Modulation of *C. elegans* Touch Sensitivity Is Integrated at Multiple Levels

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Sensory systems can adapt to different environmental signals. Here we identify four conditions that modulate anterior touch sensitivity in *Caenorhabditis elegans* after several hours and demonstrate that such sensory modulation is integrated at multiple levels to produce a single output. Prolonged vibration involving integrin signaling directly sensitizes the touch receptor neurons (TRNs). In contrast, hypoxia, the dauer state, and high salt reduce touch sensitivity by preventing the release of long-range neuroregulators, including two insulin-like proteins. Integration of these latter inputs occurs at upstream neurohormonal cells and at the insulin signaling cascade within the TRNs. These signals and those from integrin signaling converge to modulate touch sensitivity by regulating AKT kinases and DAF-16/FOXO. Thus, activation of either the integrin or insulin pathways can compensate for defects in the other pathway. This modulatory system integrates conflicting signals from different modalities, and adapts touch sensitivity to both mechanical and non-mechanical conditions.

Key words: insulin signaling; integrin signaling; long-term sensitization; mechanosensation; sensory modulation

Introduction

Various conditions modulate sensory perception and can change behavioral responses to sensory stimuli. The human sense of smell, for example, is regulated by multiple hormones that reduce the olfactory attraction of food when individuals are not hungry (Palouzier-Paulignan et al., 2012). In addition the response of songbirds to songs depends on estradiol levels, which fluctuate seasonally (Maney and Pinaud, 2011), and gustatory chemosensation in the nematode *Caenorhabditis elegans* is enhanced during hypoxic conditions because additional neurons are recruited into the circuit (Pocock and Hobert, 2010). Sensory modulation can occur through direct synaptic connections (Fex, 1967), long-range neuropeptides (Palouzier-Paulignan et al., 2012), and hormones (Maney and Pinaud, 2011; Palouzier-Paulignan et al., 2012). Sensory cells can also self-modulate, e.g., the dynamic range of mammalian cone and rod cells changes in response to overall brightness (Fain et al., 2001).

Mechanosensation, in particular, can be modulated in multiple ways. Both hearing and touch sensitivity habituate to repeated

stimuli (Pinsker et al., 1970; Weber, 1970), and touch sensitivity sensitizes when it is paired with a second obnoxious stimulus (Pinsker et al., 1973). Both mechanosensory habituation and sensitization have been attributed to synaptic plasticity. A less studied form of sensitization, produced by sustained normal stimulation, affects mammalian hearing and touch (Kujawa and Liberman, 1999; Govindaraju et al., 2006). In addition, visual and auditory inputs can modify mechanosensory perception in humans (Hötting et al., 2003; Longo et al., 2011) and motor function modulates the sensation of stretch in crustaceans (Sillar and Skorupski, 1986). How multiple and conflicting signals integrate to modulate mechanosensation, however, is unclear.

In *C. elegans*, gentle touch is sensed by six touch receptor neurons (TRNs), which detect changes in applied force and adapt quickly to constant pressure (O'Hagan et al., 2005). Transduction occurs through the MEC-4 DEG/ENaC channel complex (O'Hagan et al., 2005). The TRN touch response habituates to repeated stimuli given over several minutes and sensitizes for a short time (~2 min) following a single strong stimulus (Rankin et al., 1990).

In this paper, we show that *C. elegans* mechanosensation is sensitized by sustained vibration or tapping and reduced under several stress conditions (high salt, low oxygen, or the dauer state). Unlike previously reported habituation and short-term sensitization (Rankin et al., 1990), however, the changes we report here occur on a longer timescale (hours rather than minutes) and only affect the anterior TRNs. These conditions signal through three different sets of neurons and two molecular pathways and integrate at multiple points along these pathways. We further demonstrate the behavioral and adaptive consequences of such modulation.

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Table 1. *C. elegans* strain list

	Genotype	Comments
N2	+	Wild-type
TU3842	<i>uls109</i> (pCW2.1, <i>mec-17p::gcamp3</i>) III; <i>him-5</i> (e1490) V	Control GCaMP3 strain
TU3851	<i>uls91</i> (<i>mec-4p::Chr2::yfp</i> , pCFJ104) III; <i>sid-1</i> (pk3321) <i>him-5</i> (e1490) V	Chr2 strains, for crosses
TU3849	<i>uls94</i> (<i>mec-4p::Chr2::yfp</i> , pCFJ104); <i>sid-1</i> (pk3321) <i>him-5</i> (e1490) V	
TU4274	<i>uls156</i> (<i>unc-112p::unc-112::gfp</i>)	
TU4382	<i>uEx885</i> (<i>pat-2p::gfp</i> , pCFJ90)	
TU3836	<i>pat-2</i> (ok2148); <i>uEx829</i> (<i>pat-2p::pat-2</i> , pCFJ90, <i>mec-3p::rfp</i>)	Rescued focal adhesion mutants for mosaic analyses
TU3837	<i>pat-2</i> (ok2148); <i>uEx830</i> (<i>pat-2p::pat-2</i> , pCFJ90, <i>mec-3p::rfp</i>)	
TU3689	<i>pat-3</i> (st564); <i>uEx826</i> (<i>pat-3p::pat-3</i> , pCFJ90, <i>mec-3p::rfp</i>)	
TU3834	<i>pat-6</i> (st561); <i>uEx827</i> (<i>pat-6p::pat-6</i> , pCFJ90, <i>mec-3p::rfp</i>)	
TU3687	<i>unc-112</i> (<i>gk1</i>); <i>uEx824</i> (<i>unc-112p::unc-112</i> , pCFJ90, <i>mec-3p::rfp</i>)	
TU3839	<i>unc-97</i> (ra115) <i>dpy-8</i> (e130); <i>uEx832</i> (<i>unc-97p::unc-97</i> , pCFJ90, <i>mec-3p::rfp</i>)	
TU4365	<i>uls91</i> (<i>mec-4p::Chr2::yfp</i> , pCFJ104) III	Control Chr2 strain
TU3850	<i>pat-2</i> (ok2148) III; <i>uls94</i> ; <i>uEx830</i>	TRN::Chr2 in focal adhesion mutants and <i>egl-19</i> , made by crossing TU3849 or TU3851 into each rescued strain and <i>egl-19</i>
TU3859	<i>pat-3</i> (st564) III; <i>uls94</i> ; <i>uEx826</i>	
TU3853	<i>uls91</i> III; <i>pat-6</i> (st561) IV; <i>uEx827</i>	
TU3854	<i>uls91</i> III; <i>unc-97</i> (ra115) <i>dpy-8</i> (e130) X; <i>uEx832</i>	
TU3852	<i>uls91</i> III; <i>unc-112</i> (<i>gk1</i>) V; <i>uEx824</i>	
TU3855	<i>uls91</i> III; <i>egl-19</i> (ad1013) IV; <i>sid-1</i> (pk3321) <i>him-5</i> (e1490) V	
NG155	<i>ina-1</i> (<i>gm144</i>) III	
NG39	<i>ina-1</i> (<i>gm39</i>) III	
TU4275	<i>uls109</i> III; <i>egl-19</i> (ad1006) IV	GCaMP3 in <i>egl-19</i>
TU3844	<i>pat-2</i> (ok2148) <i>uls109</i> III; <i>uEx829</i>	GCaMP3 in <i>pat-2</i>
TU4277	<i>uls109</i> III; <i>unc-112</i> (<i>gk1</i>); <i>uEx862</i> (<i>unc-112p::unc-112</i> , pCFJ90, <i>mec-3p::rfp</i>)	GCaMP3 in <i>unc-112</i>
JT9609	<i>pdck-1</i> (sa680) X	
RB759	<i>akt-1</i> (ok525) V	
VC204	<i>akt-2</i> (ok393) X	
TU4423	<i>akt-1</i> (ok525) V; <i>akt-2</i> (ok393) X	Maintained on <i>daf-16</i> RNAi
TU4383	<i>pdck-1</i> (sa680) X; <i>uEx874</i> (<i>mec-17p::unc-112::gfp</i> , <i>mec-17p::pat-6::gfp</i> , pCFJ90, <i>mec-17p::gfp</i>)	TRN::unc-112/pat-6 overexpression
TU4384	<i>akt-1</i> (ok525) V; <i>uEx874</i>	
CB1370	<i>daf-2</i> (e1370) III	
TU4276	<i>akt-1</i> (mg144) <i>unc-112</i> (<i>gk1</i>) V; <i>uEx862</i> (<i>unc-112p::unc-112</i> , pCFJ90, <i>mec-3p::rfp</i>)	<i>akt-1gf</i> <i>unc-112</i>
TU4279	<i>daf-16</i> (mgDf50) I; <i>unc-112</i> (<i>gk1</i>) V; <i>uEx862</i> (<i>unc-112p::unc-112</i> , pCFJ90, <i>mec-3p::rfp</i>)	<i>daf-16</i> ; <i>unc-112</i>
TU4280	<i>pat-2</i> (ok2148) <i>uls109</i> III; <i>akt-1</i> (mg144); <i>uEx829</i>	<i>akt-1gf</i> ; <i>pat-2</i>
GR1310	<i>akt-1</i> (mg144) V	
RB1679	<i>cav-1</i> (ok2089) IV	
TU45	<i>mec-4</i> (u45) X	<i>mec-4</i> ts allele
TU4282	<i>akt-1</i> (mg144) V; <i>mec-4</i> (u45) X	
TU4283	<i>daf-16</i> (mgDf50) I; <i>mec-4</i> (u45) X	
TU4385	<i>mec-4</i> (u45) X; <i>uEx874</i>	<i>mec-4</i> ts + TRN::unc-112/pat-6
GR1307	<i>daf-16</i> (mgDf50) I	
TU4289	<i>him-4</i> (e1267) <i>uls157</i> (<i>mec-3p::pdck-1gf</i> , pCFJ90, <i>mec-3p::rfp</i>) X	TRN::pdck-1gf in <i>him-4</i>
TU3595	<i>sid-1</i> (pk3321) <i>him-5</i> (e1490) V; <i>lin-15B</i> (n744) X; <i>uls72</i>	Neuronally enhanced RNAi (Calixto et al., 2010)
CB1267	<i>him-4</i> (e1267) X	
JK1438	<i>daf-2</i> (m65)/ <i>qC1 dpy-19</i> (e1259) <i>glp-1</i> (q339) III	
CY401	<i>sqt-1</i> (sc13) <i>age-1</i> (mg109)/ <i>mnc1 dpy-10</i> (e128) <i>unc-52</i> (e444) II	
RB712	<i>daf-18</i> (ok480) IV	
GR1318	<i>pdck-1</i> (mg142) X	<i>pdck-1</i> gain-of-function allele
DR1942	<i>daf-2</i> (e979) III	
DR1564	<i>daf-2</i> (m41) III	
PJ1146	<i>daf-2</i> (m41) III; <i>ccls55</i> V; <i>pdck-1</i> (mg142) X	
HT1890	<i>daf-16</i> (mgDf50) I; <i>daf-2</i> (e1370) III	
DR1309	<i>daf-16</i> (m26) I; <i>daf-2</i> (e1370) II	
TU4371	<i>daf-2</i> (m65)/ <i>qC1 dpy-19</i> (e1259) <i>glp-1</i> (q339) III; <i>uEx874</i> (<i>mec-17p::unc-112::gfp</i> , <i>mec-17p::pat-6::gfp</i> , pCFJ90, <i>mec-17p::gfp</i>)	overexpresses TRN::unc-112/pat-6 in <i>daf-2</i>
TU4372	<i>daf-2</i> (m65)/ <i>qC1 dpy-19</i> (e1259) <i>glp-1</i> (q339) III; <i>uEx875</i> (<i>mec-17p::unc-112::gfp</i> , <i>mec-17p::pat-6::gfp</i> , pCFJ90, <i>mec-17p::gfp</i>)	overexpresses TRN::unc-112/pat-6 in <i>daf-2</i>
TU4278	<i>unc-112</i> (<i>gk1</i>) V; <i>pdck-1</i> (mg142) X; <i>uEx862</i>	<i>unc-112</i> ; <i>pdck-1gf</i>
TU4281	<i>pat-2</i> (ok2148) <i>uls109</i> III; <i>pdck-1</i> (mg142); <i>uEx829</i>	<i>pat-2</i> ; <i>pdck-1gf</i>
FX2988 ^a	<i>ins-33</i> (tm2988) I	
TU3927	<i>uls126</i> (<i>ins-10p::ins-10(i)</i> , <i>ins-10p::rfp</i> , pCW2.1)	<i>ins-10(i)</i> strain
TU3929	<i>uls128</i> (<i>ins-10p::ins-10(i)</i> , <i>ins-10p::rfp</i> , pCW2.1)	<i>ins-10(i)</i> strain
TU4373	<i>uls128</i> ; <i>uEx880</i> (<i>ins-10p::ins-10rec</i> , pCFJ90, <i>ins-10p::gfp</i>)	<i>ins-10</i> with alternative codons rescuing <i>ins-10(i)</i>
FX3498 ^a	<i>ins-10</i> (tm3498) V	

