Locomotion analysis identifies roles of mechanosensory neurons in governing locomotion dynamics of \textit{C. elegans}

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Abstract

The simple and well-characterized nervous system of *C. elegans* facilitates analysis of mechanisms controlling behavior. Locomotion is a major behavioral output governed by multiple external and internal signals. Here we examine the roles of low- and high-threshold mechanosensors in locomotion, using high-resolution and detailed analysis of locomotion and its dynamics. This analysis reveals a new role for touch receptor neurons in suppressing an intrinsic direction bias of locomotion. We also examine the response to noxious mechanical stimuli, showing a response entailing several locomotion properties and lasting several minutes. Effects on different locomotion properties have different half-lives and depend on different partly overlapping sets of sensory neurons. PVD and FLP, high-threshold mechanosensors, play a major role in some of these responses. Overall, our results demonstrate the power of detailed, prolonged, and high-resolution analysis of locomotion and locomotion dynamics in enabling better understanding of gene and neuron function.
Introduction

To survive in an ever-changing environment, metazoans evolved complex neuronal networks. Neurons sense, integrate, and transmit information to finally produce complex adaptive behaviors. *C. elegans*, having a simple and well characterized nervous system of only 302 neurons, is an excellent model for understanding the roles of specific molecules and neurons in nervous system function (White et al., 1986). The major behavioral output of the *C. elegans* nervous system is locomotion. Locomotion enables escape from noxious stimuli or navigation towards positive signals such as food. *C. elegans* navigates its environment by propagating a sinusoidal waveform that is generated by a well defined motor circuit (Von Stetina et al., 2006). Information perceived by sensory neurons governs this motor circuit to control reversals (tail first movement), speed, pauses, and turns (Croll, 1975; Gray et al., 2005; Tsalik and Hobert, 2003; Wakabayashi et al., 2004; Zhao et al., 2003).

As *C. elegans* is a soil dwelling nematode, its locomotion is likely to be strongly affected by collisions with soil particles. Indeed, analysis of *C. elegans* behavior identified several sets of mechanosensory neurons whose activation strongly affects locomotion (Chalfie and Sulston, 1981; Kaplan and Horvitz, 1993; Li et al., 2011). We have previously analyzed locomotion of strains defective for mechanosensation (Albeg et al., 2011). This analysis showed that the PVD and FLP high-threshold mechanosensors affect locomotion, and suggested that they activate an escape response. We note that this analysis was performed in the absence of acute sensory stimulation, thus identity of the stimulus activating this escape response remained unknown. (Albeg et al., 2011).

Here we use a toolkit that combines improved single-worm tracking and image analysis software to enable detailed analysis of multiple locomotion properties and their dynamics. This toolkit aims at providing comprehensive and detailed characterization of the function of neurons and genes in controlling behavior. For this we have modified and enhanced the image- and locomotion-analysis previously used by Albeg et al. (Albeg et al., 2011). The current software uses images and data provided by a “worm tracker” unit for higher resolution and prolonged tracking of single animals. Using this toolkit, we
analyze previously characterized strains (Albeg et al., 2011). Our results reveal novel roles for mechanosensory neurons in regulating locomotion. They also demonstrate long-lasting (minutes) effects of noxious mechanical stimuli on locomotion, showing that these long lasting effects entail changes in multiple locomotion properties. Importantly, our work shows different half-lives of effects on different locomotion properties, and suggests that different locomotion properties are controlled by different partly overlapping sets of neurons. Overall, our results demonstrate the power of detailed locomotion analysis in revealing novel functions of genes and neurons.

**Materials and methods**

**Animals and assay conditions:** Strains used in this study were previously described (Albeg et al., 2011). Briefly, the mec-4(e1611) mutation leads to degeneration of the six touch receptor neurons (ALMs, PLMs, AVM, and PVM, –T animals), the integrated ser-2prom3::deg-3(N293I) transgene eliminates, via degeneration, PVDs (–P animals), the integrated mec10p::deg-3(N293I) transgene eliminates, via degeneration, 10 body mechanosensors (six touch receptor neurons, FLPs, and PVDs), and mec-10(tm1552) is a mec-10 loss of function mutation. Animals were grown at 20°C on standard NGM plates seeded with the *E. coli* strain OP50. For each assay L4 animals were picked for overnight growth on fresh NGM plates. At the start of each experiment a single animal was transferred with a wire pick to a fresh newly seeded NGM plate and worm tracking was initiated at the moment that the animal’s image was captured, up to half a minute following transfer. All movies analyzed here track the animals for 20 minutes. Bacterial lawns on plates used for image analysis were allowed to grow to a thin regular layer surrounded with a clear unseeded border to limit wandering of animals to the edge of the plate and to enable better image identification and longer tracking.

**Worm tracking hardware:** Worm Tracker 2.0 hardware enabling image acquisition and centering of the nematode’s image as it moves on an agar plate is described in [http://www.mrc-lmb.cam.ac.uk/wormtracker/index.php?action=hardware](http://www.mrc-lmb.cam.ac.uk/wormtracker/index.php?action=hardware).
Worm tracking software: Worm Tracker 2.0 software enabling identification of the animal and centering of the microscope camera on this animal as it moves is described in http://www.mrc-lmb.cam.ac.uk/wormtracker/ and is freely available from this site. This software is compatible with all Windows operating systems from XP to Windows 7. All Ludl, Prior, and Zaber brand motorized stages are supported. All cameras with DirectShow filters (the majority of USB cameras) are supported. The software provides automatic calibration for directing the stage and converting the pixel coordinates, in its recorded movies, to real-world microns. Several other options allow the software to be configured for specialized applications, such as those requiring tracking of light objects on a dark background or using corrective manual movements (e.g., employing a joystick) in conjunction with automated tracking to follow extremely fast objects. A guide on the website provides further instructions for software configuration and use.

