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4 Touch-induced Mechanical Strain in Somatosensory Neurons is Independent of Extracellular

5 Matrix Mutations in *C. elegans*

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20 Running Head: Mechanical strain in sensory neurons

21 Abbreviations:

22 TRN: touch receptor neuron

23 ECM: extracellular matrix

24 **Abstract**

25   Cutaneous mechanosensory neurons are activated by mechanical loads applied to the

26 skin, and these stimuli are proposed to generate mechanical strain within sensory neurons. Using

27 a microfluidic device to deliver controlled stimuli to intact animals and large, immobile, and

28 fluorescent protein-tagged mitochondria as fiducial markers in the touch receptor neurons

29 (TRNs), we visualized and measured touch-induced mechanical strain in *C. elegans* worms. At

30 steady-state, touch stimuli sufficient to activate TRNs induce an average strain of 3.1% at the

31 center of the actuator and this strain decays to near zero at the edges of the actuator. We also

32 measured strain in animals carrying mutations affecting links between the extracellular matrix

33 (ECM) and the TRNs but could not detect any differences in touch-induced mechanical strain

34 between wild-type and mutant animals. Collectively, these results demonstrate that touching the

35 skin induces local mechanical strain in intact animals and suggest that a fully intact ECM is not

36 essential for transmitting mechanical strain from the skin to cutaneous mechanosensory neurons.

37

Introduction

Touch and proprioception are essential to the daily lives of all animals, including humans. Classical examples of mechanotransduction, both of these senses depend on activation of mechano-electrical transduction (MeT) channels arrayed within somatosensory neurons (Katta et al., 2015) and are thought to activate following physical deformation of sensory cells during touch and movement. Consistent with this inference, neurons innervating stretch receptor organs in vertebrates (Bewick and Banks, 2015) and arthropods (Suslak and Jarman, 2015) are activated by experimentally applied mechanical strain (stretch). High-speed volumetric imaging reveals that proprioceptors deform in crawling Drosophila larvae (He et al., 2019; Vaadia et al., 2019) and that physical deformations are correlated with calcium transients. Similarly, movement induces localized calcium transients in the tertiary dendrites of the *C. elegans* PVD proprioceptors that depend on expression of a DEG/ENaC/ASIC protein (Tao et al., 2019). These studies reinforce the idea that physical deformation of sensory neurons during movement is critical for activation of native MeT channels, but do not address whether or not similar deformations occur in response to touch or measure the extent of mechanical strain that occurs within the sensory neurons themselves.

Using *C. elegans* touch receptor neurons (TRNs), we investigated the quantitative relationship between touch and physical deformation of sensory neurons in intact living animals. The ready availability of transgenic animals expressing TRN-specific markers and their transparent body make *C. elegans* an especially useful animal for investigating touch-evoked sensory neuron deformation. Adult animals have six TRNs, consisting of two bilaterally symmetric pairs of touch receptor neurons (ALM and PLM) and two neurons that run along the ventral midline (AVM and PVM) (Goodman, 2006). These six neurons extend long, unusually straight sensory neurites (Krieg et al., 2017) that are embedded in epidermal cells and have a distinctive, electron-dense extracellular matrix or ECM as well as hemidesmosome structures attaching the TRNs to the cuticle (Chalfie and Sulston, 1981; Chalfie and Thomson, 1979).

All of the TRNs express the MEC-4 channel, which is required for touch-evoked calcium transients (Suzuki et al., 2003) and touch-evoked MeT currents (O'Hagan et al., 2005). The MEC-4 channels localize to puncta arrayed along the entire length of wild-type TRN sensory

67   neurites (Chelur et al., 2002; Cueva et al., 2007; Emtage et al., 2004; Katta et al., 2019), but are

68   disrupted in ECM mutants (Emtage et al., 2004).

69   Touch-evoked behavior (Petzold et al., 2013) and MeT channel activation (Eastwood et

70   al., 2015) depend on body indentation rather than the force applied. Slow stimuli fail to activate

71   MeT currents and their size increases with stimulus frequency, indicating that activation of MeT

72   channels in their native environment depends on tissue viscoelasticity (Eastwood et al., 2015;

73   Katta et al., 2019; Sanzeni et al., 2019). Although slow, movement-induced physical

74   deformations of the worm's body and its neurons are too slow to activate MeT currents in the

75   TRNs (Eastwood et al., 2015; Katta et al., 2019), these undulatory movements produce

76   mechanical strains of up to 40% (Krieg et al., 2017; Krieg et al., 2014), indicating that TRNs can

77   withstand significant mesoscale extension and compression *in situ*. The ability to withstand

78   movement-induced strain is shared by mammalian nerves that experience up to 30% strain

79   during limb movement (Phillips et al., 2004).

80   Here, we sought to determine whether or not body indentations sufficient to evoke

81   calcium transients in TRNs also generate local strain. To achieve this goal, we visualized steady-

82   state, touch-induced strain in TRNs in living animals restrained within a microfluidic stimulation

83   device equipped with the ability to deliver mechanical stimuli (Fehlauer et al., 2018; Nekimken

84   et al., 2017a). Our approach benefits from large, immobile mitochondria distributed within TRN

85   sensory neurites (Sure et al., 2018), exploits the ability to tag these mitochondria with a red

86   fluorescent protein (Zheng et al., 2014), and borrows analytic principles from traction force

87   microscopy (Ribeiro et al., 2016). Although mitochondria have been used to evaluate neuronal

88   mechanics in culture (O'Toole et al., 2015), we believe this study is the first to make use of

89   mitochondria as mechanical fiducial markers in living animals. We show that body indentation

90   increases local steady-state mechanical strain in the TRNs in a manner that is robust to mutations

91   known to affect attachment of the TRN to the extracellular matrix and to epidermal cells.