(Table continues.)

Table 1. Continued

	Genotype	Comments
TU4374 ^a	<i>ins-10(tm3498) V; uEx876 (ins-10p::ins-10(+), pCFJ90, ins-10p::gfp)</i>	<i>ins-10(+)</i> rescuing <i>ins-10(tm3498)</i>
FX4990 ^a	<i>ins-22(tm4990) III</i>	
TU4375 ^a	<i>ins-22(tm4990) III; uEx877 (ins-22(+), pCFJ90, ins-22p::gfp)</i>	<i>ins-22</i> rescue
TU4368	<i>uls128; rde-1(ne219) V</i>	<i>ins-10(i)</i> with <i>rde-1</i>
TU4290	<i>uls109 III; uls126</i>	GCaMP3 in <i>ins-10(i)</i>
TU4291	<i>uls109 III; uls128</i>	GCaMP3 in <i>ins-10(i)</i>
TU4292	<i>akt-1(mg144) V; uls128</i>	<i>ins-10(i); akt-1gf</i>
TU4293	<i>pdck-1(mg142) X; uls128</i>	<i>ins-10(i); pdck-1gf</i>
TU4294	<i>uls157 (mec-3p::pdck-1gf, pCFJ90, mec-3p::rfp) X; uls128</i>	<i>ins-10(i); TRN::pdck-1gf</i>
TU4376	<i>uls128; uEx880(mec-17p::unc-112::gfp, mec-17p::pat-6::gfp, pCFJ90, mec-17p::gfp)</i>	<i>ins-10(i) + TRN::unc-112/pat-6</i>
TU4377	<i>uls128; uEx881(mec-17p::unc-112::gfp, mec-17p::pat-6::gfp, pCFJ90, mec-17p::gfp)</i>	<i>ins-10(i) + TRN::unc-112/pat-6</i>
TU4378	<i>uEx878 (ins-10p::gfp, pCFJ90)</i>	
TU4379	<i>uEx879 (ins-10p::rfp, pCW2.1)</i>	
TU4296	<i>uls157 (mec-3p::pdck-1gf, pCFJ90, mec-3p::rfp) X</i>	
TU4369	<i>uls162 (ins-22p::gfp, pCFJ104)</i>	<i>ins-22p::gfp</i>
OH3679	<i>che-1(ot151) otIs114 I</i>	
TU4297	<i>che-1(ot151) otIs114 I; uls157 (mec-3p::pdck-1gf, pCFJ90, mec-3p::rfp) X</i>	<i>che-1; TRN::pdck-1gf</i>
TU4380	<i>uEx874(mec-17p::unc-112::gfp, mec-17p::pat-6::gfp, pCFJ90, mec-17p::gfp)</i>	Wild type overexpressing <i>TRN::unc-112/pat-6</i>
TU4381	<i>uEx875(mec-17p::unc-112::gfp, mec-17p::pat-6::gfp, pCFJ90, mec-17p::gfp)</i>	Wild type overexpressing <i>TRN::unc-112/pat-6</i>
RB2059 ^b	<i>ins-28(ok2722)</i>	
TU4295	<i>uls152(mec-3p::rfp)</i>	<i>mec-3p::rfp</i>
TU4473	<i>akt-1(ok525) V; uEx891(mec-17p::gfp, mec-17p::akt-1a, pCFJ90)</i>	<i>mec-17p::akt-1a</i> rescue
TU4474	<i>akt-1(ok525) V; uEx892(mec-17p::gfp, mec-17p::akt-1a, pCFJ90)</i>	<i>mec-17p::akt-1a</i> rescue
TU4475	<i>akt-1(ok525) V; uEx893(mec-17p::gfp, mec-17p::akt-1a, pCFJ90)</i>	<i>mec-17p::akt-1a</i> rescue
TU4476	<i>daf-16 (mgDf50) I; uEx894(mec-17p::gfp, mec-3p::daf-16a, pCFJ90)</i>	<i>mec-17p::daf-16a</i> rescue
TU4477	<i>daf-16 (mgDf50) I; uEx895(mec-17p::gfp, mec-3p::daf-16a, pCFJ90)</i>	<i>mec-17p::daf-16a</i> rescue
TU4478	<i>daf-16 (mgDf50) I; uEx896(mec-17p::gfp, mec-3p::daf-16a, pCFJ90)</i>	<i>mec-17p::daf-16a</i> rescue
TU4479	<i>daf-16 (mgDf50) I; uEx897(mec-17p::gfp, mec-3p::daf-16b, pCFJ90)</i>	<i>mec-17p::daf-16b</i> rescue
TU4480	<i>daf-16 (mgDf50) I; uEx898(mec-17p::gfp, mec-3p::daf-16b, pCFJ90)</i>	<i>mec-17p::daf-16b</i> rescue
TU4481	<i>daf-16 (mgDf50) I; uEx899(mec-17p::gfp, mec-3p::daf-16b, pCFJ90)</i>	<i>mec-17p::daf-16b</i> rescue
TU4523	<i>uls113(mec-3p::gcampp3, mec-3p::rfp) V; uls135</i>	GCaMP3 in interneurons
TU4521	<i>uls113 V; uls135; mec-4(u253) X</i>	<i>mec-4</i> control for GCaMP3 in interneurons

^aAll *tm* alleles were obtained from the Japan National Bioresource Project for the nematode *C. elegans*. ^bRB2059, carrying *ins-28(ok2722)*, was initially touch insensitive, but animals responded to touch after outcrossing, suggesting that the touch insensitivity was a background effect. Chr2, channelrhodopsin-2.

Materials and Methods

Strains and treatments. *C. elegans* strains (Table 1) were maintained at 15°C or 20°C as described previously (Brenner, 1974). Hermaphrodites were used for all experiments. Temperature-sensitive strains were maintained at 15°C and transferred to 25°C for one generation before testing. Most of the strains came from the Caenorhabditis Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). *pat-2(ok2148) III*, *unc-112(gk1) V*, *akt-1(ok525) V*, and *akt-2(ok393) X* were generated by the International *C. elegans* Gene Knock-out Consortium (<http://www.celeganskoconsortium.omrf.org>).

To study the importance of insulin-like peptides, we examined the effects of mutant alleles for all *ins* genes except for *ins-19*, *ins-20*, *ins-21*, *ins-24*, *ins-32*, *ins-36*, and *ins-39*, which we tested using feeding RNAi. Because the putative deletion allele, *ins-10(tm3498)*, produced detectable *ins-10* mRNA (J. Alcedo, personal communication; data not shown), we used animals carrying a construct expressing an *ins-10* hairpin from the *ins-10* promoter to induce RNAi against *ins-10* [*ins-10(i)*].

The hypoxic treatment was performed as described previously (Pock and Hobert, 2010).

Dauers were obtained by growing mixed-stage animals at 25°C until they depleted the bacteria on the plate (usually after one generation) and then further starved for 5 d. The dauer animals were then either tested for touch sensitivity directly, or separated from non-dauer animals by 1% SDS treatment for 30 min for the chemotaxis assay. Because dauers were obtained by starvation, all assays involving them used starved L3 larvae as controls.

