a 3–5 s extracellular saline perfusion, 8 s potassium depolarisation (extracellular saline solution with 110 mM KCl and 40 mM NaCl), and 7–9 s extracellular saline. The solutions were delivered with a gravity feed perfusion system, with six solution tubes reaching a manifold (Warner Instruments) and one tube entering the recording chamber with a flow rate of approximately 0.5 ml/s.

Image Analysis

Image stacks saved with MetaVue were analyzed using a custom Java-based program. Regions of interest around the cell body of each neuron were defined on the YFP channel of the first image by hand; the program then automatically copied the regions to the CFP channel and tracked the neuron through successive frames using a centroid-centering algorithm similar to one previously described (Kerr et al., 2000). The program was set to report the total intensity recorded in YFP and CFP channels. The ratio was then computed as (YFP intensity)/(CFP intensity) $-$ 0.6. The 0.6 factor corrects for emission crosstalk; CFP alone produces a signal in the YFP channel that is 60% of the signal in the CFP channel. Thus, measured YFP intensity is actually 0.6 $\times$ (CFP emission) $+$ (YFP emission).

Ratio changes were detected and parameterized using scripts for MATLAB (The Mathworks) that used a variety of criteria to find and delimit events. The primary criterion was that a rate of rise of 3%/s had to be maintained for at least three frames (typically 120 ms). Additional criteria were introduced until the results in virtually all cases were equal or superior to results produced by hand. The Mann-Whitney rank sum test was used to calculate all significance values, since the distribution of ratio changes typically did not show a Gaussian distribution. The Bonferroni correction for multiple comparisons was used for Figure 4B.

Electrophysiology

Data were recorded with an Axopatch 200B (Axon), a PC (Dell), and Clampex software (Axon), and filtered at 1 kHz and sampled at 2.5 kHz. Bath solutions were as follows: 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$, HEPES 10, 20 mM D-glucose, and 25 mM sucrose (pH 7.2 with NaOH) or 145 mM N-Methyl-D-Glucamine-Cl, 5 mM KCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 10 mM HEPES, 20 mM D-glucose, 25 mM and sucrose (pH 7.2 with HCl). Pipette solution was 145 mM KCl, 4 mM NaCl, 0.6 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, and 32 mM sucrose (pH 7.2 with KOH). The whole-cell configuration was achieved by "zapping." A 0.5 ms long voltage step at 1V was applied to the patch of membrane under the tip of the pipette after obtaining the cell-attached configuration.

Note on Quantitation of Mechanical Stimuli

A standard method for measuring sensitivity to touch in vertebrate systems is to compute the minimum force required to generate a response, either by using von Frey hairs that buckle at a reproducible force or devices that can generate and maintain constant force through a feedback control loop. Unfortunately, neither of these methods is readily applicable to studies of *C. elegans* mechanosensation due to the small size of the worm. Probes with tip areas of more than approximately 0.0001 mm^2 (10 $\times$ 10 μm) have difficulty coming in full contact with the body wall of the worm. This small contact area would result in pressures hundreds or thousands of times higher than are applied in vertebrate systems with larger tip sizes, or a requirement for forces hundreds or thousands of times smaller. Since reliably generating these small forces is difficult while avoiding perturbation by air currents, surface tension under the water immersion objective, and the like, we are restricted to using constant displacements to generate a reliable stimulation. This method is also used to study vertebrate mechanosensation (Price et al., 2000) and can be applied in worms with high precision (the probe is positioned with micromanipulators to an accuracy of 2–3 μm, as compared to a displacement of 10 μm, and is tested before imaging to make sure it contacts the worm properly). It is worth noting that the relationship between force and displacement is a function of the compressibility of the sample; for a given compressibility, a constant displacement stimulus is equal to a constant force stimulus of the force exerted by the sample on the probe at that displacement.

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