92 **Results**

93 *Mechanical stimulation in a microfluidic device*

94   To directly observe touch-induced deformation of the TRNs, we sought to develop a

95 method that combines the delivery of controlled mechanical stimuli and three-dimensional

96 optical imaging. To reach this goal, we applied mechanical stimuli to adult worms confined in a

97 microfluidic device (Nekimken *et al.*, 2017, Figure 1A). Our device has pneumatic actuators that

98 consist of a thin flexible wall that separates a channel filled with air from a worm in the trap

99 channel. When air pressure in the actuator channel is increased using a pressure controller, the

100 thin wall expands like a balloon, deforming the trapped worm and generating indentations

101 sufficient to activate the TRNs (Nekimken et al., 2017a). Other devices that deliver mechanical

102 stimuli to restrained (Cho et al., 2018) or moving (McClanahan et al., 2017) worms in

103 microfluidic chambers have been reported, but they use larger actuator channels and are thus not

104 well-matched to our goal of investigating local deformation of the TRNs.

105   Our device is designed to increase the probability that animals will enter the trap with

106 either their left or right sides in contact with the bottom of the chamber and their ventral and

107 dorsal sides near the actuator (Nekimken et al., 2017a). Consistent with this expectation and the

108 position of the AVM and ALM neurons within the worm's body, previous experiments using this

109 device resulted in a higher frequency of AVM activation compared to ALM (Nekimken et al.,

110 2017a). However, the rotational orientation of each worm was variable. We took advantage of

111 this variation to select animals oriented such that ALM (rather than AVM) was near the actuator

112 and used only ALM neurons for this study. In all cases, this variation in worm positioning leads

113 to variation in the size of the effective stimulus. To ensure that all animals received a deforming

114 stimulus, we limited our analysis to animals whose TRNs were deformed enough to observe

115 visually during the experiment.

116

117 *Mitochondria as fiducial markers for inferring strain*

118   We used fluorescent protein-tagged mitochondria as fiducial markers to measure strain,

119 an approach that uses particle tracking with large (~1µm), immobile mitochondria in the TRNs

120 (Sure et al., 2018) serving as natural fiducial markers. We imaged mitochondria position before

121   and after mechanical stimulation (Figures 1B and 1C). Next, we used the displacement between

122   adjacent mitochondria to quantify one-dimensional mechanical strain along the long axis of the

123   TRN (Figure 1D) according to: $\varepsilon = \Delta L/L_0$, where L_0 is the resting, undeformed length of an

124   object, and ΔL is the change in length of the object when deformed. For clarity, we refer to this

125   as longitudinal strain. As shown in Figure 1, mitochondria adjacent to the actuator moved more

126   than those anterior and posterior to the actuator (Figures 1B and 1C) and the inferred strain was

127   greatest near the center of the actuator (Figure 1D). This method enables direct observation of

128   touch-evoked strain in *C. elegans* TRNs in a single dimension aligned with the TRN's longest

129   dimension.

130   We note that this method is not suitable for detecting shear strain or bending strain. To

131   derive these measurements, we would need to detect angular changes (shear) or relative position

132   (bending) of the mitochondria within the TRNs. Given that the diameter of each mitochondrion

133   is only ~100 nm and the TRN is diameter is not much larger than this (200-300 nm, see Figure

134   3C, Cueva et al., 2007), such movements cannot be resolved because these objects are similar in

135   size to the estimated lateral resolution of 254 nm and axial resolution of 632 nm of our imaging

136   system. What about strain in directions orthogonal to the long axis of the TRN dendrite?

137   Although our image stacks contain three-dimensional position data (Figures 2A and 2B), the

138   initial distance between adjacent pairs of mitochondria in the *x* and *z* directions is small

139   compared to the resulting displacement. Thus, measurement resolutions lead to large

140   uncertainties in the strain calculation for these dimensions. Both limitations can be attributed to

141   the confinement of the mitochondria within the narrow caliber of the TRN and the fact that the

142   TRNs lie primarily within a single focal plane. Thus, the size of mitochondria, the geometry of

143   the TRNs and the optical resolution of the spinning-disk confocal limit this measurement to one-

144   dimensional strain along the *y*-dimension that traces the main axis of the TRN.