Image and movement analysis: The software used for image analysis is based on software described in (Albeg et al., 2011), and specifically, the calculation of posture and movement parameters remains as described there. This software was adapted for movies generated by the worm tracking module described above and utilizes the information on stage movement provided by this module to identify the relative locations of the movie frames. It is written in VisualC++ and utilizes the openCV image analysis library provided by Intel, leading to an analysis rate that is about twice real time (analyzing a 20-minute movie at 10 fps takes around 10 minutes). The main algorithms used are as follows.

Binarization. For each frame, a grey-level histogram is generated. The largest maximum in the histogram represents the background. The nearest minimum below this maximum is used as a threshold for binarization. This is combined with edge detection to improve identification of the nose and tail. Holes are closed using dilation followed by erosion. Connected-components analysis is used to identify the largest connected component and discard smaller ones.

Skeletonization. First a distance map is generated for the binary silhouette of the worm, and the maximum found. Using the maximum as a starting point a walk is performed along the main ridge of the distance map in both directions. This is done by drawing a
semi-circle with radius 4 pixels around the current point in the direction opposite from
the previous point, finding the maximal point on this semi-circle, and connecting to it by
an 8-neighbor line. Looping back at the end is prevented by disallowing two sharp turns
in a row.

Loop identification. Loops are identified when the skeleton touches or crosses itself. A
possible problem is that the hole in the middle of a loop may have been closed in the
binarization phase. This is corrected by calculating a distance map, and if the maximum
is more than 80% the average width of the worm, checking whether the location of the
maximum was actually part of the background in the original image.

Head identification. The head is identified as being brighter than the tail. Specifically,
the grey-level histogram for a 1/10 mm segment at each end of the worm is calculated.
The brightness is then defined to be the average grey level of the brightest 20% of the
pixels. If the average at one end is higher (lighter) by 2 or more it is identified as the
head. All consecutive frames in between problem frames (e.g., loops) are grouped
together. In each such group frames with consistent head orientation are identified based
on distances between locations in successive frames. The correct orientation is then
identified using majority vote. Manual inspection shows that this method is reliable
except for movie segments in which the animal pauses in close proximity to its newly
laid eggs, as the space between the worm and the egg is typically light. Such errors occur
primarily during pause segments and thus have no effect on the correct identification of
locomotion patterns (i.e., forward vs. backward movement). In contrast to the previous
version of the software the head identification is now completely automated requiring no
manual verification.

Executable, source code, sample inputs and outputs, and manual are available at
http://www.cs.huji.ac.il/~feit/worms/.

Data and statistical analysis: Data was analyzed using standard MATLAB algorithms.
Data was fitted to equations using the fit function
(http://www.mathworks.com/help/toolbox/curvefit/fit.html), specifically the
NonlinearLeastSquares method (LinearLeastSquare method was used when fitting to
linear equations). Optimal fit ($R^2$) was achieved via iteration of the analysis 100 times
and choosing values having highest $R^2$. 95% confidence intervals were calculated using Trust-Region algorithm.

**Results**

**The toolkit**

Analysis is done on a single worm moving on an agar plate. The animal is positioned above an integrated microscope camera that is connected to a computer. Tracking initiates once the animal is found within the camera’s frame. To track the animal, the system controls the camera’s position using a motorized stage. Only the camera moves—the worm’s platform is kept motionless so as not to disrupt the animal’s behavior. This tracking system produces three outputs: a movie of the animal, information on stage movements, and information on camera and stage calibrations. The first two outputs are used as inputs to the image analysis software (Figure 1).

The image analysis software starts by identifying the animal using thresholding and edge-detection to create a binary image of the animal. This is followed by skeleton identification and head assignment. Binarization and skeletonization are done on each frame separately; head assignment also uses information from surrounding frames and information on position of the worm derived from both the movie and the stage movement outputs. Frames in which the skeleton is ill-formed (as in a loop) or its length deviates by more than 9% from the median are considered inconsistent and discarded from the analysis. The skeleton and head assignment for each frame combined with the information on stage movements are used for classifying each frame into a locomotion pattern (forward movement, backwards movement, pause, omega, or loop) (Albeg et al., 2011). Position of the skeleton’s mid-point from each frame and information on stage movements are used for speed and displacement analysis. The skeleton is also used for analysis of postural properties. A short description of the outputs generated by this analysis program is provided in Table 1. Output files are in CSV format enabling further analysis using Matlab or Excel.
Characterization of locomotion patterns

The wild-type *C. elegans* (N2) lies on its side generating an anterior to posterior propagating sinusoidal waveform in order to move forward (head first). This forward locomotion is interspersed with reversals (backwards movement), pauses, and loops that in some cases lead to altered direction of movement (Croll, 1975). Sensory cues are known to control the frequency and duration of these locomotion patterns (Croll, 1975; Gray et al., 2005; Tsalik and Hobert, 2003; Wakabayashi et al., 2004; Zhao et al., 2003). Thus several “movement analysis” programs classify locomotion into forward, backwards, pause, and loops (curls) (Albeg et al., 2011; Geng et al., 2004; Hoshi and Shingai, 2006). We slightly modified this classification by separately analyzing omega-shaped worms (animals showing an exaggerated body bend (Albeg et al., 2011)) and loops (animals whose skeleton forms a closed loop). Loops pose a problem for analysis, since characterization of the skeleton and head identification in this configuration are not possible using our current software. However, identification of this postural configuration is important since it is often associated with direction changes. Using this classification method we show that in N2 animals the reversal frequency (initiation of backwards movement) is 3.9/minute±0.34 and the average duration of backwards movement is 0.69 seconds. Similarly, pauses are 13/minute±0.81 and last on average 1.19 seconds. Both omegas and loops are rarely seen in N2, showing a frequency of 0.3/minute±0.09 and 0.19/minute±0.05, respectively. These numbers represent averages for 14 animals during 20-minute movies. We note that the high variability of these numbers is a result of the dynamic nature of locomotion, as both frequency and duration of each locomotion pattern are regulated by multiple signals including time from transfer to a new plate. For example, both the frequency and duration of backwards-movement increase with time from start of analysis (see below).

