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Phospholipids that Contain Polyunsaturated Fatty Acids Enhance Neuronal Cell Mechanics and Touch Sensation

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SUMMARY

Mechanoelectrical transduction (MeT) channels embedded in neuronal cell membranes are essential for touch and proprioception. Little is understood about the interplay between native MeT channels and membrane phospholipids, in part because few techniques are available for altering plasma membrane composition in vivo. Here, we leverage genetic dissection, chemical complementation, and optogenetics to establish that arachidonic acid (AA), an omega-6 polyunsaturated fatty acid, enhances touch sensation and mechanoelectrical transduction activity while incorporated into membrane phospholipids in *C. elegans* touch receptor neurons (TRNs). Because dynamic force spectroscopy reveals that AA modulates the mechanical properties of TRN plasma membranes, we propose that this polyunsaturated fatty acid (PUFA) is needed for MeT channel activity. These findings establish that polyunsaturated phospholipids are crucial determinants of both the biochemistry and mechanics of mechanoreceptor neurons and reinforce the idea that sensory mechanotransduction in animals relies on a cellular machine composed of both proteins and membrane lipids.

INTRODUCTION

The sensory neurons embedded in our skin use mechanoelectrical transduction (MeT) channels to detect mechanical stimuli delivered by a feather's brush, a pin's prick, or a mobile phone's buzz. Analogous sensory neurons in joints and muscles help to maintain balance and posture, whereas others innervate the aorta and regulate heart rate on a beat-by-beat basis. Most, if not all, MeT channels belong to macromolecular complexes whose protein constituents have been identified only for a few mechanoreceptor cells. In *C. elegans*, it has been shown that members of the degenerin/epithelial sodium channel (DEG/ENaC) (MEC-4, MEC-10, and DEG-1) and transient receptor potential (TRP) channel (TRP-4, also known as CeNOMPC) families are pore-forming subunits of MeT channels in vivo (Arnadóttir et al., 2011; Geffeney et al., 2011; Kang et al., 2010; O'Hagan et al., 2005). Additional MeT channel subunits have been identified in fruit flies and mice: NOMPC is thought to be a pore-forming subunit in fly mechanoreceptor neurons (Gong et al., 2013; Yan et al., 2013; Zhang et al., 2013); piezo proteins are thought to function as MeT channels in body mechanoreceptor neurons and in red blood cells (Bae et al., 2013; Coste et al., 2010; Kim et al., 2012); TMHS and TMC proteins are required for mechanotransduction by inner-ear hair cells in mice (Pan et al., 2013; Xiong et al., 2012). Thus, whereas many of the proteins needed to form MeT channels in mechanoreceptor cells have been identified, very little is known about how the composition of the membrane modulates MeT channel function.

One of the many advantages of studying touch sensitivity in *C. elegans* touch receptor neurons (TRNs) is that they generate electrical signals in response to cuticle deflection and transmit signals mainly via electrical synapses onto interneurons that control locomotion (Chalfie and Sulston, 1981; Chalfie et al., 1985; Goodman, 2006). At least five genes encode membrane proteins required to form the MeT channel complex in the TRNs. Four are *mec* (*mec*hanosensory abnormal) genes that can mutate to disrupt gentle touch sensation, and mutations in the fifth disrupt both touch sensation and locomotion (Chalfie and Sulston, 1981; Zhang et al., 2004): *mec-4, mec-10, mec-2, mec-6,* and *unc-24*. These genes encode two DEG/ENaC ion channel subunits (MEC-4 and MEC-10), two stomatin homologs (MEC-2 and UNC-24), and a paraoxonase-like protein (MEC-6) (Arnadóttir and Chalfie, 2010). Both MEC-2 and MEC-6 are thought to interact with cholesterol (Brown et al., 2008; Chelur et al., 2002; Huber et al., 2006). Indeed, MEC-2 binds cholesterol, and its function as an auxiliary channel subunit depends on this ability (Brown et al., 2008; Huber et al., 2006). Therefore, it is likely that membrane composition plays an important role in modulating the response of TRNs to mechanical stimuli.

When incorporated into phospholipids, polyunsaturated fatty acids (PUFAs) confer fluidity and flexibility to membranes (Rawicz et al., 2000; Rajamoorthi et al., 2005); consequently, they could modulate membrane protein function. Free PUFAs have been shown to modulate voltage-gated and mechanosensitive