For high-salt treatments, the animals were grown for one generation on NGM plates (Brenner, 1974) supplemented with an additional 180 mM NaCl to produce a final NaCl concentration of 230 mM. For amiloride treatment, *bus-17* animals, which are more permeable to drugs

as adults (Gravato-Nobre et al., 2005; Bounoutas et al., 2009), were transferred to 230 mM NaCl plates supplemented with 2 mM amiloride hydrochloride hydrate (Sigma-Aldrich) for at least 2 h, vibrated for 2 h, and recovered for 1 h on 230 mM NaCl plates without amiloride before testing. For channelrhodopsin-2 activation during culture, TU3851 animals were grown on 230 mM NaCl plates supplemented with 500 μ M all-trans-retinal (Sigma-Aldrich) until the L4 stage, wrapped in aluminum foil, and illuminated by an LXML-PB01–0040 Luxeon Rebel 470 nm LED (Philips Lighting) at 3.4 V and 700 mA continuously for 2 h, then rested for 30–40 min before testing.

For all vibrations, we played .wav files containing the appropriate waveforms through 3.5" (for culture) or 5.25" (for observation) dual cone speakers amplified by a digital amplifier. The outer cone of the speaker was removed, leaving only the inner cone as a stand for the plates. A lid of a 3.5 cm Petri dish (BD Falcon 351008) was glued to the top of the cone facing up, and the bottom of the dish was glued to a test plate, also facing up. The test plate was then put on the speaker by fitting the dish bottom to the lid, which produced enough friction to hold the plate in place. The .wav files were written with MATLAB (MathWorks). The average acceleration of the vibration was quantified using a DE-ACCM6G accelerometer (Dimension Engineering) on top of the plates. To observe the plates under vibration, we illuminated the plates on one side from underneath and recorded at a resolution of 1920 $\times$ 1080 with a Canon EOS 60D camera (Canon USA) with an Olympus OM 50 mm f/2 Macro lens (Olympus Imaging America).

For prolonged vibration, the animals were vibrated with 50 Hz square waves for 24 h with an average acceleration of 1.5 $\times$ g, and recovered from habituation for 30–40 min before testing except for calcium imaging experiments and the habituation recovery assay, in which the animals were tested after resting for times indicated in the following sections.

Constructs. The full list of primers and plasmids used is available upon request. Most constructs were made using the three-fragment Gateway system (Life Technologies) according to the manufacturer's instructions using the pDONRP4-P1r, pDONR221, pDONRP2r-P3, and pDEST4-R3 vectors unless noted otherwise.

Behavioral assays. All behavioral assays were performed blind with regard to genotype. At least three biologically independent tests were performed for each strain. For mosaic analyses and laser ablations, the average and SEM of the response of animals were reported. For vibration-induced behaviors, the number of animals from independent tests were summed together and reported. For all other tests, the average and SEM of the means from the independent tests were reported.

The touch response was assayed by gently touching the side of the animal (see below for validations). Each animal was tested five times anteriorly and five times posteriorly unless noted (Hobert et al., 1999). Ten to 20 animals were tested each time, and each strain was tested at least three times independently.

To test activation by channelrhodopsin-2 (Nagel et al., 2005), bleached eggs were put on plates seeded with 100 μ l *Escherichia coli* OP50 in LB broth at the stationary phase ($OD_{595} = 0.45$; Brenner, 1974) with 500 μ M all *trans*-retinal (Sigma-Aldrich). Animals grown for 5 d at 15°C were tested as young adults under a Leica M12 stereoscope modified with a M2 Bio Quad fluorescent attachment (Kramer Scientific) and an EXFO X-cite 120 metal halide light source (EXFO). We then exposed each worm to a <0.5 s flash of blue light coming from the GFP filter under a 20 $\times$ f = 0.6 objective and 12 $\times$ zoom aimed at the ALM cell body. A fast backward movement within the 0.5 s exposure was counted as a positive response. Each animal was tested three times instead of five times because considerable habituation occurs after three tests (Nagel et al., 2005). The vibration response assays (Fig. 10A) were performed using 50 Hz, 0.5 s square pulses with an average plate acceleration of 1.7 $\times$ g. Each plate was tested twice with at least 10 min in between, and the data from independent plates were pooled for analysis.

To test the response to nonlocalized vibration, animals were stimulated with a 0.5 s 0 dB 50 Hz square wave pulse vibration reaching an average acceleration of 1.7 $\times$ g. We videotaped the experiments and then counted the number of animals that backed within 0.5 s of the stimulus on the video and measured the turning angle of the animals that backed. The animals that did not back were given a turning angle of 0° and the average turning angle was calculated for all animals.

To test touch sensitivity during background vibration (Fig. 10D), we inserted a 0.5 s, 0 dB 50 Hz square wave pulse in a background of −4 dB 50 Hz square wave vibration, reaching average accelerations of 1.7 and 0.7 $\times$ g in the plates, respectively. We videotaped the animals and counted the number of animals that backed within 0.5 s of the stimulus on the videos.

To test recovery from habituation (Fig. 10E,F), we tested animals at various times after cessation of vibration with a sustained 50 Hz square wave with an average acceleration of 1 $\times$ g. Responses of animals were pooled from multiple plates.

The chemotaxis assay with tapping was performed using a mechanical tapper driven by a magnetic relay as described previously (Rankin et al., 1990). Animals were then allowed to move toward a spot of diacetyl for a fixed amount of time. The ratio of the chemotaxis efficiency index (CEI; percentage of animals that had reached the spot) with or without tapping was then calculated, and the means and SEMs of the ratios were reported.

Touch test validation. Originally, the touch assay was done by stroking a thin hair (eyebrow hair) across the animal (Chalfie and Sulston, 1981). However, by only touching the side of the animal, we were able to detect more subtle changes in touch sensitivity. For *akt-1(ok525)* animals, five anterior touches produced on average 4.4 ± 0.2 responses (mean $\pm$ SEM of responses of individual animals, $n = 20$) by stroking a thin hair across the animal, and 1.7 ± 0.2 ($n = 19$) by touching the side of the animal. Both methods produced similar scores for wild-type animals (4.9 ± 0.1 for the original method and 4.6 ± 0.1 for side touch, $n = 16$ and $n = 18$, respectively).

Feeding RNAi. Feeding RNAi was performed as described previously (Calixto et al., 2010). All RNAi bacteria used were from the Ahringer

Table 2. Integrin signaling genes affecting mechanosensation

Focal adhesion components	
<i>unc-112^a</i>	Mig-2 like
<i>pat-2^a</i>	α -Integrin
<i>unc-97^a</i>	PINCH
<i>pat-4^a</i>	ILK
<i>pxl-1</i>	Paxillin
<i>pat-6^a</i>	Actopaxin
<i>nck-1</i>	NCK adapter protein 2
<i>Y71G12B0.11^a</i>	Talin
<i>tag-224</i>	Ortholog of LMCD1 and TESTIN
Rho GTPase related	
<i>rga-5</i>	RHO-GTPase activating protein
<i>let-502</i>	Rho-binding ser/thr kinase ortholog (ROCK)
<i>unc-103</i>	ERG-like K ⁺ channel, activated by rho
<i>unc-73</i>	Rho-GEF for <i>ced-10</i> , <i>mig-2</i> , <i>rho-1</i>
<i>ced-2</i>	SH2/3 adapter protein
<i>pkc-1</i>	Ortholog to protein kinase C ϵ
<i>Y106G6H0.14</i>	Homolog of Yeast BEM1, which binds Cdc24p
Insulin signaling pathway	
<i>daf-2</i>	Insulin receptor
<i>age-1</i>	PI3k p110 catalytic subunit
<i>pdh-1</i>	3-Phosphoinositide-dependent kinase 1 ortholog
Ras/MAP kinase pathway	
<i>arf-1.1</i>	ADP-ribosylation factor, Ras superfamily GTPase
<i>let-60</i>	K-RAS
<i>mpk-1</i>	MAPK
<i>let-92</i>	PP2A catalytic subunit
<i>sos-1</i>	Son of sevenless, RAS-GEF
Cytoskeleton	
<i>F42H10.3</i>	Yeast homolog binds Arp2/3
<i>tba-1</i>	α -Tubulin
<i>tsp-8</i>	Ortholog of KANGAL 1

Twenty-nine of 87 conserved integrin signal genes (Zaidel-Bar, 2009) affected touch sensitivity through feeding RNAi. Two of the candidates, *cav-1* and *daf-18*, were subsequently identified as false positives. The remaining genes are listed here each followed by a concise description. ^aGenes known to affect touch sensitivity (Calixto et al., 2010) and were included as positive controls.

library (Source Bioscience) except for *ins-20* and *ins-36*, which were made in L4440 as described. Except for the feeding RNAi screen, we examined at least three independent plates at the same time.

For the feeding RNAi screen, we scored genes that caused touch insensitivity in three of four tests as positive. As a control we included the seven dense body genes found in our initial study (Calixto et al., 2010) in our list of 87 candidate genes, and we were able to identify six of them blindly (the remaining gene, *pat-3*, produced lethality in TU3595). RNAi for 23 of the remaining 80 genes reduced touch insensitivity (Table 2). Based on known functions or homology, the 23 genes included additional focal adhesion genes, genes in the Ras/MAPK pathway, insulin signaling pathway, Rho-GTPase-related genes, and cytoskeleton related genes. *unc-73*, which encodes a guanine exchange factor for several Rho-GTPases, and *mec-12*, which is a secondary target for *tba-1* RNAi, were previously reported to affect TRN functions and/or development (Hedgecock et al., 1987; Chalfie and Thomson, 1982).

For a single test, we estimated the false positive rate to be <20% from a larger screen for ~1000 genes. Therefore, the false positive rate of the screen was estimated to be $C_4^1 \times 0.2^3 \times 0.8 + 0.2^4 = 2.7\%$, corresponding to $2.7\% \times 80 = 2$ false positives out of the 23 genes obtained from the screen (7 genes from the list of 87 genes did not have RNAi bacteria, and were not tested). Subsequent tests of all identified insulin and Ras/MAPK pathway genes using mutant alleles identified *cav-1* and *daf-18* as false positives in these two pathways.