145

146   *Local indentation induces local mechanical strain in TRNs*

147   Next, we estimated the spatially averaged distribution of touch-induced strain in the

148   TRN. The mitochondria are sparsely distributed in the TRN and strain is a pairwise measurement

149   between adjacent mitochondria, so our strain measurements result in a series of discontinuous

150   step functions for a single trial rather than a smooth curve (Figure 1D). Each segment of the plot

151   indicates the average strain between two markers, so the maximum local strain on each interval

152   may be larger than our measured strain. The average distance between mitochondria in control

153   *jsIs1073* animals was 26 μm (min: 5 μm; max: 64 μm) and we were able to analyze an average

154   of 8.9 intervals per trial (Table S1). To obtain averages across trials, we plotted deformation

155   against position in the longitudinal or *y* direction, fit this profile with a Gaussian function and

156   defined the position of the maximum as the center of the actuator and *y*=0. (see dotted line in

157   Figure 1C). In this coordinate system, positions anterior to *y=0* are negative and positions

158   posterior to *y=0* are positive. Finally, we determined the average strain at each longitudinal

159   position along the neurite across actuation trials (see Figure 2C). Note that the average strain is

160   both larger and noisier for anterior (negative) positions. This is likely to reflect the fact that the

161   head is less constrained in the microfluidic channel than other parts of the body and suggests that

162   displacement and strain on the anterior side of the actuator include both touch- and movement-

163   induced mechanical strain on the neuron.

164   As expected, strain at the center of the actuator (*y=0*) increases with TRN deformation

165   (Figure 2D), but the dependence on TRN deformation was weak. The observed, but modest

166   variation in TRN deformation arises from two aspects of our method. First, because the actuator

167   is fixed to the surrounding material of the device on all sides, its center is the location of

168   maximum deformation. Second, although most animals are trapped with either their left or right

169   side in contact with the coverslip at the bottom of the device, some are rotated along their

170   anterior-posterior axis such that the position of the imaged TRN varies with respect to the

171   actuator (Figure 2A). Thus, the average strain induced between the pair of mitochondria at the

172   center of the actuator was 0.031±0.005 (mean ± SEM, *n* = 61 trials, *N*=15 animals) in transgenic

173   *jsIs1073* TRN neurons. Although additional studies exploring a wider range of deformations will

174   be needed to determine the nature relationship between TRN deformation and strain, to our

175   knowledge, these are the first *in vivo* measurements of touch-induced longitudinal strain in touch

176   receptor neurons, and they link local body indentation to local cellular deformation.

177

178   *Do tagged mitochondria affect TRN function?*

179   We used classical touch assays to address this question (Chalfie et al., 2014; Nekimken et

180   al., 2017b) by measuring touch sensitivity in wild-type (N2) and transgenic worms carrying the

181   transgenic mitochondria marker, *jsIs1073*. In blinded assays of three independent cohorts of 25

182   animals for each genotype, wild-type and *jsIs1073* animals had touch response rates of 0.869 and

183   0.865, respectively. The difference between the means was 0.00667 [95% CI (-0.0347, 0.048)].

184   Thus, the *jsIs1073* transgene does not decrease touch sensitivity.

185

186   *Selected ECM mutants and their touch sensation and anatomical phenotypes*

187   Having measured touch-induced mechanical strain in control TRNs, next we measured

188   longitudinal strain in four existing ECM mutants: *him-4(e1267), mec-1(e1738)*, *mec-1(e1066),*

189   and *mec-5(u440)*. One of these mutants, *him-4(e1267),* has a partial defect in touch-sensitivity,

190   and the others are touch-insensitive (Du et al., 1996; Emtage et al., 2004; Vogel and Hedgecock,

191   2001). We reproduced these previous results using a ten-touch assay (Table 1). Whereas *him-4*

192   mutants retain MEC-4 puncta that are grossly wild-type, TRN neurites in *mec-1* and *mec-5*

193   mutants lack prominent MEC-4 puncta (Emtage et al., 2004). In *him-4* and in *mec-1(e1738)*

194   mutants analyzed here, the ALM and PLM neurons are displaced from their normal body

195   position near the lateral midlines and are not properly embedded in the epidermis (Emtage et al.,

196   2004; Vogel and Hedgecock, 2001). This effect is inferred to arise from a defective TRN-ECM

197   attachment (Vogel and Hedgecock, 2001). Consistent with this idea, the electron-dense ECM is

198   not detected in *mec-1* mutants (Chalfie and Sulston, 1981). Analyzing these four mutants enables

199   us to evaluate the relationship, if any, between touch-induced longitudinal strain and three other

200   phenotypes: behavioral responses to touch, the distribution of MEC-4 puncta, and TRN-ECM

201   attachment.

202   The *him-4* gene encodes hemicentin, a conserved ECM protein that is rich in Ig and EGF

203   domains and is expressed by body wall muscles (Vogel and Hedgecock, 2001). The *him-4* locus

204   is >30 kb, which complicated determination of the molecular defect encoded by *e1267* using

205   classical sequencing methods. We used whole-genome sequencing to circumvent this limitation

206   and found that *e1267* encodes a single base indel in the third intron of the *him-4* gene,

207   introducing a shift in the reading frame of the encoded protein (Figure S1). A second

208   polymorphism was detected in the intron following the 48th exon. Thus, the *e1267* allele is a null

209   allele and animals carrying this mutant are likely to lack the HIM-4 protein. The *mec-1* gene

210   encodes a large secreted protein rich in kunitz-like domains, is expressed by the TRNs, and many

211 alleles of this gene were found in forward genetic screens for touch-insensitive animals (Chalfie

212 and Au, 1989; Chalfie and Sulston, 1981). The *e1066* allele is a null and animals carrying this

213 allele are likely to lack the MEC-1 protein, whereas the *e1738* allele encodes a premature stop

214 codon and is proposed to express a truncated MEC-1 protein. Both *mec-1* mutants are touch

215 insensitive, but only *e1066* lacks proper TRN-ECM attachments (Emtage et al., 2004). *mec-5*

216 encodes an atypical collagen and, unlike *mec-1*, it is not made by the TRNs (Du et al., 1996).