An important feature of our software is that locomotion and posture properties such as speed, bending angle, bend amplitude are analyzed separately for different locomotion patterns. This distinction between locomotion patterns is unique to this toolkit, and was first introduced in (Albeg et al., 2011). This feature is important in order to reduce
confounding effects such as the effects of including pauses in analyses of speed or of
including omegas and loops in analyses of bending angle. Such confounding effects will
increase variability and thus reduce sensitivity to small phenotypic differences when
comparing mutant strains. In addition, averaging locomotion properties from all
locomotion patterns may confound effects on frequency or duration of locomotion
patterns with effects on speed or posture.

For a graphical illustration of the major postural properties measured by this program see
Figure 2A. Bend angle, normalized bend amplitude, and cut-point number are analyzed
for each frame in which a consistent skeleton is identified, while normalized wavelength
is only calculated for skeletons having a posture with sufficient wavelike features (Albeg
et al., 2011). Analysis of these properties in N2 (Figure 2) demonstrates significant
differences in posture between locomotion patterns not defined by posture (omegas and
loops are defined by posture while forward, backwards, and pauses are not). This analysis
shows that cut point number, bending angle, and bend amplitude differ between
backward and forward movement, while posture of animals during pauses is similar to
what is seen during backward movement. The clear differences between forward and
backward locomotion and the low variation of the results provide strong and unbiased
support for our software’s ability to correctly classify locomotion patterns. Differences in
posture between forward and backward movement are associated with differences in
speed: speed is higher for forward movement (Figure 2B). Importantly, these results are
consistent with previous results showing that backwards movement is characterized by
increased bending amplitude and angle (Croll, 1975; Gray et al., 2005). In addition,
similarity in normalized wavelength between forward and backward movement also
reproduces previous results (Figure 2F, (Cronin et al., 2005)). Last, our current high-
resolution analysis provides a sensitive method for posture analysis showing that
elimination of PVD neurons leads to a significant (p<0.01) increase in normalized
wavelength (0.5±0.005 relative to 0.45±0.005 during forward movement in animals
lacking PVD relative to N2 (n=10 and n=14 respectively)). We have previously
suggested that elimination of PVD leads to increased wavelength, but those previous
results suffered from high variability and the differences were not significant (Albeg et
We believe that the increased resolution afforded by the worm-tracking unit has reduced inaccuracies enabling increased sensitivity of this analysis.

Visualization tools reveal phenotypic differences in the dynamics of locomotion

To facilitate identification of new locomotion phenotypes our software provides two visualization tools: detailed traces of the animal’s locomotion and images of wave propagation (Figures 3 and 4). Both tools provide visual representations for locomotion dynamics and allow for unbiased inspection by the sensitive human eye for altered locomotion patterns.

The first tool shows superimposed images of the animal as it traverses the plate. In these images grey scale is used to indicate the time spent at each location, with light grey indicating short time duration (i.e., high speed) and black indicating prolonged duration (i.e., pause) (Figure 3). This tool is a modified version of a tool presented in previous work (Albeg et al., 2011). The second tool is also an adaptation from previous work (Korta et al., 2007). In the resulting images the x-axis is time and the y-axis is position along the animal’s skeleton. The grey scale indicates bending angle, with white indicating maximal bend in one direction and black in the other. Resulting diagonal lines depict propagation of body bends over time. The density of these lines and their slopes indicate speed and movement direction respectively (top is head and thus diagonals sloping down to the right indicate forward movement). In these images, consistent forward movement results in regularly spaced diagonal lines, reversals are associated with a zigzag pattern (segments of backward movement are shorter than those of forward movement (Croll, 1975)), and pauses with horizontal smears (Figure 4).

Using the first tool, we traced movement of N2 and of strains lacking specific mechanosensory neurons (Albeg et al., 2011). Traces of N2 started immediately after transfer to an agar plate seeded with bacteria reproducibly show animals starting with a roaming type of locomotion, with high speed and few if any directional changes (escape or dispersal have also been used as terms to describe similar locomotion patterns (Gray et al., 2005; Wakabayashi et al., 2004; Zhao et al., 2003)). As time progresses these animals...
shift to a dwelling type of movement typified by increased pauses and directional changes (* indicates start of trace and x end of trace, Figure 3A). Similarly, others have shown that transfer with a wire pick leads to a transient inhibition of reversals that depends on body mechanosensors (Zhao et al., 2003). Indeed, traces showing movement of animals lacking all body mechanosensors (touch receptor neurons, PVDs, and FLPs (–TPF) (Albeg et al., 2011), Figure 3B) support involvement of body mechanosensors in generating this dynamic behavior following transfer to a new plate. Further visual evidence for this shift from roaming to dwelling is provided by bend propagation images (Figure 4A compared to 4E, first and last minute of a representative movie, respectively). These changes in locomotion properties are quantified in detail below (Figure 6).

Interestingly, dynamics of this behavior are opposite of what is seen in the absence of food: upon transfer to an unseeded plate animals start by initiating local search behavior (similar to dwelling) and with time shift to dispersal promoting behavior (roaming) (Gray et al., 2005; Hoshi and Shingai, 2006).

