MEASURING THRESHOLDS FOR TOUCH SENSATION IN C. ELEGANS

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ABSTRACT

The transduction of force governs many complex biological processes, however the precise molecular mechanisms are still poorly understood. Mechanotransduction is widely studied in *Caenorhabditis elegans*, but the precise range of force that mediates a behavioral locomotion response in freely moving *C. elegans* has not been quantified to date. We measured the touch response threshold of the animals to forces applied tens to hundreds of microns away from the nose using a micromachined silicon capacitive force sensor. The response threshold was measured for wild-type animals (N2, HA1134) and mutants that fail to respond to mechanical stimuli applied to the nose (*osm-9*, *ocr-2*). The mean force required to evoke a behavioral response was elevated in osm-9 mutants, which may indicate a role for osm-9 in the function of the OLQ and PVD neurons which innervate the lateral aspect of the nose and the body wall, respectively, and express osm-9, but not ocr-2

KEY WORDS: Caenorhabditis elegans, mechanosensation, force sensor

INTRODUCTION

Touch sensation is one of our least understood senses, however it has a role in many vital biological processes such as locomotion [1] and embryonic development [2]. *Caenorhabditis elegans* is a model organism for the study of locomotion and mechanotransduction [3]. Although the behavioral response of *C. elegans* to touch is used for genetic screening, the forces involved have only recently been quantified [4], however the range of force which mediates a behavioral response is still unclear.

We used the capacitive force sensor of a MEMS based microgripper with force feedback (FT-G100, FemtoTools GmBH, Figure 1) to measure the touch response threshold of wild-type *C. elegans* and several mutant strains. The design, fabrication and characterization of the sensor have been previously reported [5]. A tungsten wire was glued to the end of the sensor and used as the force probe in order to more easily visualize the contact point of the probe and the worm (Figure 2).

EXPERIMENTAL METHODS

Wild-type *C. elegans* (N2) as well as two strains with defective nose touch (*osm-9* and *ocr-2*) and a strain with the ASH neuron tagged with GFP (HA1134*)* were synchronized and grown at room temperature (approx. 20℃) for 44 hours to obtain L4 animals, which were then placed on an agar pad and mounted on the motorized stage (x- and y-axes) of an inverted microscope. Both of the nose touch mutants also expressed GFP in ASH. The force sensor was mounted on a separate actuator (z-axis) and positioned above the agar pad. The force sensor was calibrated with the tungsten wire attached. For each force measurement, the sensor was positioned in front of a worm and left stationary until the worm contacted the probe. Video was captured with a camera attached to the microscope, and a synchronized video containing the microscope view and the force data was recorded to aid later analysis.

Analysis was performed by manually reviewing the video and identifying the time of each interaction, the distance from the nose, and the angle between the probe and worm. duration and amplitude were automatically calculated (MATLAB, Mathworks) from the interaction times and results were analyzed. A behavioral response of the animal was considered positive if the animal reversed upon contact with the probe.

RESULTS AND DISCUSSION

The touch response threshold is plotted in Figure 3 for each strain. The mean contact forces recorded for N2, HA1134, osm-9 and ocr-2 were 1.70, 1.70, 2.61, and 1.83 μ N, respectively. The force threshold for N2 agrees with previous work [4, 6]. Response thresholds were elevated for osm-9 mutants compared to wild type (N2, HA1134) and ocr-2 mutants. This may indicate a role for osm-9 in the function of the OLQ and PVD neurons which innervate the lateral aspect of the nose and the body wall, respectively, and express osm-9, but not ocr-2.

The touch threshold force plotted against distance from the nose in Figure 4. The data suggest that touch sensitivity is higher close to the nose than far away, possibly due to the greater number of mechanosensory neurons which innervate the head near the nose (ASH, IL1, OLQ, CEP, FLP) than in the head posterior to the nose (ALM, PVD). In the future, measuring the relationship between touch sensitivity and parameters such as force, velocity, and position could provide a powerful tool for modeling the activation of mechanosensitive cells.

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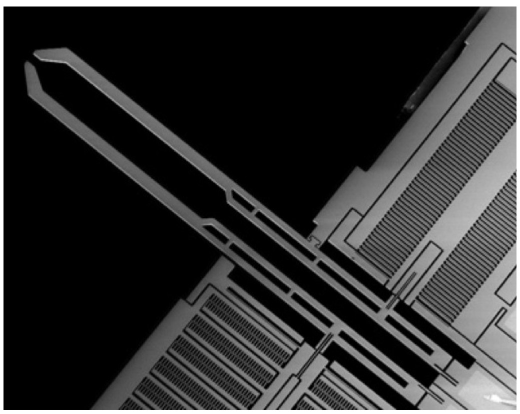

Figure 1: MEMS force sensor used in this work. The gap between the grippers is 100 μ m. The actuator is on the left side and the sensor is on the right. The actuator was removed for these experiments and only the sensor was used.

Figure 2: Force measurement probe with a tip radius <0.1 μ m positioned above the agar surface with a worm in the field of view. A low magnification objective was used to view the experiments through the agar gel with the inverted microscope.

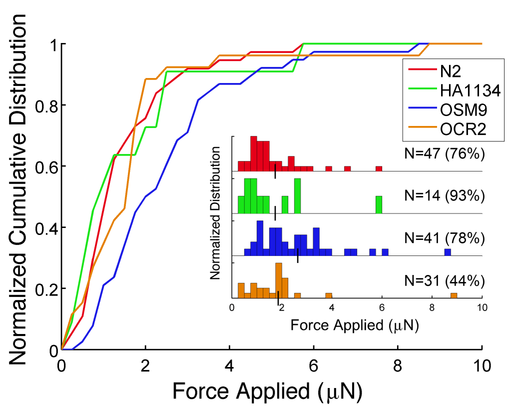

Figure 3: Cumulative force distribution and normalized force distribution (inset) for events which yielded a positive behavioral response for each strain. The mean force is noted by the black line in the inset figure. The number of positive responses measured and positive response rate percentage for each strain are noted in the histogram. 6, 4, 5 and 6 animals were tested for N2, HA1134, osm-9 and ocr-2, respectively.

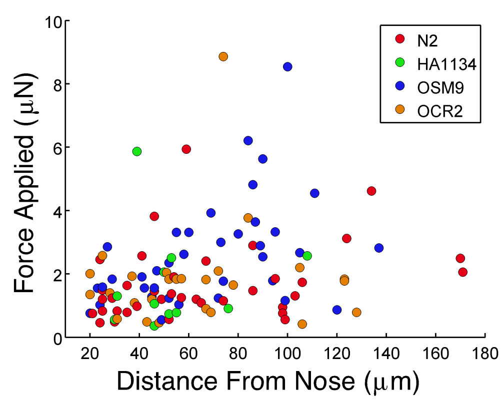

Figure 4: Variation between response force and distance from the nose of the animal. Although a broad range is observed, the touch sensitivity of the animal appears to decrease slightly as the force is applied further from the nose.