217 Like other ECM mutants we analyzed here, the *u440* allele is null and *u440* mutants do not

218 express the MEC-5 protein. The TRN neurites in *mec-5* mutants are positioned near the lateral

219 midlines, but are less straight than they are in wild-type animals, meandering such that some

220 segments of the neurite are close to the muscle and other parts closer to the lateral midline

221 (Emtage et al., 2004).

222

223 *Touch-induced longitudinal strain in TRNs with ECM mutants*

224   To analyze transmission of mechanical energy from the skin in animals lacking proper

225 TRN-ECM attachment, we introduced the *jsIs1073* transgene into *him-4* and *mec-1* mutants and

226 applied mechanical stimuli to animals trapped in our pneumatic microfluidic device. Touch-

227 induced longitudinal mechanical strain in *him-4(e1267)* TRNs was indistinguishable from that

228 observed in control animals (Figure 3A, Table 2). Touch-induced longitudinal strain was

229 likewise similar to control in both *mec-1* mutants (Figure 3B) and in *mec-5* mutants (Figure 3C).

230 Figure 3D shows that the average longitudinal strain profiles for control, *him-4*, *mec-1*, and

231 *mec-5* mutants are similar across the entire 200 μm segment of the TRN neurite analyzed here.

232 Values for strain measured at the actuator center ($y=0$) had similar mean values and distribution

233 for all genotypes tested (Figure 4A) and estimation graphics (Ho et al., 2019) indicate that the

234 mean values for all genotypes are not different than control values (Figure 4B). Except for *mec-5*

235 mutants, the number of mitochondria available for tracking and the average distance between

236 mitochondria was similar in control transgenic and mutant animals (Table S1). *mec-5* mutants

237 had fewer, more widely spaced mitochondria than control animals, an effect would be expected

238 to impair the spatial resolution of longitudinal strain measurements. Collectively, these findings

239 suggest that neither severe nor mild defects in TRN-ECM attachment play a significant role in

240 the generation of touch-induced longitudinal mechanical strain at steady-state. Future studies

241 and techniques will be needed to resolve dynamic changes in local strain that are expected to

242 occur on the millisecond timescale of MEC-4-dependent MeT channel activation.

243

Discussion

245   Using mitochondria as natural fiducial markers in *C. elegans* TRNs, we showed for the

246 first time that body indentation sufficient to activate the TRN causes an increase in longitudinal

247 strain. The magnitude of this strain increases with TRN displacement. In wild-type animals, the

248 touch-induced strain closest to the point of maximum indentation was roughly 3% (Figure 3,

249 Table 2). This change in strain is unlikely to damage these sensory neurons, since they are

250 subjected to mechanical strain on the order of 40% during locomotion (Krieg et al., 2017; Krieg

251 et al., 2014). Such global changes in strain are too slow to activate the TRNs (Eastwood et al.,

252 2015; Katta et al., 2019).

253   The 3% strain we measured may be a lower bound on the true value for longitudinal

254 strain. Simulations predicted an elongated strain field with a peak value of 12% for longitudinal

255 strain (Sanzeni et al., 2019). Unlike our measurements, which used a wide (50µm), flexible

256 PDMS membrane to indent the worm's body and have limited spatial resolution, the simulations

257 employed a stiff spherical bead (10µm diameter) to deliver touch stimuli and a continuous

258 deformation function. The simulations (Sanzeni et al., 2019) predict strains are present in the

259 other two dimensions (along the direction of the stimulus and tangential to the circumference of

260 the worm at the point of the stimulus). Due to the nature of our measurement, however, we were

261 only able to measure one-dimensional strain along the length of the TRN. Nevertheless, these

262 measurements of touch-induced strain can be incorporated into future models of *C. elegans* touch

263 sensation to improve understanding of the mechanical state of the TRN and mechano-sensitive

264 ion channel complex upon touch stimulation.

265

266 *Origins of mechanical coupling between the skin surface and somatosensory neurons*

267   We tested the idea that strain transmission would depend on TRN-ECM attachment, be

268 correlated with impaired touch sensation, and be independent of the proper distribution of

269 MEC-4 channels. To our surprise, we found that touch-induced longitudinal mechanical strain

270   was similar in control TRNs and all ECM mutants tested here, including the *him-4(e1267)* and

271   *mec-1(e1066)* mutants exhibiting severe defects in TRN-ECM attachments. Thus, touch-induced

272   longitudinal strain we observed in the ALM neurons is independent of their attachment to other

273   tissues or the expression of ECM proteins MEC-1 and MEC-5, at least at steady state.

274   This finding implies that mechanical coupling between the skin and sensory neurons

275   persists in mutants with ECM defects. Friction is one alternative source of mechanical coupling

276   between TRNs and surrounding tissues. In this scenario, all of the worm's tissues are compacted

277   together by a high hydrostatic pressure and this would elevate friction between tissues. Further

278   compression applied to the outside of the worm during touch stimulation could lead to stiffening

279   of the worm's body that might be caused by internal structures jamming together (Gilpin et al.,

280   2015). When we and others immobilize worms in microfluidic devices, we fabricate channels

281   small enough to apply gentle compressive forces that the worm cannot overcome. As a result, the

282   worm is compressed on all sides except the nose and tail when in the microfluidic trap,

283   potentially jamming the TRNs against surrounding tissues and further increasing friction.