Traces of –T animals lacking low-threshold body mechanosensors (in *mec-4(e1611)* animals, all 6 touch receptor neurons degenerate (Driscoll and Chalfie, 1991)) show circular tracks suggesting a bias in movement direction (Figure 3C). In 13 traces, 10 show more than one full circle. Interestingly, similar bias, although much weaker, is sometimes seen in tracks of N2 (Croll, 1975; Stephens et al., 2010; Zhao et al., 2003). To quantify this bias we analyzed curvature of the track in N2 relative to –T mutants (Ben Arous et al., 2009). For this analysis curvature is only measured during segments of rapid forward movement in order to avoid the confounding effects of high curvatures measured following reversals or loops. Analysis of curvature angles over time for individual animals show that while the track of a single N2 usually contains both negative and positive curve angles (Figure 5A), tracks of –T animals have higher curve angles that are mostly positive or mostly negative (Figure 5B compared with Figure 5C). The average of the track curvatures for –T animals is 25.9±4.06 degrees compared to 12.6±1.2 degrees for N2 (n=13 and 14 respectively). Because the average track curvature for individual animals is either negative or positive, we averaged the absolute values of these averages. Careful examination of changes in track curvature over time (Figure 5A-C) suggests that
it may decay with time. To better examine this observation we averaged absolute curvature angle for all N2 or –T animals in successive one minute segments. This analysis shows that track curvature angle decays over the first 5 minutes of the analysis (Figure 5D). Indeed, data on track curvature fits well with a power function having a negative exponent. Only the first 5 minutes are analyzed as at later times curvature angles become highly variable or cannot be measured due to frequent reversals. We conclude that track curvature is controlled by touch receptor neurons and external stimuli, likely to be the noxious mechanical stimuli inflicted on the animal as it is being transferred to the plate used for analysis.

To further examine this curve angle preference we used egg-laying events to identify the ventral side of each animal (the vulva is on the ventral side and since animals lie on either their left or right side, orientation of the ventral side in individual animals needs to be examined). This analysis shows that in all nine –T animals for which the ventral side could be identified, tracks always curved towards the ventral side. N2, while showing smaller average curve angles, also show some bias (i.e., negative or positive average curvature). Analysis of these animals showed that in 10 of 14 animals the tracks curved towards the ventral side. Based on this analysis we suggest that C. elegans have an intrinsic ventral curve angle bias during forward movement, a bias that is normally suppressed by touch receptor neurons. Previous work suggested that asymmetric innervation of head muscles leads to ventral direction bias following reversals (Gray et al., 2005). This asymmetry may also underlie the bias seen here for forward movement.

The phenotypes demonstrated above can be quantified using standard indicators. However, visualization tools also enable subclassification of locomotion patterns based on subtle, not routinely measured, differences. For example pauses may represent complete quiescence (Figure 4F) or an active pause consisting of short back and forth movements, with active head foraging, seen as rapid changes in shading at the top (Figure 4E). Figure 3 also contains several examples of pauses indicated by arrows: an almost complete pause in Figure 3D and additional pauses varying in degree of quiscence in the other panels. Sensitivity of the bend propagation visualization tool is also demonstrated
by the ability of this tool to identify anatomical details. Specifically the dark band indicated by the arrow in Figure 4A indicates a kink in the waveform that corresponds to the vulva region. Anatomy of this region is likely to affect tissue rigidity thus producing this small kink. Importantly, subtle differences identified by the visualization tools can be used to classify mutants. For example, in \( -T \) animals we often see a pattern indicating irregular bend propagation during forward movement which is not seen in N2 (Figure 4D compared with 4B). This difference is not obvious when comparing earlier time points at which animals move much faster (Figure 4C compared with 4A).

Characterizing the dynamic response to transfer

Image analysis presented here is initiated quickly (<0.5 minutes) after transferring a single animal, using a wire pick, to the plate used for imaging. In previous analysis we allowed animals to recover from this procedure for 10 minutes following transfer (Albeg et al., 2011). Here, however, we examined the behavioral response to what is likely to be an extremely noxious mechanical insult. As described above visual tools show that this procedure is associated with a dynamic shift from roaming, in the first minutes following transfer, to dwelling as time progresses. In order to enable analysis of locomotion dynamics, our program generates several outputs describing locomotion dynamics, in addition to summaries of locomotion and posture properties from the entire movie. Using these outputs it is possible to analyze how locomotion properties change over time.

End-to-end displacement over short time segments (0.5 minute) is a sensitive measure that integrates information on speed and on efficiency of movement (reversals will reduce end to end displacement). Analysis of such end to end displacement in N2 shows that initially it is relatively high, and declines slowly over several minutes to finally reach a variable and low steady state level lasting until the end of our movies (20 minutes, Figure 6A). Results of this analysis can be fitted by an exponential function having a \( t_{1/2} (\tau) \) of 3.29 minutes \((R^2=0.935, 95\% \text{ confidence interval } 2.69:4.24, \text{ Figure } 6A)\). Difference in the end-to-end displacement during the first minute of analysis relative to the last minute of analysis is significant \((p<0.01)\). Similar decreasing trends were shown for speed and for fraction of time spent in forward movement, while an opposite (increasing) trend was
seen for fraction of time spent in backwards movement and for fraction of time spent in pauses (Figure 6). Frequency of reversals also increases with time; however, reversal frequency is highly variable and, thus, was not further analyzed. The strongest effect seen in this analysis is the suppression of pauses (a >20-fold effect, last minute relative to first minute). Previous analysis of the effects of noxious mechanical stimuli on locomotion showed a shorter 100-second effect that was limited to suppression of reversals (Zhao et al., 2003). Differences between our data and previously reported data are likely to be a result of the higher sensitivity of our analysis, but may also represent differences in the assay conditions. Notably, both the previously published results and our results were obtained in the presence of food and thus are not confounded by the effects of food withdrawal (Zhao et al., 2003).