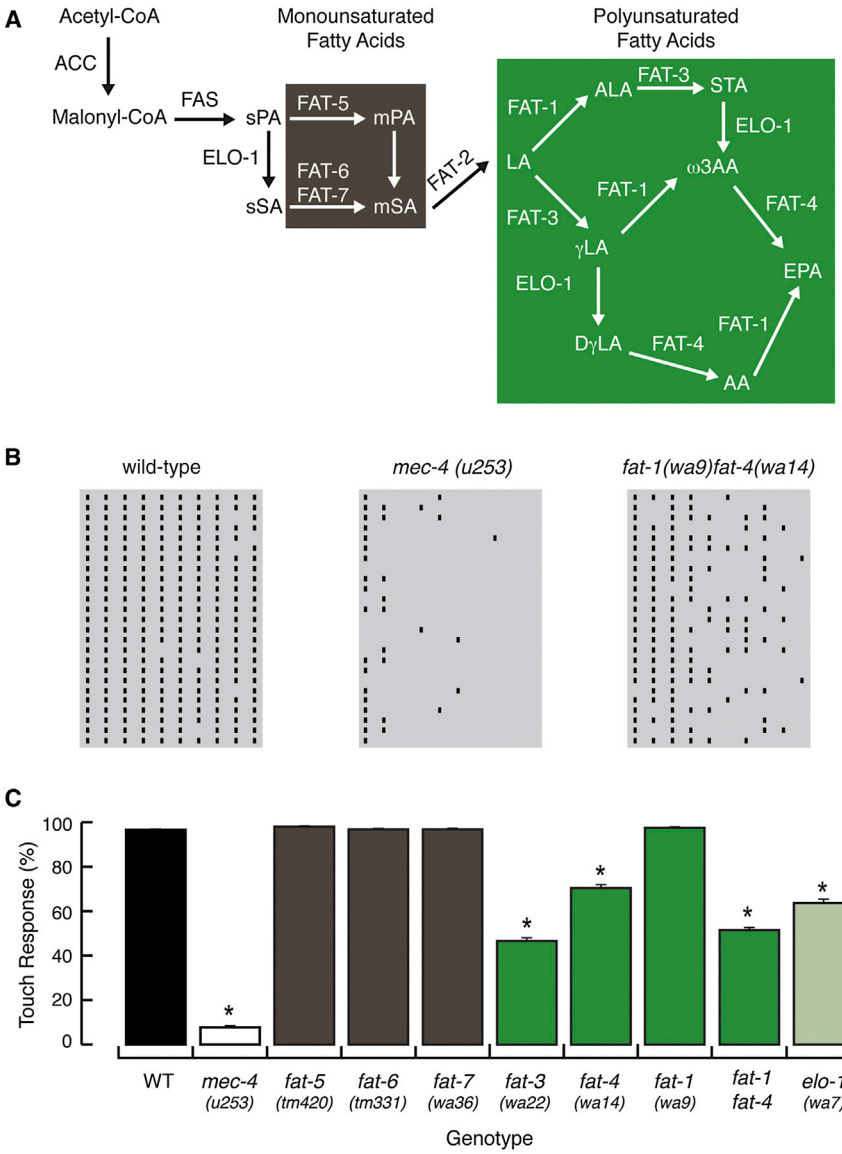

Figure 1. Arachidonic and Eicosapentaenoic Acids Are Required for Touch Sensitivity

(A) Fatty acid synthesis in *C. elegans*, adapted from Watts (2009). Brown and green boxes denote the enzymatic cascade needed to synthesize monounsaturated and polyunsaturated fatty acids, respectively. AA, arachidonic acid (20:4n-6); ALA, α-linolenic acid (18:3n-3); DγLA, dihomo-linolenic acid (20:3n-6); EPA, eicosapentaenoic acid (20:5n-3); FAS, fatty acid synthase; LA, linolenic acid (18:2n-6); STA, stearidonic acid (18:4n-3); γLA, γ-linolenic acid (18:3n-6); ω3AA, ω-3 AA (20:4n-3).

(B) Raster plots displaying the response to gentle touch of cohorts of 25 worms. Bars indicate trials that elicited reversals or pauses. Columns and rows represent trials and worms, respectively.

(C) Touch response in wild-type (WT), *mec*-4, and *fat* mutants. At least 75 animals were tested blind to genotype. Bars are mean ± SEM. The asterisk indicates values significantly different from WT; Kruskall-Wallis and Dunn's multiple comparisons tests; $p < 0.001$.

See also Figure S1.

ion channels in heterologous cells (Balleza et al., 2010; Kim, 1992; Maingret et al., 2000). Genetic dissection in *C. elegans* shows that sensory and motor neurons depend on PUFAs for their function (Kahn-Kirby et al., 2004; Lesa et al., 2003). However, the physiological role of this modulation remains to be determined. Unlike mammals, worms can synthesize PUFAs de novo from acetyl-coenzyme A using a series of fatty acid desaturase and elongase enzymes, known as FAT and ELO proteins, respectively (Wallis et al., 2002; Watts and Browse, 2002). Mutants defective in *fat* and *elo* genes have altered PUFA content (Watts and Browse, 2002) and grow into normal adults with mild phenotypes, except for *fat-2* mutants. We exploited this knowledge to ask whether PUFAs are needed for touch sensation and the normal function of the *C. elegans* TRNs.

Here, we demonstrate that disrupting arachidonic acid (AA) content or its incorporation into phospholipids impairs TRN-dependent behavioral responses, thereby identifying membrane phospholipids containing AA as critical for touch sensitivity. Arachidonic acid is likely synthesized within TRNs in vivo, because we show that enzymes needed for its synthesis are expressed in TRNs. We used an optogenetic approach to show that the defect in touch sensation likely reflects a loss of mechanotransduction rather than lack of excitability or downstream signaling. Finally, we found that the membrane viscoelastic properties of TRNs lacking C20 PUFAs are altered (i.e., membrane bending and viscosity), yielding less flexible membranes than wild-type, as determined by atomic force microscopy (AFM)-based single-tether extrusion. Our findings demonstrate that, when AA is part of membrane phospholipids, it modulates the mechanical properties of *C. elegans* TRN membranes and is crucial for touch sensation.

RESULTS

Arachidonic Acid Is Required for Touch Sensation

We used a behavioral test to investigate the contribution of lipids to touch sensitivity, exploiting the well-characterized enzymatic cascade (Figure 1A) that leads to the production of C20 PUFAs in *C. elegans* (Watts, 2009; Watts and Browse, 2002). Touch sensitivity was measured with a ten-trial touch assay that consisted of stroking an eyebrow hair across the body of young adults, alternating between the anterior and posterior part of the worm (Hart, 2006). Trials that elicited a pause or avoidance