284   Another possibility is that the generation of touch-induced local mechanical strain is dominated

285   by the cytoskeleton. In support of this idea, mutations that disrupt the expression of MEC-12 α-

286   tubulin and MEC-7 β-tubulin decrease mechanoreceptor currents and increase stimulus

287   amplitude needed to activate these currents (Bounoutas et al., 2009; O'Hagan, 2005). Additional

288   experimental work and methods with improved spatial and temporal resolution will be needed to

289   differentiate among these possibilities.

290

291   *Spatial and temporal resolution of mechanical strain transmission*

292   The spatial resolution of our strain measurements is limited by the average distance

293   between adjacent, immobile mitochondria, which was 26μm (Table S1). Thus, this method may

294   lack sufficient spatial resolution to detect micro- or nanoscale variations in mechanical strain

295   transmission at steady state. The spatial resolution of strain measurements could be improved by

296   using more closely spaced fiducial markers. In principle, MEC-4 channels (with an average

297   spacing of 2-3 μm) tagged with a very bright fluorescent protein would provide a ten-fold

298   increase in resolution. Independent of potential improvements in spatial resolution, the present

299   approach is limited to steady-state measurements because our volume imaging rate is low (15

300   seconds per stack). In this regard, it is important to note that wild-type *C. elegans* TRNs are

301   preferentially activated mostly by high-velocity stimuli (Eastwood et al., 2015; Katta et al., 2019;

302   Nekimken et al., 2017a; Suzuki et al., 2003). Temporal resolution could be improved using other

303   imaging techniques enabling rapid acquisition of imaging volumes. If volumes could be acquired

304   at 20 Hz or faster, it would possible to observe mechanical strain in a 10 Hz buzz stimulus that

305   we previously used to activate the TRNs (Nekimken et al., 2017a).

306   *Conclusion*

307   We performed the first *in vivo* measurements of touch-induced mechanical strain in *C.*

308   *elegans* TRNs. We used mechanically stable mitochondria in the TRNs as natural fiducial

309   markers to observe deformation of the TRN and found that local touch stimuli applied in a

310   microfluidic device induces local strain in the TRN. Defects in the ECM surrounding the TRN

311   did not alter the steady-state mechanical strain in the TRN, suggesting that explicit attachments

312   are not necessary for deformation of the TRN and that the bulk properties of tissues are sufficient

313   to sustain significant mechanical energy transfer from the skin surface to the embedded neurons.

314   In light of the temporal limitations of our measurements, however, we cannot exclude the

315   possibility that TRN-ECM attachments contribute to dynamic aspects of mechanical strain

316   transmission. Collectively, these findings provide an empirical basis for the idea that mechanical

317   stimuli applied to the skin stretch embedded sensory neurons that may be shared by the sensory

318   neurons that innervate the skin and other tissue in mammals.

319

320 **Methods and Materials**

321

322 *Nematode strains*

323 For all experiments measuring mechanical strain, we used animals carrying *jsIs1073*

324 [mec-7p::TagRFP-mito::CBunc-119], a transgene that drives expression of TagRFP in the

325 mitochondria in the TRNs (Zheng et al., 2014), alone or together with ECM mutants (Table 3).

326 We relied on visualization of the *jsIs1073* transgene and behavioral phenotypes (for *mec* genes)

327 or anatomical defects (for *him-4*) to perform genetic crosses. We used gene sequencing to verify

328 the presence of ECM mutants in all strains created for this study. The primer pairs we used to

329 amplify the relevant segments of each gene are:

330 *mec-1(e1066)*: Forward–catcttccacgccgcaaagtc, Reverse—aatcctctctgccctcatgttcc

331 *mec-1(e1738)*: Forward—tcacagtcagacgtgcctcg, Reverse—cattgcctcacaccaacttccac

332 *mec-5(u444)*: Forward—cagaatactatgtacgtaacttgggatc; Reverse—ctcatgggtacgcaaatgatactc

333 *him-4(e1267)*: Forward—tttcgtgatgactggtgactgtgg; Reverse—ttaaagtcaacagcaccgtgacc

334

335 *Immobilization and mechanical stimulation with a pneumatic microfluidics device*

336 To provide a repeatable mechanical stimulus that is compatible with imaging the mitochondria of

337 the TRN, we used a microfluidic device made of the transparent elastomer PDMS for

338 simultaneous mechanical stimulation and imaging of *C. elegans* (Nekimken et al., 2017a). Using

339 a pressure controller (Elveflow OB1), we applied 300 kPa of pressure to one of the device's

340 actuators to create a mechanical stimulus. We fabricated and operated devices designed for use

341 with young adult worms as described in our previous work (Fehlauer et al., 2018; Nekimken et

342 al., 2017a). Because worms immobilized in this trap are uniformly and partially deformed by the

343 channel, we refer to this as the rest configuration.