Dynamics of the response to transfer, shown in figure 6, reflect three factors: the starting point (locomotion immediately following transfer), the rate at which the response decays with time, and the end-point, likely to reflect steady-state behavior. To enable comparison between different strains we need to characterize each of these factors. For this we fitted the results to an exponential function or to a linear function. In the exponential function \( f(x)=ae^{-bx}+c \): “a” is the difference between the start point (time zero) and end point of the function (an estimate for the magnitude of the response to transfer), “ln2/b” is \( \tau \) (t1/2, half life of the response), and “c” gives the end point, an estimate for steady-state behavior (basal locomotion). The resulting values are shown on the right side of Figure 6. We do not show values derived from fitting data to linear functions as these functions show lower fits (R2). Examples for fitted curves are shown in figure 7.

Previous work and results shown in Figure 3 (compare Figure 3A to 3B) suggest a role for body mechanosensors in the response to transfer; evidence for this comes from analysis of \( mec-3(lf) \) mutants that are defective for differentiation of all 10 body mechanosensors and from analysis of animals in which all 10 cells degenerate ((Zhao et al., 2003) and Figure 3). To further examine which specific neurons and molecular mechanisms are required for this effect we analyzed previously described strains in which
all or some of these neurons degenerate and a mec-10(lf) strain lacking the proposed PVD mechanosensory channel (Albeg et al., 2011; Chatzigeorgiou et al., 2010). The following results clearly show that eliminating all body mechanosensors eliminates or greatly reduces the effects of transfer on locomotion. First, b for speed of animals lacking all body mechanosensors (-TPF) is very small (figure 6) and therefore data on speed can also be fitted by a straight line with a slope of −0.0008 (95% confidence intervals -0.0012:-0.0004, R^2=0.49). Second, effects of transfer on other locomotion features of –TPF animals, as measured by “a”, are significantly reduced relative to N2 (Figure 6). We note that although fits for N2 are excellent (R^2>0.85) fits for –TPF animals are always lower, sometimes much lower, when compared to N2. This difference between N2 and –TPF serves as additional evidence for effects of mechanosensory neurons on the behavioral response to transfer (Figure 6). Interestingly, our results also show that PVD has a significant role in the response to transfer; in its absence “a” of end-to-end displacement is significantly reduced relative to N2. Touch receptors, however, contribute little to this response, and in their absence “a” is not reduced and is even sometimes increased (fraction of time in backwards movement). Thus differences in the response (“a”) between animals lacking all body mechanosensors and these lacking only PVD can be attributed to FLPs. In addition, PVD is likely to express receptors for noxious mechanical stimuli in addition to mec-10, as was suggested by (Li et al., 2011). mec-10(lf) was shown to eliminate the harsh touch response in PVDs, suggesting that mec-10 is the harsh touch receptor in these neurons (Chatzigeorgiou et al., 2010). However, mec-10(lf) mutants behave differently from –P animals, even showing increased “a” relative to N2 for some measurements (Figure 6).

One important outcome of fitting the response to transfer data with exponential functions is our ability to estimate half-lives for these responses. This analysis shows different half-lives for different locomotion features. Specifically, the half-life of effect of transfer on speed for N2 is 2.35 minutes, and differs significantly from the half-life of the effects on pauses, which is 8.4 minutes (Figures 6 and 7). This correlates with results showing that in the absence of body mechanosensors (-TPF animals) effects on speed are eliminated while effects on pauses are only reduced (“a” for N2 is -0.84 relative to -0.61 for –TPF...
for pauses) (Figure 6). Thus our results suggest that pauses are governed by additional
eurons, yet unidentified, whose effects are longer lasting.

Previous analysis suggested effects of PVD and FLP on basal locomotion (Albeg et al.,
2011). Those findings are supported by estimates of basal locomotion properties provided
by “c” (Figure 6). However, estimates given here are not identical to previously measured
data, suggesting for example, a significant effect of \textit{mec-10} on basal locomotion
properties. Importantly, the previous analysis characterized locomotion 10 minutes
following transfer while the current analysis shows that some responses to transfer do not
fully decay after 10 minutes. Validation of the new estimates for basal locomotion will,
therefore, require analysis following longer intervals or a less intrusive means of
transferring animals for analysis. Interestingly, values of “c” for \textminus{T} animals suggest a
significant reduction in basal speed. This reduction correlates with the irregular bend
propagation seen in the same animals (figure 4D).

\textbf{Discussion}

The analysis presented here relies on a new toolkit for analyzing \textit{C. elegans} locomotion.
Like previously described “worm tracking” toolkits, this toolkit enables high-resolution
prolonged analysis of a single animal’s locomotion (Baek et al., 2002; Cronin et al.,
2005; Hoshi and Shingai, 2006; Tsibidis and Tavernarakis, 2007). This toolkit includes
several improvements relative to previous toolkits. First, the worm-tracking hardware is
cheaper and easier to assemble. Moreover, tracking is performed by moving the camera
and not the stage thus reducing mechanical perturbation which may affect behavior.
Second, analysis of locomotion and posture properties is performed separately for
different locomotion patterns (forward, backwards, pauses, and omegas). This feature
increases sensitivity of the analysis and facilitates interpretation of the results. Last, this
analysis produces several visual and frame by frame data outputs that enable
identification and detailed analysis of locomotion and posture dynamics. Many of the
analysis features of this toolkit were previously described (Albeg et al., 2011). However,
they are now combined with the “worm-tracking” hardware to enable higher resolution
and prolonged analysis.
Using this toolkit we examine the roles of genes and neurons known to function in mechanosensation. Results of this analysis support some of our previous findings, add new functions to previously characterized neurons, and enable better interpretation of previous results. Specifically, this analysis produced the following novel findings: 1) Demonstration of a bias in movement direction of animals lacking the 6 touch receptors. 
2) Showing that the behavioral response to transfer is prolonged, lasting several minutes. 
3) Identifying neurons participating in the response to transfer and showing a non-redundant role for PVD in this response.