Mosaic analysis. All tests on focal adhesion mutants were done on mosaic animals, except for RNAi treatments as indicated. Rescued *pat-2*, *pat-3*, *pat-6*, *unc-97*, and *unc-112* strains all have *mec-3p::rff* in the extra-chromosomal rescuing array as a TRN marker. Animals were grown to the L4 stage and observed under a Leica M12 stereoscope modified with a M2 Bio Quad fluorescent attachment (Kramer Scientific) and an EXFO X-cite 120 metal halide light source. Animals lacking RFP in ALM and

AVM cells, the two PLM cells, or having RFP in all six TRNs (controls) were selected and scored blindly for both anterior and posterior touch sensitivity. The anterior touch response of animals that had lost the rescuing array in the anterior TRNs and the posterior touch response of animals that had lost the rescuing array in the posterior TRNs were compared with both the anterior and posterior touch response of the controls. For calcium imaging experiments, the absence of RFP in the ALM cells was additionally confirmed under a 40×0.95 lens. For all experiments involving mosaic animals, at least two independent tests were done with the data pooled for analysis.

Laser ablations. Laser ablations were performed as described previously (Tsalik and Hobert, 2003). The M4 and I5 cells were labeled with GFP and ablated in L4 larvae. All animals were anesthetized with 30 mM sodium azide. Control animals were left in sodium azide on a slide for the same time as ablated animals, and mock-ablated animals had adjacent pharyngeal neurons ablated instead of M4 and I5. After 24 h, the ablation was confirmed by the lack of GFP recovery in the M4 and I5 cells. The confirmed animals were then tested for touch sensitivity in blind tests. Ablating the M4 neuron prevented feeding, so the ablated animals were compared with mock ablated animals that were starved for 24 h.

Calcium imaging. Calcium imaging was performed as described previously (Suzuki et al., 2003) using GCaMP3 with minor modifications on a Zeiss Observer Z1 microscope with a Photometrics Evolve 512 camera (Photometrics). Each animal was glued on its ventral surface using Dermabond (Ethicon) to a 4% agarose pad in M9 buffer on a 24×60 mm No. 1 coverslip and covered with 100 μ l M9 buffer. We mounted the coverslip on a rotating stage on a Zeiss Observer Z1 microscope equipped with an Eppendorf TransferMan NK2 micromanipulator (Eppendorf North America). A glass probe with a round tip with a diameter of 22 μ m driven by a two-layer piezoelectric rectangular bending actuator (Piezo Systems) was mounted on the micromanipulator, and carefully placed next to the animal without pressing it and parallel to the animal. The piezo lever was driven by 250 ms square waves from a 33221A waveform generator (Agilent Technologies) through a Piezo Linear Amplifier (Piezo Systems). The square waves cause the probe to bend sideways in the x - y plane, thus pressing the animal. During the recording, we illuminated the animal with a 470 nm LED from Colibri 2 (Carl Zeiss Microscopy) at 10% intensity through a GFP filter cube, and recorded the images through a Zeiss Apochromat 40×0.95 lens with a Photometrics Evolve 512 camera at ~ 10 fps and analyzed with AxioVision (Carl Zeiss Microscopy).

Only late L4 larvae or young adult animals were used for calcium imaging in the TRNs, although L3 larvae had similar touch sensitivity and calcium response (data not shown). In a typical experiment, we touched an animal with a piezo probe and varied the stimulus by increasing the voltage from 0.01 to 1.6 V, once or twice at each voltage, and recorded the calcium signal. Each stimulus was given when calcium re-

Figure 1. Sensitization to touch by vibration. **A**, Anterior (red) and posterior (blue) response (mean $\pm$ SEM) of wild-type to touch after sustained vibration for the indicated time. $P < 0.005$ comparing responses with 0 h (*) or 1.5 h (**). $N \geq 3$ for each time point. For all figures, N represents the number of independent sets of animals tested, each with at least 10 animals, and n represents the number of animals. **B–D**, Anterior touch sensitivity (mean $\pm$ SEM) in animals vibrated at various frequencies, strengths, and times. The enhancement of touch sensitivity by prolonged vibration was greatest when animals were (**B**) vibrated for more than 2 h at (**C**) a frequency of 50 Hz with (**D**) an average acceleration $> 1\times g$. Values for anterior sensitivity are given as mean $\pm$ SEM. The data for no vibration (—) and 2 h, 50 Hz, $1.5\times g$ vibration from all the experiments were pooled and used for all three figures. Optima of the other parameters were used when testing a particular parameter; * $p < 0.02$, ** $p < 0.005$, *** $p < 0.001$, $N \geq 3$ for all conditions tested. **E**, Normalized calcium responses of control (black), vibrated (red) animals, and animals recovered from vibration (blue) and their corresponding Boltzmann fits; $n \geq 6$ for all strains. Error bars represent SEM of responses at each displacement. **F**, Mean $\pm$ SEM of the D_{50} and k values of the indicated cells in wild-type animals. **G**, Sample calcium responses (blue) from control (top) and vibrated (bottom) animals. The GCaMP3 fluorescence levels are shown with arbitrary units (au). The displacement of each stimulus (black cross, in micrometers) is marked at each peak. Arrows indicate calcium peaks without stimuli. **H–K**, Statistics of calcium responses in AVA and AVD neurons with (white, V) or without (black, C) sustained vibration. Maximum fluorescence changes (**H**) were shown instead of $\Delta F/F_0$ because baseline GCaMP3 fluorescence in the AVA neurons was reduced after vibration (**J**), complicating the interpretation of $\Delta F/F_0$. This reduction of baseline fluorescence was likely due to a change in the baseline calcium level because antibody staining against GCaMP3 in vibrated animals showed no change in the amount of GCaMP3 expressed (**K**). D_{10} was shown (**I**) instead of D_{50} because estimation of the D_{10} values are less sensitive to the fast habituation in these cells.

sponse had reached the baseline or had plateaued. The timing between two stimuli could thus be different, but such timing differences did not change the response over the small number of trials in each experiment (data not shown). Calcium responses (after background subtraction) at different displacements were normalized to the maximum calcium response for each animal. All calcium responses from multiple animals of the same genotype and treatment were then pooled together and fitted

with a Boltzmann equation using Microsoft Excel in the form of the following:

$$R = \frac{1}{1 + e^{k(D-D_{50})}}$$

where R is the normalized calcium response, and D is the probe displacement. Additionally, calcium responses from the same animal were fitted with a Boltzmann equation to calculate D_{50} , the slope factor k , and maximum calcium response for each animal tested, and their means and SEMs were used for evaluating statistical significance of the changes in D_{50} , k , and maximum calcium response across different strains. The D_{50} and k obtained by fitting all data points for a particular strain showed only small discrepancies from the mean D_{50} and k calculated from individual animals and falls within 1 SEM from the mean D_{50} and k values. Calcium imaging in the interneurons was performed similarly except that only adult animals were used. Animals were stimulated every 20 s once a response was observed to minimize the effect of habituation. Each animal was tested once at each voltage because of stronger habituation than the signals in the TRNs. The actual displacement of the probe at a particular voltage was measured using DIC images from multiple animals that were acquired after the calcium recordings were finished. The displacements of the probe were essentially identical (within 10%) across individual animals and genotypes.

Calcium imaging of TRNs cultured on coverslips (Topalidou and Chalfie, 2011) was performed according to Suzuki et al. (2003). *pat-2* or *unc-112* ALM cells were selected by finding GCaMP3-positive and RFP-negative cells with one long process only. ALM cells from other strains were selected by finding GCaMP3-positive cells with one long process only. Potassium depolarization was performed according to Suzuki et al. (2003): cells were perfused using an extracellular saline (145 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 10 mM D-glucose, pH 7.2, and adjusted to 340 mOsm with sucrose) and depolarized with extracellular saline with 110 mM KCl and 40 mM NaCl. The maximum calcium change within five seconds from the initiation of the response was measured.

Scoring of TRN morphological defects. A TRN process was scored as ensheathment defective if it was close to the muscle in adults for more than half its length when examined under 20× magnification. For RNAi-treated animals (Fig. 3F), an ALM process was scored as attachment defective if the ALM cell body was squeezed into a half-circle shape (an indication of its adjacency to muscle) instead of the normal raindrop shape. We scored an ALM cell as migration defective when the cell body was anterior either to the mid-point between terminal bulb of the pharynx and the vulva, or to the AVM cell body. P values were calculated using Fisher's exact test.

Imaging and single-molecule mRNA FISH. All images were taken on a Zeiss Observer Z1 microscope with a Photometrics CoolSnap HQ² camera. We performed single-molecule mRNA FISH as described previously (Topalidou et al., 2011). The numbers of transcripts per cell were counted manually. Three independent tests were performed and the results were pooled.

Statistics. Results from touch assays, channelrhodopsin-2 assays, and antibody staining were compared using Student's t test. Categorical data from vibration assays, attachment defects, and migration defects were compared using Fisher's exact test. smFISH data were compared using Mann–Whitney's U test. All p values reported are after Bonferroni corrections.

Results

Multiple conditions alter touch sensitivity

The response to gentle touch in *C. elegans* habituates with repeated stimuli (Chalfie and Sulston, 1981; Rankin et al., 1990). We induced habituation by placing plates of animals on speakers producing a 50 Hz vibration ($1.5 \times g$ average acceleration), subjecting them to varying periods of stimulation, and testing their touch sensitivity 5 min after the end of stimulation. The anterior and posterior responses habituated with different time courses

Figure 2. Modulation of touch sensitivity by stress and vibration. **A**, Anterior touch sensitivity (mean ± SEM) in control animals (–), dauer larvae (dauer), or animals subjected to sustained vibration (vib), tapping (tap), or sustained channelrhodopsin-2 activation (ChR2). Additionally animals were treated with 230 mM NaCl (NaCl), 230 mM NaCl with 2 mM amiloride (NaCl + Amil), or 1% O₂ (hyp). The amiloride-treated animal contained a *bus-17* mutation; ** $p < 0.005$ compared with control, and * $p < 0.05$, *** $p < 0.005$ compared with the respective control under each condition, $N \geq 3$ for all strains. **B**, Anterior touch response (mean ± SEM) of wild-type animals at the noted time points after they were transferred from 50 mM NaCl to 230 mM NaCl (blue) or from 230 mM NaCl to 50 mM NaCl (red). $N \geq 4$ for all time points; * $p < 0.01$, ** $p < 0.005$. **C**, Anterior touch response (mean ± SEM) of wild-type animals grown on NGM plates with the specified concentration of NaCl or with 50 mM NaCl and 380 mM sucrose; $N = 3$, * $p < 0.05$. **D**, Anterior touch response (mean ± SEM) of wild-type animals grown on NGM plates supplemented with 180 mM of the specified salts or 380 mM sucrose; $N \geq 6$, * $p < 0.05$, ** $p < 0.005$.