344

345 *Sample preparation and inclusion criteria*

346 We performed all experiments using young adult animals that were synchronized by

347 hypochlorite treatment (Stiernagle, 2006) and cultured for three days on NGM agar plates seeded

348 with OP50 bacteria for food. We transferred animals from the agar plates with a platinum wire

349   pick to a drop of imaging medium (see below) in a small Petri dish. After using the pick to push

350   away large particles that might clog the microfluidic device, we aspirated worms into

351   polyethylene tubing (PE50, Intramedic™ brand, Becton-Dickson) with a syringe and connected

352   the tubing to the device's inlet. For each trial, we pushed a worm into the trap channel using the

353   syringe and then evaluated whether to use this worm for an experiment based on our inclusion

354   criteria. We included animals that fit in the microfluidic trap with minimal movement and could

355   be ejected through the narrow opening at the head of the trap. When performing experiments

356   with *him-4* mutants, we chose animals whose gross morphology was wild-type and avoided

357   animals whose intestines were everted. In all cases, we only acquired images from animals

358   whose ALM neurons were oriented adjacent to the mechanical actuation channels.

359   To improve image quality by reducing reflections generated by the walls of the

360   microfluidic trap, we designed a non-toxic imaging medium with a refractive index similar to

361   PDMS (1.4). The imaging medium was a 70%:30% (vol:vol) mixture of physiological saline

362   and iodixanol (Optiprep™, Sigma-Aldrich), a non-toxic density-gradient medium (Boothe et al.,

363   2017). We used the same physiological saline as that used for electrophysiological recordings

364   from *C. elegans* neurons (O'Hagan et al., 2005), which contains (in mM): NaCl (145), KCl (5),

365   $MgCl_2$ (5), $CaCl_2$ (1), and Na-HEPES (10), adjusted to a pH of 7.2 with NaOH. The imaging

366   medium has an osmolality of 300-325 mOsm (Fiske Micro-Osmometer Model 210), so it does

367   not cause large osmotic shocks to *C. elegans*. By contrast with other fluids we tested (e.g.

368   glycerol, halocarbon oil), this medium has a viscosity that appeared to be similar to that of

369   physiological saline.

370

371   *Image acquisition*

372   Although the mechanical stimulus is mostly in the horizontal direction, there is enough

373   deformation in the z-direction to move the neuron out of plane during stimulation in some cases.

374   In initial experiments with a traditional epifluorescence microscope, the mitochondria often

375   moved out of focus during stimulation due to movement in the z-direction. To account for this

376   problem, we used a spinning disk confocal microscope (Nikon TiE, Yokogawa CSU-X1,

377   40x/NA 1.4 oil objective, and Photometrics Prime95B sCMOS camera) to acquire z-stacks,

378   which provided adequate resolution in the z-direction.

379   For each trial, we acquired eleven (11) z-stacks containing the neuron of interest, with
380   300 kPa of pressure applied during the even-numbered stacks, and 0 kPa applied during the odd-
381   numbered stacks. We started acquisition of a stack every 15 seconds, although the time to
382   acquire each given stack was approximately 12 seconds. The exact time varied depending on the
383   height of the z-stack, which was manually set to accommodate observed motion in the z direction
384   during the test actuation. During the short delay between acquisition of stacks, we toggled the
385   applied pressure.

386

387   *Image analysis*

388   To detect the mitochondria, we used a particle-tracking algorithm implemented in Python
389   (Allan et al., 2018), based on work by Crocker and Grier (Crocker and Grier, 1996). Briefly, the
390   algorithm involves applying a spatial band pass filter, finding peaks, refining the position of
391   peaks by finding their center of mass, and linking particles across timepoints into trajectories. In
392   some stacks, the algorithm failed to detect the mitochondria because they were too close to the
393   top or bottom of the stack, were not bright enough, overlapped with the mitochondria of another
394   TRN, or were blurred due to the motion of the worm. We discarded all stacks subsequent to a
395   stack where the image processing failed, because the strain measurement requires comparison to
396   a previous stack. Additionally, not all of the stacks from each trial were usable, since the
397   fluorophores bleached over time. As a result, not all actuation events yielded strain
398   measurements (see Supplementary Figure S2).

399   Due to variability across trials, we manually selected a region of interest around the TRN
400   and tuned parameters in the particle tracking algorithm. Primarily, we changed the minmass
401   threshold, which filters out particles where the sum of pixel values within the boundaries of the
402   particle is below the chosen threshold, and the search radius for the linking step, which specifies
403   how far a particle can travel between timepoints and still be identified as the same particle. Less
404   frequently, we changed the cutoff size for the bandpass filter or the brightness percentile
405   threshold, which sets a minimum value for the brightest pixel in a particle as a percentile of the
406   brightness of pixels in the image. We tuned these parameters until the particles found and linked
407   included only particles along the location of the neuron and not autofluorescent spots.
408   Supplemental Figure S3 shows the range of parameters we used.

409

Touch assays

411   To test the touch-sensitivity of the mutants used in our strain transmission experiments,
412 we performed touch assays by lightly stroking an eyebrow hair across the body of a worm and
413 scoring its behavioral response (Goodman, 2006). For each session of touch assays, we tested 25
414 worms from a plate, performing 10 touches per worm. We performed the touch assays blinded
415 with respect to genotype. For each touch event, we counted a response consisting of reversing
416 direction or speeding up to move away from the stimulus as a positive response.