The six touch receptor neurons are a set of well-characterized neurons mediating the response to low threshold mechanical stimuli to the nematode’s body (Chalfie and Sulston, 1981; Chalfie et al., 1985; Wicks and Rankin, 1995). Mutations interfering with function of these neurons were also shown to affect locomotion within a structured environment, to control forward thrashing frequency in liquid, and to control speed and wave propagation (Korta et al., 2007; Lebois et al., 2012; Park et al., 2008). Here we show for the first time that touch receptor neurons function to regulate direction bias. Our results suggest that during forward movement wild-type animals have a ventral bias in movement direction, possibly a result of asymmetric innervations, as previously shown for backward movement (Gray et al., 2005). We show that in the absence of touch receptor neurons this bias is enhanced. Thus we suggest that touch receptor neurons also have a role in regulating locomotion in the absence of obvious extrinsic inputs and thus may sense intrinsic signals such as subtle differences in posture.

Previous studies looking at the response to noxious mechanical stimuli applied to the body focused on the response to prodding with a wire pick (Albeg et al., 2011; Chatzigeorgiou et al., 2010; Li et al., 2011; Way and Chalfie, 1989; Zhao et al., 2003). These studies identified PVD and touch receptor neurons as mechanosensors functioning redundantly to mediate the response to harsh touch. Our work focuses on the behavioral response to transfer with a wire pick. This behavioral response was previously characterized in a single study (Zhao et al., 2003). Like the response to harsh touch this
behavior requires activity of body mechanosensors and thus is likely to represent the
response to a particularly noxious mechanical stimulation. We show that in wild-type
animals this response, unlike the transient response to harsh-touch or to optogenetic
stimulation of PVD, is a sustained response (half-life 2-8 minutes relative to 5 head
swings for anterior prodding, i.e. several seconds, or ~5 seconds for brief optogenetic
stimulation (Husson et al., 2012; Li et al., 2011)). We also show that in this response,
unlike in the harsh-touch response, PVD has a non-redundant role. Moreover, touch
receptor neurons that function redundantly with PVD in the harsh touch response have a
minor role in the response to transfer.

Previous work suggested that PVD utilizes the DEG/ENaC channel MEC-10 as a
mechanosensory channel (Chatzigeorgiou et al., 2010). This conclusion was disputed in
later publications (Arnadóttir et al., 2011; Li et al., 2011). For example, results of
electrophysiological recordings from PVD following harsh touch were unaffected by
mutation of mec-10 (Li et al., 2011). Similarly, our analysis shows that PVD mediated
responses to transfer are unaffected by mutation in mec-10. The conflicting results
concerning the role of MEC-10 in PVD-mediated responses to high threshold mechanical
stimuli can be explained by differences in intensity of the applied stimulus or by
differences in the assay used to examine the response. Based on these conflicting results
we suggest that PVD, like Drosophila DA-IV nociceptors, employs more than one
mechanosensor (Kim et al., 2012).

The response to high-threshold mechanical stimulation (prodding with a wire pick) is
either forward movement or reversals (Li et al., 2011; Way and Chalfie, 1989). Careful
analysis showed that the response to such prodding depends on the body region receiving
the stimulus; prodding the anterior region led to reversals and prodding to the posterior
region to forward movement (Li et al., 2011). Similar conclusions were obtained from
optogenetic analysis (Husson et al., 2012). Thus the behavioral response to mechanical
inputs depends on identity of the responding sensory neurons. Specifically, FLPs,
nervating the head, and not PVDs, innervating the body, are required for the response
to anterior stimulation and thus for reversals (Li et al., 2011). Transfer with a wire pick,
unlike prodding with a wire pick, appears to activate both anterior and posterior
mechanosensors. This difference in the stimulation protocol, combined with detailed and
prolonged analysis of multiple locomotion properties, shows that body mechanosensors
can function together to produce a response that appears to be more than the sum of
responses elicited by activating each neuron individually.

Our analysis, while showing the importance of PVDs and FLPs in the response to
transfer, also supports involvement of additional neurons, as animals lacking PVDs,
FLPs, and touch receptor neurons (–TPF animals) still show many alterations in
movement properties following transfer. Thus our results are in agreement with previous
results suggesting involvement of additional neurons in this behavior (Zhao et al., 2003).
Indeed, even the response to harsh touch appears to require additional cells (Li et al.,
2011). Importantly, we show that transfer affects multiple locomotion properties having
different half-lives and depending on the activity of overlapping but not identical sets of
neurons. Specifically, effects of transfer on pauses are much more prolonged than effects
of speed. And while speed is strongly affected by PVD and FLP, pauses are only weakly
affected and are instead governed by a distinct yet unidentified set of neurons.

Some of the results obtained in this study reproduce previous results (Albeg et al., 2011),
but the current more detailed analysis leads to interesting differences and to better
understanding. In particular, some of the results have now been reinterpreted. For
example, PVD and FLP were previously shown to affect locomotion. But this analysis
was conducted 10 minutes after transfer at which time the effects of transfer have not yet
fully decayed. The current analysis shows that differences between strains lacking PVD
and FLP and wild-type animals are partly attributed to the long-lasting effects of transfer
to a new plate. Importantly, the current analysis also identifies phenotypes that were
previously masked by the effects of transfer. For example, we now show reduced
“steady-state” speed of *mec-10(lf)* and animals lacking touch receptor neurons (-T).
Effects of transfer on speed of these strains are higher than effects on wild-type animals
masking their “steady-state” phenotype. We conclude that characterization of behavioral
dynamics enables better interpretation and more sensitive detection of behavioral phenotypes.
References


Figure legends

Figure 1. Schematic of the toolkit.
Hardware is on the left and software modules on right. The tracking, data extraction, and analysis modules are implemented in software. In the current implementation the data extraction and analysis are bundled together.