(Fig. 1A). Vibration caused posterior touch sensitivity to be increasingly lost over 24 h. In contrast, although animals lost touch sensitivity to anterior touch if stimulated for less than 2 h, they were sensitized back to nearly wild-type levels after 2 h (Fig. 1A). Longer period of vibration did not further increase touch sensitivity. A similar increase in posterior touch sensitivity after habituation was not seen (Fig. 1A). The greatest sensitization occurred when animals were vibrated for more than 2 h at a frequency of 50 Hz and with average acceleration $\geq 1 \times g$ (Fig. 1B–D). We further substantiated these findings using GCaMP3 imaging: vibration reversibly reduced the displacement required for 50% activation (D_{50}) in the ALM neurons ($p < 0.02$), but not in the PLM neurons (Fig. 1E,F). In addition, ALM neurons of treated animals produced spontaneous calcium spikes without a mechanical stimulus; no such spikes were observed in untreated animals (Fig. 1G). The appearance of the spikes suggests that the vibrated animals detected either background vibrations from the microscope stage or stimuli induced by the contraction of body wall muscles. Thus, prolonged vibration specifically increased the touch sensitivity of ALM neurons and counteracted habituation to maintain a normal response.

In addition to increased sensitivity of the TRNs, the sensitized behavioral response could also be caused by changes in the downstream circuits. The anterior ALM neurons synapse onto the AVD interneurons neurons, which synapse onto the AVA interneurons; these interneurons drive backward movement

(Chalfie et al., 1985). Calcium imaging in these cells revealed a similar reduction in the displacement needed to reproducibly elicit a calcium response as in ALM neurons, and no increase in the maximum amplitude of calcium response by saturated stimulation except for a reduced baseline level in the AVA cells following sustained vibration (Fig. 1H–K). Thus, synaptic changes are unlikely to contribute to sensitization.

In contrast to the stimulating effect of prolonged vibration, several stress conditions reduced the anterior touch response (Fig. 2A). Wild-type animals grown on high salt (230 mM NaCl, but not 380 mM sucrose + 50 mM NaCl; Fig. 2B–D) or under hypoxic conditions (1% O₂) or animals that had developed into dauer larvae had reduced anterior touch sensitivity (Fig. 2A). The touch insensitivity, however, was restored when the animals were subjected to several hours of sustained vibration or repeated tapping (1 Hz; Fig. 2A). To test if activation of the MEC-4 mechanotransduction channel was needed for sensitization by vibration, we blocked the activation of MEC-4 mechanotransduction channels by treating *bus-17* animals with amiloride during prolonged vibration and tested the animals after recovery from amiloride (the amiloride treatment effectively reduced anterior touch sensitivity of *bus-17* animals from wild-type level to 0.3 ± 0.1 responses out of five touches, $N = 3$). Prolonged vibration in the presence of amiloride restored touch sensitivity (Fig. 2A), indicating that activation of the MEC-4 channel is not required for sensitization. In addition sensitization was not induced by continuous activation of the TRNs through channelrhodopsin-2 (Nagel et al., 2005; Fig. 2A), suggesting that activation of the TRNs without mechanical stimulation is not sufficient to induce sensitization. These data suggest that vibration-induced sensitization requires a different force sensor.

Integrin signaling modulates mechanical sensitivity

Integrins and focal adhesion proteins sense cellular stretching forces and induce long-term cellular changes (Roca-Cusachs et al., 2012). Even though the focal adhesion proteins are expressed in mechanosensory cells, including the TRNs and vertebrate hair cells (Fig. 3A), they are unlikely to mediate the rapid, submillisecond mechanosensory transduction seen in neurons (Chalfie, 2009) because integrin-mediated mechanosensation is usually slow (tens of seconds; Vogel and Sheetz, 2009). Nonetheless, a role of these proteins in mechanosensation is suggested by the loss of touch sensitivity that occurs when focal adhesion proteins are reduced using cell-

Figure 3. Effect of focal adhesion mutants on TRN function. **A**, The expression of *pat-2p::gfp* or *unc-112p::unc-112::gfp* in ALM, AVM, and PLM TRNs of animals fed bacteria containing dsRNA against *gfp*. Because of the inefficient systemic RNAi in the nervous system, *gfp* expression in the body-wall muscle, but not in the neurons, is reduced, allowing visualization of GFP in the TRNs. Other focal adhesion proteins have been shown to be expressed in the TRNs and the vertebrate hair cells (Gettner et al., 1995; Hobert et al., 1999; Littlewood Evans and Muller, 2000; Mackinnon et al., 2002; Lin et al., 2003). **B**, Anterior (A, black) and posterior (P, white) responses (mean $\pm$ SEM of responses of individual animals) of mosaic animals with (+) or without (–) the rescuing arrays of the indicated genes in the TRNs. For the focal adhesion genes, $n > 20$ for anterior responses, and $n > 15$ for posterior responses. For *mec-4*, $n > 10$. For all anterior responses, $p < 0.005$ between (+) and (–) animals. **C**, Response (mean $\pm$ SEM of responses of individual animals) to three light pulses from focal adhesion mosaic animals lacking the rescuing arrays of the indicated genes in the TRNs but expressing channelrhodopsin-2 (ChR2) in the TRNs and from *egl-19* animals expressing channelrhodopsin-2 in the TRNs; $n \geq 20$ for all strains tested, $p < 0.05$ compared with the wild-type. **D**, ALM processes (green) and the body-wall muscle (red) in *unc-112* mosaic animals with or without *unc-112* in the ALM cells. The ALM process is normally ensheathed by the hypodermis, which separates the ALM process from the body-wall muscle in adults. ALM processes that are not ensheathed appear adjacent to the body-wall muscle. The ALM cell body would also be pressed against the body-wall muscle, assuming a half-circle shape instead of the normal raindrop shape. The PLM processes had similar defects, yet retained the touch sensitivity, suggesting that the ensheathment defect alone did not cause touch insensitivity. **E**, Fractions of TRNs showing ensheathment defects (black) or migration defects (white); $n > 15$ for ensheathment data and $n \geq 20$ for migration data; $p < 0.002$ compared with wild-type. The migration defect seen in *pat-3* animals could not account for the reduced touch sensitivity either, because animals lacking the second *C. elegans* α -integrin gene, *ina-1*, were touch sensitive despite having similar migration defects (Baum and Garriga, 1997; data not shown). **F**, Fractions of ensheathment-defective ALM cells in TU3595 animals fed with neuron-enhanced RNAi against *gfp*, *mec-4*, *unc-112*, *pat-6*, or *mec-1*. See Materials and Methods for detailed scoring standard. $N \geq 3$, $p < 0.05$ compared with *gfp* RNAi control. The RNAi-treated animals, except for the *gfp* control, had reduced touch sensitivity, suggesting that the ensheathment defect cannot solely account for the reduced touch sensitivity.

specific RNAi or partial loss-of-function mutations (Hobert et al., 1999; Calixto et al., 2010). We wondered, however, whether the focal adhesion proteins contributed to force sensing for sensitization.

To circumvent the embryonic lethality associated with the complete loss of the focal adhesion genes, we tested touch sensitivity in animals that were mosaic for null alleles (Fig. 3B). Loss of *pat-2*/ α -integrin, *pat-3*/ β -integrin, *unc-97*/PINCH, *unc-112*/Mig-2, or *pat-6*/actopaxin in the anterior TRNs (the two ALM

cells and the AVM cell) yielded animals that were partially insensitive to anterior touch. (Loss of these genes in the posterior TRNs, the two PLM neurons, did not reduce touch sensitivity.) The partial loss of anterior sensitivity was not caused by general cellular dysfunction or changes in downstream circuits, because ALM neurons expressing channelrhodopsin-2 (Nagel et al., 2005) and containing or lacking the rescuing arrays of the focal adhesion genes were equally capable of inducing backing when activated by blue light (Fig. 3C). In contrast, animals with reduced activity of the L-type voltage-gated calcium channel EGL-19 responded less to blue light activation of channelrhodopsin-2. These data indicate that the focal adhesion proteins do not disrupt the channelrhodopsin-2 response and are likely to affect an early stage of mechanosensation.

The TRNs lacking rescuing arrays had normal morphology except for minor migration defects seen in ALM cells lacking *pat-3* and the failure of the ALM and PLM processes to separate from the muscle that normally occurs after the TRN processes are ensheathed by the surrounding hypodermis in adults (Fig. 3D, E; Gettner et al., 1995). A similar role for integrins in the anchoring of neuronal processes to epidermal cells occurs in *Drosophila* (Kim et al., 2012). These defects, however, cannot solely account for the reduced touch sensitivity (Fig. 3F).

Focal adhesion proteins did, however, modulate the sensitivity of the ALM neurons as measured by an increase in D_{50} in *pat-2* ($2.6 \pm 0.2 \mu\text{m}$, mean $\pm$ SEM, $n = 11$, $p < 0.0001$, $k = 1.2 \pm 0.1$) and *unc-112* mutants ($1.7 \pm 0.2 \mu\text{m}$, $n = 6$, $p < 0.005$, $k = 1.6 \pm 0.1$) compared with wild-type animals ($1.1 \pm 0.1 \mu\text{m}$, $n = 26$, $k = 3.3 \pm 0.3$; Fig. 4A). Loss of *pat-2*, but not *unc-112*, also reduced maximum calcium response both *in vivo* and in cultured cells depolarized by high potassium (Fig. 4B, C), suggesting an additional role in regulating calcium influx.