417

Whole-genome sequencing of him-4(e1267)

419 Our whole-genome sequencing protocol involved four sub-protocols: 1) DNA extraction, 2)
420 sequencing library preparation, 3) sequencing, and 4) analysis. We isolated DNA from CB1267
421 *him-4(e1267) X* animals with a phenol chloroform isoamyl alcohol (PCI) extraction. First, we
422 washed worms off a mostly starved plate using M9 buffer, rinsed the animals twice in M9 buffer,
423 resuspended them in EN buffer (0.1M NaCl and 20 mM EDTA), removed the supernatant, and
424 flash-froze the sample in liquid nitrogen. Next, we added 450 µL of worm lysis buffer (0.1 M
425 TRIS pH 8.5, 0.1 M NaCl, 50 mM EDTA, and 1% SDS) and 40 µL of proteinase K (10 mg/ml)
426 to 50 µL of frozen worms and incubated at 62°C for 45 minutes, vortexing occasionally. Then,
427 we performed the PCI extraction in a phase lock gel tube (VWR) by adding 500 µL of PCI to the
428 sample, vortexing, spinning for 5 minutes at 10,000 rpm, and then collecting the upper phase.
429 We repeated the PCI extraction step, and then extracted twice using chloroform. We precipitated
430 the DNA by adding 40 µL of 5M sodium acetate and 1 mL of ethanol, spinning for 5 minutes at
431 10,000 rpm, removing the supernatant, washing in 70% ethanol, and resuspending in 50 µL of
432 TE buffer at pH 7.4.

433   We created a sequencing library according to manufacturer instructions (Nextera DNA
434 Library Prep Kit, Illumina). Briefly, this includes tagmentation of the DNA using the Illumina
435 Tagment DNA Buffer and Enzyme, clean-up of the tagmented DNA using a Zymo DNA Clean
436 and Concentrator Kit, PCR to add the library indices to the tagmented DNA, and PCR cleanup
437 by gel extraction using a Qiagen MinElute Kit. We did a quality control step to confirm that the
438 average length of DNA fragments was in the expected range of 300-500 bp using the Agilent

439   Bioanalyzer at the Stanford Protein and Nucleic Acid Biotechnology Facility. Sequencing was

440   completed on an Illumina NextSeq sequencer in the Stanford Functional Genomics Facility.

441   We analyzed the sequencing data using the computing cluster of the Stanford Center for

442   Genomics and Personalized Medicine. Briefly, we mapped reads using Bowtie2, used Picard to

443   sort reads, mark duplicates, and prepare read groups, then used GATK to select high quality

444   SNPs and INDELs, and SnpEff to annotate the results.

445

446   *Code availability*

447   Two kinds of data were analyzed using custom code: image analysis (Python,

448   https://github.com/anekimken/SSN_ImageAnalysis) and whole-genome sequence analysis

449   available (scripts, https://github.com/wormsenseLab/whole_genome_sequencing).

450

451   **Acknowledgments**

462

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581

582 **Figure Legends**

583

584 **Figure 1: Touch-induced mechanical strain measured using mitochondria as fiducial**

585 **markers in *C. elegans* TRNs *in vivo*.**

586 A) Brightfield image of worm in the microfluidic device. Scale bar 20 μm. B) Maximum

587 projection image of TRN mitochondria before (magenta) and during (green) mechanical

588 stimulus. Mitochondria that did not move appreciably appear white due to the overlap of

589 magenta and green. Image intensity and contrast was adjusted to improve visualization. Scale bar

590 20 μm. C) Displacement in the direction of actuation before (magenta) and during (green)

591 stimulation. The smooth line (green) is a Gaussian fit used to infer the center of actuator. D)

592 Distribution of touch-induced strain in the TRN for a single actuation trial.

593

594   **Figure 2: Indentation induces local longitudinal strain in *C. elegans* TRNs.**

595   A) Three-dimensional diagram the positioning of the worm in the microfluidics trap, the animal-

596   centric coordinate system used to characterize strain, and the consequences of trapping animals

597   in different orientations. TRN is red, and muscles are included in brown as visual aid. B) Touch-

598   induced displacement in three dimensions. C) Touch-induced strain, anterior is to the left and

599   posterior is to the right. $y=0$ is the center of the actuator. $n=61$ trials from $N=15$ worms. D)

600   Mechanical strain at $y=0$ as a function of maximum neuron displacement. The line is a linear fit

601   to the data: $\varepsilon = 0.0061\mathrm{x} - 0.0067$, where ε is the strain and x is the maximum displacement. The

602   shaded area indicates 95% confidence intervals of the fit. $R^2 = 0.04$.

603

604

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606

607 **Figure 3: Touch-induced mechanical strain profiles are similar in control animals and**

608 **ECM mutants.**

609 A) Spatially averaged strain in control ALM neurons with normal TRN attachment (15 animals)

610 and *him-4(e1267)* with attachment defects (16 animals). The data for control animals are the

611 same data as Figure 2. B) Spatially averaged strain in *mec-1* mutants. 14 independent animals for

612 *mec-1(1066)* and 16 animals for *mec-1(e1738)*. C) Spatially averaged strain in *mec-5(u444)*

613 mutants (17 animals). D) Overlay of all spatially averaged strain profiles. Smooth lines are the

614 averages across all trials and shaded areas show the error (95% confidence intervals). One ALM

615 neuron tested in each animal.