Figure 2. Properties of locomotion patterns.
A) Diagramatic representation of parameters used for postural analysis. Circles surround cut-points between the animal’s skeleton and a straight line connecting the head and tail. B) Speed. C) Average cut-point number D) Average bending angle. E) Average normalized amplitude. F) Average normalized wavelength. Each number represents the average from 14 N2 animals using the median result of analysis for all frames classified as forward, backward, or pause. Asterisks indicate significant difference relative to forward movement ( * - P<0.05, ** - P<0.01, t-test).

Figure 3. Traces of C. elegans locomotion.

Figure 4. Body-bend propagation images.
A) N2, first minute - roaming. B). N2, 5-6th minute reduced speed. C) –T, first minute. D) –T, 5-6th minute showing irregular speed. E) N2, Last minute (19th) - dwelling. E) N2, quiescent pause. Indicated at the bottom is time from beginning of analysis. Lines indicate 100-frame intervals (10 seconds).

Figure 5. Track curvature in N2 compared with –T animals.
A) Curvature angles over time for a representative N2 animal. B and C) Curvature angles over time for two representative -T animals. In B curvature is mostly positive and in C mostly negative. Positive is clockwise or to the right, and negative is counterclockwise or
Figure 6. Quantitative analysis of movement dynamics.

Analysis of wild-type animals (N2, n=14), animals lacking all body mechanosensors (–TPF, n=13), animals lacking PVD (–P, n=10), animals lacking touch receptor neurons (–T, n=13), and *mec-10(lf)* (n=11). Left panels show how locomotion properties change with time (standard error interval is shaded) and right panels show values derived from fitting the data with an exponential function \( f(x) = ae^{-bx} + c \) (“a” and “c” are in the same units as the y-axis on the left, “x” and “ln2/b” (\( \tau \)) are in minutes). 95% confidence interval is given below each number. A) End to end displacement measured for 0.5 minute segments (mm/0.5 minute). B) Speed (averaged over 1 minute (mm/sec)). C) Fraction of time in forward movement. D) Fraction of time in backwards movement. E) Fraction of time in pauses. Asterisks indicate significant difference relative to N2. (\( p<0.05 \), based on lack of overlap between 95% confidence intervals calculated using MATLAB, Trust-Region algorithm). # half life value is irrelevant as this dataset does not fit the exponential function, low \( R^2 \).

Figure 7. Dynamics of speed and pause frequency are altered in –P and –TPF animals.

Examples from data in Figure 6 are shown with the addition of a fitted curve (thick lines) using an exponential function \( f(x) = ae^{-bx} + c \). A) Speed. B) Fraction of time in pause. Only three strains are shown: wild-type animals (N2, n=14), animals lacking all body mechanosensors (–TPF, n=13), and animals lacking PVD (–P, n=10). Half life estimates for N2 are indicated by arrows.
### Table 1: Summary of Parameter Estimates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Estimate (95% CI)</th>
<th>Standard Error</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>a</td>
<td>4.367 (3.944:4.789)</td>
<td>0.210 (0.163:0.258)</td>
<td>3.294</td>
<td>0.800 (0.583:1.017)</td>
</tr>
<tr>
<td>mec10(lf)</td>
<td>b</td>
<td>5.282 (4.828:5.736)</td>
<td>0.230 (0.187:0.272)</td>
<td>3.020</td>
<td>0.148* (0.053:0.348)</td>
</tr>
<tr>
<td>-T</td>
<td>τ(ln(2)/b)</td>
<td>4.316 (3.959:4.674)</td>
<td>0.144 (0.106:0.182)</td>
<td>4.825</td>
<td>0.341 (-0.012:0.694)</td>
</tr>
<tr>
<td>-TPF</td>
<td>c</td>
<td>1.543 (1.219:1.866)</td>
<td>0.207 (0.105:0.310)</td>
<td>3.341</td>
<td>0.628 (0.458:0.799)</td>
</tr>
<tr>
<td>-P</td>
<td>R²</td>
<td>2.519 (2.223:2.815)</td>
<td>0.235 (0.177:0.294)</td>
<td>2.944</td>
<td>0.398* (0.272:0.523)</td>
</tr>
</tbody>
</table>

### Table 2: Summary of Parameter Estimates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Estimate (95% CI)</th>
<th>Standard Error</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>a</td>
<td>0.107 (0.083:0.131)</td>
<td>0.294 (0.185:0.403)</td>
<td>2.358</td>
<td>0.106 (0.100:0.112)</td>
</tr>
<tr>
<td>mec10(lf)</td>
<td>b</td>
<td>0.173 (0.154:0.192)</td>
<td>0.249 (0.196:0.301)</td>
<td>2.788</td>
<td>0.079* (0.073:0.086)</td>
</tr>
<tr>
<td>-T</td>
<td>τ(ln(2)/b)</td>
<td>0.133 (0.115:0.151)</td>
<td>0.204 (0.141:0.267)</td>
<td>3.398</td>
<td>0.091* (0.082:0.100)</td>
</tr>
<tr>
<td>-TPF</td>
<td>c</td>
<td>-0.011* (-0.086:0.064)</td>
<td>-0.046 (-0.252:0.160)</td>
<td>-15.060 #</td>
<td>0.124 (0.043:0.205)</td>
</tr>
<tr>
<td>-P</td>
<td>R²</td>
<td>0.082 (0.063:0.102)</td>
<td>0.284 (0.170:0.398)</td>
<td>2.439</td>
<td>0.085* (0.080:0.090)</td>
</tr>
</tbody>
</table>