Integrins mediate vibration-induced sensitization through AKT and FOXO

Using neuron-enhanced feeding RNAi, we screened conserved signaling genes whose products may interact with or affect integrin signaling (Zaidel-Bar, 2009) and found that insulin signaling and several other signaling pathways were required for optimal touch sensitivity (Table 2). Insulin signaling prevents dauer formation in *C. elegans* through the successive activation of the DAF-2/insulin receptor, the AGE-1/PI3 kinase, the PDK-1/3-phosphoinositide-dependent kinase, and the redundantly acting AKT-1 and AKT-2/AKT, which inhibit the activity of the DAF-16/FOXO transcription factor (Hu, 2007). Mutations in *daf-2*, *age-1*, *pdk-1*, and *akt-1* reduced anterior touch sensitivity (Fig. 5A). Both *akt-1* and *akt-2* are expressed in the TRNs (Fig. 5B). Loss of *akt-2*, however, did not produce touch insensitivity by itself, but further reduced anterior touch sensitivity of *akt-1* mutant (from 2.5 ± 0.1 responses to 0.7 ± 0.2 responses, $N \geq 3$, $p < 0.0001$), suggesting that *akt-2* plays a minor role in regulating touch sensitivity. None of these mutations affected posterior touch sensitivity except for the *daf-2(m65)*-null mutation (other temperature-sensitive *daf-2* mutations, including *e979*, *m41*, and *sa193*, which produce similar dauer formation and lifespan phenotypes as *m65*, only reduced anterior touch sensitivity; Fig. 5C). As expected from the known pathway, the touch insensitivity of *daf-2* animals was suppressed by the loss of *daf-16* (Fig. 5D) or a gain-of-function mutation in *pdk-1* [*mg142*; *pdk-1(gf)*] (from 0.9 ± 0.2 responses to 4.1 ± 0.1 , $N \geq 4$, $p < 0.0001$). Moreover TRN-specific expression of *akt-1(+)* restored the anterior touch sensitivity of *akt-1* animals (from 2.6 ± 0.3 responses to 4.0 ± 0.3

Figure 4. The calcium response to touch in focal adhesion mutants. **A**, Normalized calcium response (mean $\pm$ SEM at each given displacement) of wild-type, *pat-2* mosaic animals, *pat-2* mosaic animals subjected to sustained vibration, *unc-112* mosaic animals, and *egl-19* animals and their corresponding Boltzmann fits using all data points for a particular strain; $n \geq 6$ for all strains. Wild-type data is reused from Figure 1B. **B**, Maximum calcium response (Max $\Delta F/F_0$) of ALM neurons to saturated stimulation from wild-type, *pat-2* mosaic, *unc-112* mosaic, and *egl-19* animals; $*p < 0.05$ compared with wild-type, $n \geq 6$ for all strains. **C**, Calcium responses (mean $\Delta F/F_0 \pm$ SEM) of cultured ALM cells from wild-type, *unc-112*, *pat-2*, and *egl-19* animals to potassium depolarization; $*p < 0.05$ compared with wild-type response, $n \geq 9$ for all groups. ALM neurons from both *pat-2* and *egl-19* mutants, either *in vivo* or cultured, gave a decreased maximum calcium signal without a decrease in sensitivity ($D_{50(egl-19)} = 1.0 \pm 0.3 \mu\text{m}$, $n = 5$, $k = 2.0 \pm 0.6$), indicating that the maximum calcium response and sensitivity do not depend on each other. These results suggest that *pat-2* and *egl-19* mutations changed the calcium response independently of mechanotransduction and of UNC-112. Therefore, PAT-2 additionally modulates the calcium response.

responses, $N \geq 3$, $p < 0.0001$), indicating that the pathway was active in the TRNs.

Focal adhesion complexes phosphorylate and activate AKT kinases in mammalian cells (Persad et al., 2001). To test if integrin signaling modulates *C. elegans* touch sensitivity through AKT-1, we overexpressed focal adhesion genes in the TRNs in *pdk-1* and *akt-1* mutants. Overexpressing *unc-112* and *pat-6* in the TRNs of *daf-2(m65)*, *pdk-1(sa680)*, but not *akt-1(ok525)* animals restored their touch sensitivity (Fig. 6A). Increasing insulin signaling by *akt-1(mg144)*, *pdk-1(mg142)*, or *daf-16(mgDf50)* restored the anterior touch sensitivity of *unc-112* and *pat-2* mosaic animals (Fig. 6B). These data indicate that insulin and integrin signaling converge on AKT-1 to modulate touch sensitivity and compensate for each other. The focal adhesion proteins are unlikely to act downstream of insulin signaling, as suggested by quantitative PCR results of Suzuki and Han (2006), because single molecule mRNA fluorescent *in situ* hybridization (smFISH) revealed no change in the number of *unc-112* transcripts in *daf-2(e1370)* animals (data not shown).

Sustained vibration restored anterior touch sensitivity to *daf-2* and *pdk-1* animals, but not to *unc-112*, *pat-2* or *akt-1* mutants (Fig. 6C), indicating that vibration compensates for loss of insulin signaling through the focal adhesion proteins and AKT-1, but not PDK-1. Calcium imaging confirms that vibration did not change the sensitivity of ALM neurons lacking *pat-2* (Fig. 4A). Because focal adhesions are implicated in sensing force, these data suggest that the focal adhesions act as secondary mechano-

sensors in the ALM neurons, although we cannot rule out that they act immediately downstream of the receptor.

If integrins act as secondary mechanosensors, they must bind to ECM components so external force can be transmitted through the hypodermis to the TRNs (Roca-Cusachs et al., 2012). *him-4* mutant animals lack most of the ECM around the TRN processes and the attachment between the processes and the hypodermis (Vogel and Hedgecock, 2001), and should prevent integrin activation. Although *him-4* animals respond to touch (Vogel and Hedgecock, 2001), using our modified touch test (see Materials and Methods), we detected a reduction in touch sensitivity (Fig. 6C,D). Anterior sensitivity was restored to *him-4* animals by expression of the gain-of-function *pdk-1* allele in the TRNs (Fig. 6D), but not by sustained vibration (Fig. 6C), suggesting that integrin signaling cannot be activated by external force without the ECM and/or attachment to the hypodermis.

Hypoxia and the dauer state regulate TRN touch sensitivity through the reduction in INS-10

TRNs are unlikely to sense hypoxia, high salt, or the dauer state directly. These conditions must be sensed by other neurons that then signal to the TRNs. Because insulin signaling alters the anterior touch response, we tested the effect on touch sensitivity of the loss of the 40 genes encoding insulin-like peptides (Pierce et al., 2001) using mutants with loss-of-function alleles or RNAi-treated animals. Mutation or reduction of *ins-10* and *ins-22* caused anterior touch insensitivity (a third gene, *ins-33*, may affect TRN development; Fig. 7A).

INS-10 modulates anterior touch sensitivity through its release from two pharyngeal neurons: the M4 motor neuron and the I5 interneuron (Fig. 7B). Laser ablation of both the M4 and I5 neurons in late L4 stage animals resulted in adults whose anterior touch sensitivity mimicked the loss of *ins-10* within 24 h of ablation (Fig. 7C), but whose posterior touch sensitivity did not change (data not shown). Ablation of either cell alone resulted in little or no reduction of anterior touch sensitivity compared with controls. Because neither M4 nor I5 connects synaptically to the TRNs or their downstream interneurons (Albertson and Thomson, 1976; Chalfie et al., 1985), INS-10 acts hormonally on the TRNs.

INS-10 expression was greatly reduced by hypoxic conditions and dauer arrest (Fig. 7D–G). Under the same conditions, GFP or RFP expressed in the pharyngeal muscles (driven by *ceh-22p* or

Figure 5. Insulin signaling affects touch sensitivity. **A**, Anterior (A, black) and posterior (P, white) response (mean ± SEM) of animals with the indicated phenotypes; * $p < 0.005$ compared with wild-type, $N \geq 3$ for all strains. **B**, smFISH of *akt-1* (left) or *akt-2* (right; red), and *mec-18* (green) in wild-type (+) or the respective null (null) mutants. The positions of the *mec-18* dots delineate the shape of the ALM cell body. **C**, Anterior (black) and posterior (white) response (mean ± SEM) of *daf-2(e1370)*, *daf-2(e979)*, and *daf-2(m41)* animals; $N = 3$, * $p < 0.001$ compared with wild-type. **D**, Anterior touch response (mean ± SEM) of *daf-2(e1370)*, *daf-16(m26)*; *daf-2(e1370)*, and *daf-16(mgDf50)*; *daf-2(e1370)* animals; * $p < 0.05$, ** $p < 0.0005$ compared with *daf-2* alone, $N \geq 3$ for all strains.

Figure 6. Integrin and insulin signaling converge to affect touch sensitivity. **A**, Anterior response of *daf-2(m65)*, *pdk-1*, and *akt-1* animals with (white) or without (black) added focal adhesion (FA) proteins UNC-112 and PAT-6 in the TRNs; * $p < 0.005$ with or without UNC-112/PAT-6, $N \geq 3$. **B**, Effect of *akt-1(gf)*, *pdk-1(gf)*, and *daf-16* on the anterior touch response of *unc-112* or *pat-2* mosaic animals. Values are the means ± SEM of responses of individual animals; $n > 10$, * $p < 0.005$ compared with *unc-112* or *pat-2* alone. **C**, Anterior touch sensitivity of animals with the indicated phenotypes grown with (V, white) or without (C, black) vibration; * $p < 0.05$, ** $p < 0.002$ with or without vibration, $N \geq 3$ for all strains. **D**, Anterior (A, black) and posterior (P, white) touch response of wild-type, *him-4* animals, and *him-4* animals carrying *mec-3p::pdk-1(gf)*; * $p < 0.005$ compared with wild-type anterior response and $p < 0.05$ compared with *him-4* + *mec-3p::pdk-1(gf)*; $N \geq 3$.

myo-2p) or the TRNs (*mec-3p*) changed much less. These changes in expression were not caused by starvation because starved L3 animals did not show them (Fig. 7D). These data suggest that the reduction of INS-10 expression was not caused solely by a general