616

617 **Figure 4: Strain at the center of the actuator is similar in control animals and ECM**

618 **mutants**

619 A) Strain at the center of the actuator. Each point is the result of a single trial. The vertical lines

620 next to the swarm plots indicate the median and quartiles of the data. Data collected from 15, 16,

621 14, 16, and 17 animals (left to right). B) Difference in the mean strain between control and each

622 mutant with a bootstrapped resampled distribution of the data. Estimation plots generated using

623 the DABEST plotting package (Ho et al., 2019).

624

Table 1: Touch sensitivity of ECM mutants.

626

Genotype	Response probability	Mean difference from control	95% confidence interval of mean difference
control	0.742		
him-4(e1267)	0.527	-0.215	(-0.275, -0.163)
mec-1(e1066)	0.083	-0.659	(-0.698, -0.619)
mec-1(e1738)	0.237	-0.505	(-0.551, -0.461)
mec-5(u444)	0.208	-0.534	(-0.580, -0.487)

627 All mutants we tested were less sensitive to touch than control animals; effect size was smallest

628 for *him-4(e1267)* and largest for *mec-1(e1066)*. All strains tested contained the transgene

629 *jsIs1073* that tags mitochondria in the TRNs with RFP. *N*=100 worms for each genotype, tested

630 in four independent cohorts of 25 blinded to genotype.

631

632

633

Table 2: Touch-evoked longitudinal strain in the TRNs as a function of genotype.

635

Genotype	Mean strain ± SEM	Mean difference from control	95% confidence interval of mean difference	stimulation trials	animals
control	0.031 ± 0.005			61	15
him-4(e1267)	0.024 ± 0.005	-0.007	(-0.020, 0.007)	61	16
mec-1(e1066)	0.034 ± 0.007	0.003	(-0.012 0.022)	49	14
mec-1(e1738)	0.035 ± 0.005	0.004	(-0.009 0.018)	68	16
mec-5(u444)	0.026 ± 0.009	-0.005	(-0.028 0.013)	44	17

636 There was no detectable difference in the magnitude of the touch-evoked strain in control and

637 ECM mutant animals.

638

639   **Table 3:** *C. elegans* **strains**

640

Strain Name	Genotype	Source
N2 (Bristol)	wild-type	CGC
NM3573	*jsIs1073*	(Zheng et al., 2014)
GN885	*jsIs1073;him-4(e1267)*	this study
GN886	*jsIs1073;mec-1(e1066)*	this study
GN887	*jsIs1073;mec-1(e1738)*	this study
GN906	*jsIs1073;mec-5(u444)*	this study

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649   Figure S1: *him-4(e1267)* is likely to be a null allele. A) Map of *him-4* with *e1267* allele

650   annotated. B) Close-up of insertion in sequence as indicated by dotted lines in Panel A. C)

651   Sequences of both Indels found by sequencing. The first indel is likely to cause the null

652   phenotype because it causes a frameshift in an early exon, whereas the second indel is in a later

653   intron.

654

655   Figure S2: Quality control for image (z) stacks as a function of genotype. Eleven (11) image

656   stacks were collected from each TRN analyzed for touch-induced strain. This plot shows the

657   number of stacks that passed quality for each experiment. Note: The final stack was not used as

658   it was acquired in the resting configuration and could not be compared to a subsequent stack

659   collected in the indented configuration.

660

661   Figure S3: We tuned parameters of the particle tracking algorithm to account for variability

662   across images.

663

664 **Table S1: Average number of detected mitochondria and distance between adjacent**

665 **detected mitochondria as a function of genotype.**

666

Number of mitochondria					
Genotype	Mean number ± SEM	Mean difference from control	95% confidence interval of mean difference	Minimum	Maximum
Control	8.9 ± 0.6			5	15
him-4(e1267)	9.1 ± 0.5	0.2	(-1.5, 1.6)	6	13
mec-1(e1066)	8.8 ± 0.5	-0.1	(-1.7, 1.5)	6	13
mec-1(e1738)	8.9 ± 0.5	0.01	(-1.6, 1.5)	6	15
mec-5(u444)	7.2 ± 0.5	-1.7	(-3.3, -0.3)	5	12
Distance between adjacent mitochondria					
Genotype	Mean distance ± SEM (μm)	Mean difference from control (μm)	95% confidence interval of mean difference (μm)	Minimum (μm)	Maximum (μm)
Control	26.0± 0.4			5.4	63.7
him-4(e1267)	27.3±0.4	1.4	(-2.0, 4.8)	5.0	76.1
mec-1(e1066)	27.0±0.5	1.0	(-2.6, 4.7)	5.3	65.1
mec-1(e1738)	26.9±0.4	0.9	(-2.4, 4.3)	6.0	64.1
mec-5(u444)	31.1±0.6	5.2	(1.2, 9.3)	5.5	84.1

667

668 The number of detected mitochondria and distance between adjacent mitochondria in the ALM

669 neurons was similar in control and the ECM mutants, except for *mec-5(u444)* which had fewer

670 detected mitochondria and larger distances between adjacent mitochondria, on average. Number

671 of animals is the same as Table 2.

672

673