### Table 3: Summary of Parameter Estimates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Estimate (95% CI)</th>
<th>Standard Error</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>a</td>
<td>0.926 (0.801:1.051)</td>
<td>0.102 (0.057:0.148)</td>
<td>6.772</td>
<td>0.163 (0.001:0.324)</td>
</tr>
<tr>
<td>mec10(lf)</td>
<td>b</td>
<td>1.114 (1.002:1.227)</td>
<td>0.134 (0.088:0.180)</td>
<td>5.170</td>
<td>0.027 (-0.093:0.148)</td>
</tr>
<tr>
<td>-T</td>
<td>τ(ln(2)/b)</td>
<td>1.030 (0.885:1.175)</td>
<td>0.104 (0.056:0.153)</td>
<td>6.638</td>
<td>0.102 (-0.085:0.289)</td>
</tr>
<tr>
<td>-TPF</td>
<td>c</td>
<td>0.465* (0.322:0.608)</td>
<td>0.094 (0.005:0.182)</td>
<td>7.390</td>
<td>0.162 (-0.022:0.347)</td>
</tr>
<tr>
<td>-P</td>
<td>R²</td>
<td>0.721 (0.609:0.834)</td>
<td>0.114 (0.053:0.175)</td>
<td>6.093</td>
<td>0.090 (-0.051:0.231)</td>
</tr>
</tbody>
</table>

### Table 4: Summary of Parameter Estimates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Estimate (95% CI)</th>
<th>Standard Error</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>a</td>
<td>-0.120 (-0.147:0.092)</td>
<td>0.213 (0.107:0.320)</td>
<td>3.250</td>
<td>0.103 (0.091:0.115)</td>
</tr>
<tr>
<td>mec10(lf)</td>
<td>b</td>
<td>-0.173* (-0.250:0.096)</td>
<td>0.318 (0.096:0.540)</td>
<td>2.179</td>
<td>0.144 (0.127:0.161)</td>
</tr>
<tr>
<td>-T</td>
<td>τ(ln(2)/b)</td>
<td>-0.191* (-0.228:0.154)</td>
<td>0.143 (0.054:0.232)</td>
<td>4.843</td>
<td>0.167 (0.131:0.202)</td>
</tr>
<tr>
<td>-TPF</td>
<td>c</td>
<td>-0.001* (-0.006:0.004)</td>
<td>-0.205 (-0.609:0.200)</td>
<td>-3.385 #</td>
<td>0.119 (0.100:0.138)</td>
</tr>
<tr>
<td>-P</td>
<td>R²</td>
<td>-0.118 (-0.183:0.053)</td>
<td>0.290 (0.023:0.557)</td>
<td>2.390</td>
<td>0.132 (0.115:0.149)</td>
</tr>
</tbody>
</table>

### Table 5: Summary of Parameter Estimates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Estimate (95% CI)</th>
<th>Standard Error</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>a</td>
<td>-0.841 (-1.005:0.678)</td>
<td>0.082 (0.039:0.125)</td>
<td>8.445</td>
<td>0.769 (0.564:0.974)</td>
</tr>
<tr>
<td>mec10(lf)</td>
<td>b</td>
<td>-0.926 (-1.049:0.803)</td>
<td>0.114 (0.062:0.167)</td>
<td>6.058</td>
<td>0.793 (0.640:0.946)</td>
</tr>
<tr>
<td>-T</td>
<td>τ(ln(2)/b)</td>
<td>-0.832 (-0.983:0.681)</td>
<td>0.094 (0.042:0.147)</td>
<td>7.367</td>
<td>0.723 (0.528:0.918)</td>
</tr>
<tr>
<td>-TPF</td>
<td>c</td>
<td>-0.618 (-1.052:0.184)</td>
<td>0.053 (-0.017:0.123)</td>
<td>13.034</td>
<td>0.876 (0.392:1.361)</td>
</tr>
<tr>
<td>-P</td>
<td>R²</td>
<td>-0.720 (-1.029:0.412)</td>
<td>0.067 (0.003:0.131)</td>
<td>10.394</td>
<td>0.894 (0.529:1.259)</td>
</tr>
</tbody>
</table>
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A

Fraction of time in pause

B

Avg. speed

Time (min)

N2
TPF
P

A

Fraction of time in pause

B

Avg. speed

Time (min)
Table 1. Outputs generated by the data extraction and analysis modules.

<table>
<thead>
<tr>
<th>Output file</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>wxo</td>
<td>Distributions of posture parameters for the whole movie and separately for each locomotion pattern</td>
</tr>
<tr>
<td>wxd</td>
<td>Full frame-by-frame dump of measured posture parameters</td>
</tr>
<tr>
<td>wxs</td>
<td>Distribution and statistics of locomotion patterns</td>
</tr>
<tr>
<td>spd</td>
<td>Speed of movement per frame and per 0.5 sec segment</td>
</tr>
<tr>
<td>dsp</td>
<td>Displacement in successive 0.5 sec segments</td>
</tr>
<tr>
<td>ang</td>
<td>Changes in angle of movement in successive 10 sec segments</td>
</tr>
<tr>
<td>track</td>
<td>Composite image of worm’s track throughout the movie</td>
</tr>
<tr>
<td>wave</td>
<td>Map of wave propagation along the worm’s skeleton</td>
</tr>
<tr>
<td>skel</td>
<td>Full listing of skeleton points in each frame for future analysis</td>
</tr>
</tbody>
</table>