Figure 7. Regulation of touch sensitivity through insulin-like peptides. **A**, The anterior touch sensitivity (mean ± SEM) of *ins-10(i)* animals, *ins-10(i)* animals carrying either wild-type copies of *ins-10* with different codons or an *rde-1* mutation, *ins-10(tm3498)* animals with or without wild-type copies of *ins-10*, and *ins-22(tm4990)* animals with or without wild-type copies of *ins-22*. $N > 3$ for all strains tested; $*p < 0.01$ between the indicated strains, and comparing *ins-10(i)*, *ins-10*, and *ins-22* to wild-type. *ins-10(i)* had no effect on touch sensitivity in a *rde-1* background, which abolishes RNAi. Loss of INS-33, which is needed for larva development (Hristova et al., 2005), also resulted in a weak reduction of touch sensitivity in some abnormally developed animals (data not shown). INS-33, however, probably acts generally, since *ins-33* larvae that had normal growth were touch sensitive (data not shown). In addition, the difference between *ins-33* and wild-type animals was statistically insignificant after Bonferroni correction. **B**, *ins-10p::gfp* expression in well fed and starved animals. The green image (GFP), the red image (*myo-2p::mCherry*), and the DIC image were merged. Strong GFP expression was seen throughout larval development in M4 and I5 and weakly and sporadically in the MC pharyngeal interneurons, RIS interneurons, and an additional pair of nerve ring interneurons tentatively identified as either the RMF cells or RMH cells. **C**, Anterior touch response (mean ± SEM of individual animals) of animals with no ablation (—), mock ablation (mock), or with I5 and/or M4 ablated; $n \geq 12$ for all, $*p < 0.001$. **D**, Expression of *ins-10p::gfp* or *ins-10p::rfp* in well fed L3 animals, starved L3 animals, dauer larvae, or (**E**) adult animals in normoxic or hypoxic conditions. Images of *myo-2p::mCherry* and *ceh-22p::gfp* expression in the pharyngeal muscles were merged with the green *ins-10p::gfp* (**D**) and red *ins-10p::rfp* (**E**). **F**, **G**, The intensities (mean ± SEM of individual animals) of *ins-10p::gfp* or *ins-10p::rfp* in the M4 and I5 neurons, *ceh-22p::gfp* or *myo-2p::mCherry* in the pharynx, and *mec-3p::rfp* in the TRNs in (**F**) starved L3 or dauer larvae and in (**G**) adult animals under normoxic or hypoxic conditions; $n \geq 5$ for all conditions.

reduction of translation during hypoxia (Connolly et al., 2006) or of transcription in dauer larvae (Dalley and Golomb, 1992).

Animals generating RNAi against *ins-10* [*ins-10(i)*] were less sensitive to mechanical stimulation ($D_{50} = 2.1 \pm 0.3 \mu\text{m}$, $n = 16$, $p < 0.001$, $k = 2.2 \pm 0.4$; Fig. 8A) than wild-type animals ($D_{50} = 1.1 \pm 0.1 \mu\text{m}$, $k = 3.3 \pm 0.3$, as noted above) without changing the maximum calcium response (data not shown). The anterior touch insensitivity of *ins-10(i)* animals was restored by *akt-1(gf)*, *pdh-1(gf)*, or by an equivalent construct of *pdh-1(gf)* expressed only in the TRNs [*TRN::pdh-1(gf)*] (Fig. 8B), suggesting that INS-10 acts via the insulin signaling pathway in the ALM neurons. Because activation of integrin signaling compensates for the loss of insulin signaling in the TRNs (Fig. 6A), we expected and saw that the touch insensitivity of *ins-10(i)* was restored by overexpressing UNC-112/PAT-2 in the TRNs and by vibration (Figs. 6C, 8B). Moreover, hypoxia and the dauer state did not cause anterior touch insensitivity in *akt-1(gf)*, *daf-16*, and *TRN::pdh-1(gf)* animals (Fig. 8C,D). These data suggest that hypoxia and the dauer state prevent the release of INS-10 from M4 and I5, causing a

reduction of insulin signaling in the anterior TRNs and the subsequent loss of touch sensitivity.

ASE releases *ins-22* to modulate touch sensation

The loss of *ins-22(tm4990)* also reduced anterior touch sensitivity (Fig. 7A) without affecting normal development or dauer formation (data not shown). As with *ins-10*, the *ins-22* touch insensitivity was rescued by the wild-type gene (Fig. 7A), *daf-16*, *TRN::pdh-1(gf)* (Fig. 9A), and vibration (Fig. 6C).

ins-22 is expressed strongly in both ASE cells (Fig. 9B), sensory neurons that sense the ions (Na^+ , Cl^- , but not CH_3COO^- and NH_4^+ ; Ortiz et al., 2009) whose elevation reduces anterior touch sensitivity (Fig. 2D). *che-1* animals, which lack both ASE cells, have reduced anterior touch sensitivity that can be restored by *TRN::pdh-1(gf)* (Fig. 9C), suggesting that INS-22 from the ASE cells regulates TRN touch sensitivity. Consistent with this hypothesis, INS-22 expression was reduced when the animals were grown in high salt (Fig. 9B,D), and the reduced touch sensitivity produced by high salt was rescued by *akt-1(gf)*, *pdh-1(gf)*, *TRN::pdh-1(gf)*, and *daf-16* or TRN overexpression of UNC-112/PAT-6 (Fig. 9E). Expressing *daf-16a* but not *daf-16b* in the TRNs of *daf-16* animals caused them to reduce anterior touch sensitivity on high salt.

Animals without *ins-22*, however, were more sensitive to anterior touch than either *che-1* mutants or wild-type animals grown on high salt (Fig. 9A,C,E). Moreover, we were unable to detect significant changes in the sensitivity of *ins-22* animals ($D_{50} = 0.9 \pm 0.2 \mu\text{m}$, $n = 10$, $k = 3.7 \pm 0.8$). These results suggest that loss of additional components other than INS-

22, perhaps other neuropeptides from the ASE neurons, signals high salt conditions to the TRNs.

Modulation of touch sensitivity adapts animals to diverse conditions

A striking aspect of the effects of high salt, hypoxia, dauer, and prolonged vibration is that they change only anterior touch sensitivity. Anterior touch, but not posterior touch, causes an initial backing usually followed by a turn so animals go in a new forward direction (Croll, 1975; Chalfie and Sulston, 1981). Therefore, reducing anterior touch sensitivity may cause wild-type animals to change movement directions less in response to nonlocalized mechanical distractions, such as short vibratory pulse, which activates both anterior and posterior TRNs. Indeed, *ins-10(i)* animals moved backward less often than wild-type animals (Fig. 10A) when given a short pulse of vibration (0.5 s, 50 Hz), and as a result changed directions less than wild-type (average turning angles are $22.5 \pm 2.6^\circ$ in wild-type vs $6.24 \pm 1.8^\circ$ in *akt-1* animals; $N = 4$, $p < 0.005$).

Such increased resistance to mechanical distraction may facilitate the completion of non-mechanosensory tasks. We tested how fast animals moved to a source of an attractant (diacetyl) when the plates were tapped once every 30 s (see Materials and Methods). This stimulus activates the TRNs. Although some habituation was seen, many animals still responded to the stimulus at the end of the trial. Fewer wild-type animals reached the diacetyl spot when the plate was tapped than when it was not tapped (Fig. 10*B,C*), suggesting that the mechanosensory response interfered with the efficiency of chemotaxis. Wild-type dauers and *ins-10(i)* animals moved slower (data not shown) in general, but they were slightly more efficient at chemotaxis when tapped (Fig. 10*B,C*). To compensate for the slowing of the animals, we looked at *ins-10(i)* animals in which *pdk-1(gf)* was expressed in the TRNs (Fig. 10*B,C*). These animals were sensitive to touch and, like wild-type animals, chemotaxed much slower with intermittent tapping. These results suggest that reduction of touch sensitivity in the TRNs was responsible for the change in chemotaxis efficiency when the plates were tapped. The change in anterior touch sensitivity allows animals to be less distracted by mechanical signals while they are responding to other senses.

Sensitization, in contrast, partially counteracts the effect of habituation that also occurs during sustained vibration: ~10% more animals responded to a stronger vibratory pulse (0.5 s) given at 2 h during sustained vibration, when the cells were sensitized, rather than at 1 h (Fig. 10*D*). This subtle yet statistically significant ($p < 0.01$) difference in responsiveness became more significant during the recovery following habituation. Wild-type animals recovered 95% of the unhabituated response to anterior touch after 10 min of rest following 3 h of vibration compared with 35% for *akt-1* mutants (Fig. 10*E*). In contrast the posterior touch response showed no significant recovery by 30 min (Fig. 10*F*). Because *akt-1* mutation blocks sensitization, these data suggest that sensitization facilitates the detection of stimuli during and after habituation.

Discussion

We have described and characterized the mechanisms and biological functions of several modulators of touch sensitivity (Fig. 11). Sustained stimulation sensitizes the TRNs, which relies not on the activation of the MEC-4 mechanosensory chan-

Figure 8. INS-10 regulates touch sensitivity through insulin signaling. **A**, Normalized calcium responses (mean $\pm$ SEM) of wild-type (black) and *ins-10(i)* (red) animals with different probe displacements, and their corresponding Boltzmann fits; $n \geq 16$ for both strains. The wild-type data were reused from Figure 1*B*. **B**, Anterior touch response (mean $\pm$ SEM) of *ins-10(i)* animals with or without *akt-1(gf)*, *pdk-1(gf)*, *mec-3p::pdk-1(gf)*, or *mec-17p::unc-112::gfp* and *mec-17p::pat-6::gfp*; $p < 0.005$ for all other strains compared with *ins-10(i)* alone, $N \geq 3$. **C**, Anterior touch sensitivity (mean $\pm$ SEM) of wild-type, *daf-16*, or *akt-1(gf)* animals, or animals carrying *mec-3p::pdk-1(gf)* grown under normoxic (N, black) or hypoxic (H, white) conditions; $N \geq 4$ for all strains and conditions tested, $p < 0.005$ comparing hypoxic wild-type to other hypoxic strains. **D**, Anterior touch sensitivity (mean $\pm$ SEM) of wild-type starved L3 larvae, wild-type dauer larvae, or dauer larvae expressing *mec-3p::pdk-1(gf)*; $N \geq 3$, $*p < 0.001$, $**p < 0.0005$ compared with wild-type dauer larvae.

Figure 9. The ASE neurons and INS-22 regulates touch sensitivity. **A**, Anterior touch response (mean $\pm$ SEM) of *ins-22* animals, *ins-22* animals expressing *mec-3p::pdk-1(gf)*, and *daf-16; ins-22* animals; $*p < 0.05$ compared with *ins-22*, $N \geq 3$. **B**, Expression of *ins-22p::gfp* in animals grown on high sucrose or high salt (NaCl). Images of *myo-3p::mcherry* were merged with green *ins-22p::gfp* images. **C**, Anterior touch response (mean $\pm$ SEM) of wild-type and *che-1* animals with or without *mec-3p::pdk-1(gf)*; $N \geq 3$, $p < 0.01$ between *che-1* and the other two strains. **D**, Quantification of *ins-22p::gfp* in animals grown on control NGM plates and NGM plates supplemented with 180 mM NaCl or 380 mM sucrose; $*p < 0.05$ compared with control and $p < 0.005$ compared with sucrose, $n > 15$ for all samples. **E**, Anterior touch response (mean $\pm$ SEM) of the indicated animals grown with 50 mM NaCl (black, C) or with 230 mM NaCl (white, S); $*p < 0.05$ and $**p < 0.01$ comparing the respective strain on high salt to wild-type grown on high salt, $***p < 0.01$, $N \geq 3$ for all strains tested. AU, arbitrary units.

Figure 10. Behavioral consequences of mechanosensory modulation. **A**, The number of animals responding to a pulse vibration by backward movement (black) or nonbackward movement (forward or no movement, white) in wild-type and *ins-10(i)* animals. The data are pooled from four independent trials; $p < 0.0001$ comparing *ins-10(i)* to wild-type. **B**, The ratio of chemotaxis efficiency index (mean $\pm$ SEM; CEI, the fractions of animals that have reached the diacetyl spot) with or without tapping every 30 s during a 12 min chemotaxis assay; $*p < 0.05$ comparing animals with or without *TRN::pdk-1(gf)*, or comparing *ins-10(i)* or wild-type dauer with wild-type adults, $N \geq 3$ for all strains. **C**, The ratio of CEI (the fractions of animals that have reached the diacetyl spot; mean $\pm$ SEM) with or without tapping. The data used are the same as in **B**. **D**, Fraction of animals moving backward in response to a 0.5 s vibratory pulse without sustained vibration (no vib) or with background sustained vibration for the indicated time. The total number of animals tested is noted at each point; $*p < 0.01$ compared with no vib and 2 h using Fisher's exact test. **E**, **F**, Anterior (**E**) and posterior (**F**) response (mean $\pm$ SEM) of wild-type (blue) or *akt-1* (red) animals to touch before habituation (pre) or after vibration for more than 2 h and rested for the indicated amount of time, normalized to the response before habituation; $*p < 0.005$ comparing wild-type and *akt-1* responses, $N > 10$ for all time points for each strain.

nel, but on a secondary mechanosensor involving integrins and other focal adhesion proteins to detect sustained stimuli. Long-term sensitization acts via a pathway that joins the insulin signaling pathway, which is affected by several stress signals. The joining of these pathways allows the integration of several different modulatory inputs into a single output. In addition, these various responses occur only in the anterior TRNs and not in the posterior cells. Kindt et al. (2007) also found a difference between the anterior and posterior cells with regard to short-term habituation. Together these results suggest that these very similar looking touch-sensing cells actually function quite differently.

Integrins sensitize the anterior TRNs

Integrins anchor cells to the ECM and serve as cellular mechanosensors. The activation of integrins by mechanical force induces diverse changes such as cell proliferation and differentiation

(Roca-Cusachs et al., 2012). Integrin signaling has been implicated in the function of mechanosensory cells. For example, rats exposed to strong auditory stimuli increased the expression of integrins in the cochlear sensory epithelium; this change correlated with a shift in hearing threshold (Cai et al., 2012). In addition, mechanical hyperalgesia, where normal touch elicits a painful response, is reduced by blocking integrin signaling (Dina et al., 2004). The role of integrin signaling, however, in neuronal mechanosensation is unclear in these examples.

Our results show that the integrins are needed for the long-term sensitization of the anterior TRNs. The initial detection of prolonged stimulation does not require the MEC-4 channel. Given their involvement in this sensitization and their actions in other cells, the integrins are prime candidates for the secondary mechanotransducer in the TRNs. This hypothesis does not exclude the possibility that integrins detect changes in the ECM caused by prolonged stimulation and thus act as a signaling molecule (indeed, change in ECM proteins usually accompanies or leads to integrin activation; Roca-Cusachs et al., 2012). Because integrins respond less quickly than the MEC-4 channels, they are more suited for detecting sustained stimuli. We propose that these two systems respond to different mechanical signals; the MEC-4 channels are activated by changes in displacement, whereas the focal adhesions respond to sustained or repeated force. In addition MEC-4 channels do not require the *him-4*-dependent anchorage of the TRNs to the hypodermis to function, but the integrins and sensitization do.

Sensitization counters habituation during sustained stimulation

Similar sensitization is seen in mammalian hearing and mechanical nociception

(Kujawa and Liberman, 1999; Govindaraju et al., 2006; Chen et al., 2010), suggesting that sensitization following sustained stimulation may be a conserved and common characteristic of mechanosensory systems. The counteracting effects of sensitization and habituation, both occurring after sustained stimuli, pose an intriguing dilemma: why would the animal reduce the behavioral response but concurrently become more sensitive to the stimulus? Our data suggest that sensitization allows animals to respond better during and after habituation.

Habituation attenuates the response to repeated stimulation. This process allows the animal to ignore background stimuli, but also reduces its sensitivity to specific stimuli, making the animal more vulnerable to predators (Maguire et al., 2011). Sensitized animals respond slightly better during background stimulation (Fig. 10D), but much better after the cessation of background stimulation (Fig. 10E). Therefore, sensitized animals could main-

Figure 11. Regulation of TRN mechanosensation. Sustained vibration (green pathway) sensed by integrins, and hypoxia, dauer formation, and high salt (red pathway) sensed by insulin peptides, converge on AKT-1 and DAF-16 to modulate touch sensitivity in the anterior TRNs. Dashed connectors indicate that the signaling strength is attenuated by the corresponding signals.

tain their defenses against predators in the face of sustained background stimulation. Because sensitization occurs over hours, but habituation can occur within minutes (Rankin et al., 1990), sensitization may be a further adaptation to prolonged habituation to reduce risks associated with attenuated touch response. Long-term sensitization, thus, could provide a strong survival advantage in the wild under conditions that might stimulate the animals mechanically for prolonged periods, such as rain, which is simulated by our 1 Hz stimulation experiment.

Neurohormones coordinate senses under stress conditions

Sensory modulation optimizes behavioral outcomes in many species (Sillar and Skorupski, 1986; Maney and Pinaud, 2011). These changes in sensory perception allow different behavioral responses depending on environmental cues. Here we show that stress conditions sensed by several other cells modulate *C. elegans* touch sensitivity through insulin-like neurohormones. If insulin-based regulation of touch sensitivity is more general, the loss of insulin signaling may underlie neuropathic numbness in humans, a common early symptom during prediabetic neuropathy (Vincent et al., 2011).

Mechanosensory responses are robust and faster than those of other senses, such as olfaction or thermal sensitivity; they, thus, take priority over other senses in determining an animal's behavior. Our data suggest, however, that this neuronal priority can be reordered through neurohormonal modulation. We saw, for example, that animals with reduced anterior touch sensitivity performed better when chemotaxing in the presence of mechanical distraction. Therefore, the reduction of touch sensitivity under stress conditions may facilitate escape from the stress environ-

ment by lowering the priority of mechanosensation. Conversely, we imagine that under good conditions, increased touch sensitivity would prepare the animals for escape from mechanical signals in the face of favorable environmental signals that would otherwise have them remain in place. Previous studies by Kindt et al. (2007) on dopamine modulation of touch habituation also support this hypothesis.

Such a change in priority may be an important adaptive change in the wild. In particular, we find that dauer larvae actually accumulate more rapidly at a source of an attractive chemo-sensory signal when mechanical stimuli are present (Fig. 10D). Thus, in this instance mechanosensory signals can attract rather than repel, a reversal of the normal sensory behavior. Interestingly, Torr et al. (2004) found that infectious larvae (the equivalent to the *C. elegans* dauer larvae) from three different nematodes, *Steinernema carpocapsae*, *S. feltiae*, and *Heterorhabditis megidis*, which parasitize insects, were better able to find prey (greater wax moth larvae) when vibratory signals were present.

The organization of sensory modulatory networks

Although many sensory cells (Fex, 1967; Sillar and Skorupski, 1986; Massey and Redburn, 1987) receive synaptic inputs from efferent neurons, the TRNs receive no obvious synaptic inputs (Chalfie et al., 1985). Our data indicate, however, that a complex, nonsynaptic signaling network integrates multiple non-mechanical signals to modify the activity of the TRNs.

Modulatory signals from different neurons converge on the TRNs through different neuropeptides: the M4/I5 cells secrete INS-10, and the ASE cells rely on INS-22 and possibly other neuropeptides. The M4/I5 cells are pharyngeal interneuron/motor neurons that control pharyngeal pumping. These cells, however, also secrete multiple neuropeptides (Rogers et al., 2003) and receive neurohormonal inputs (Pocock and Hobert, 2010) from outside of the pharynx. These observations and our data suggest that the M4 and I5 neurons act as neurohormonal hubs that function similarly to interneurons in a synaptic network: they integrate information and modulate multiple downstream neurons through diverse neurohormonal peptides. These cells may constitute a primitive neuroendocrine system similar to the *Drosophila* pars intercerebralis and pars lateralis and the mammalian hypothalamus (Hartenstein, 2006), which also secrete neuropeptides and hormones to regulate animal behavior and development. Convergence of modulatory signals through neurohormonal hubs and at the TRNs through insulin signaling allows multiple non-mechanical stress conditions to regulate touch sensitivity through a common mechanism. This nonsynaptic network may potentially mediate modulation of touch sensitivity through additional neuropeptides, including the FLPs, NLPs, oxytocin/vasopressin-like neuropeptides, and other redundant or negatively acting insulin-like peptides.

Subsequently, the input of the insulin signals is integrated with that of integrin signaling, which respond to mechanical activation, by AKT-1 and AKT-2 in the TRNs. The AKT kinases then suppress the activity of DAF-16/FOXO transcription factor, which provides a common mechanism to integrate both mechanical and non-mechanical modulatory signals. The relatively slow rate of transcriptional regulation probably explains why all four conditions require several hours to affect touch sensitivity. This convergence allows compensation between conflicting modulatory signals from different modalities: the reduced touch sensitivity under non-mechanical stress conditions or in insulin signaling mutants can be restored by mechanical stimulation, and the reduced touch sensitivity caused by loss of integrin

signaling can be compensated by increasing insulin signaling. Together, the signaling convergence on AKT kinases and the nonsynaptic networks unify multisensory cues at multiple levels to modify sensory perception through a common mechanism.

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