Piezo2 is the principal mechanotransduction channel for proprioception

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Proprioception, the perception of body and limb position, is mediated by proprioceptors, specialized mechanosensory neurons that convey information about the stretch and tension experienced by muscles, tendons, skin and joints. In mammals, the molecular identity of the stretch-sensitive channel that mediates proprioception is unknown. We found that the mechanically activated nonselective cation channel Piezo2 was expressed in sensory endings of proprioceptors innervating muscle spindles and Golgi tendon organs in mice. Two independent mouse lines that lack Piezo2 in proprioceptive neurons showed severely uncoordinated body movements and abnormal limb positions. Moreover, the mechanosensitivity of parvalbumin-expressing neurons that predominantly mark proprioceptors was dependent on Piezo2 expression in vitro, and the stretch-induced firing of proprioceptors in muscle-nerve recordings was markedly reduced in Piezo2-deficient mice. Together, our results indicate that Piezo2 is the major mechanotransducer of mammalian proprioceptors.

Proprioception is the sense of body and limb position and is transduced by proprioceptive sensory neurons1,2. The information encoded by proprioceptors contributes to both unconscious (for example, knee jerk reflex) and conscious (for example, the ability to touch one’s nose with eyes closed) sensations and is required for basic motor functions such as standing and walking3. In mammals, proprioceptors represent anatomically distinct sensory neurons that have cell bodies in dorsal root ganglia (DRG) and innervate two distinct mechanoreceptors in skeletal muscles: muscle spindles (MSs) and Golgi tendon organs (GTOs)2,4. MS afferents innervate intrafusal muscle fibers and detect changes in muscle length, whereas GTO afferents innervate the tendon organs at the myo-tendinous junction and respond to changes in muscle tone2,4.

The molecular mechanism(s) underlying proprioception has been a long-standing question. In particular, the identification of ion channel(s) that are thought to transduce mechanical strain experienced by muscles and joints into electrical signals has been a major topic of research, and study has focused on the transient receptor potential (TRP) and Degenerin/Epithelial Na+ Channel (DEG/ENaC) families4. In Drosophila, the TRPN/NompC channel is expressed in both bipolar dendrite (bd) and class I dendritic arborization (da) proprioceptive neurons, and is required for proper larval crawling and behavioral coordination in adult flies5,6. In C. elegans, trp-4 (a TRPN/NompC homolog) and unc-8 (a DEG/ENaC family member) have been implicated in proprioception, as mutations in these genes cause impaired movement in worms7,8. More recently, Piezo has also been shown to mediate stretch-activated firing of larval Drosophila dorsal bipolar dendritic (dbd) neurons9.

In mammals, however, the molecular mechanism underlying proprioception has remained largely elusive. Previous studies have suggested that mechanically activated (MA) currents in mammalian proprioceptive neurons are largely mediated by Na+ ions, with Ca2+ ions having a minor role10. Consistent with this observation, ENaC proteins are expressed in rat MSs11. However, no strong evidence has been provided for ENaC proteins in mammalian proprioception8,11. Piezo family members are nonselective cation channels with diverse roles in mechanotransduction and volume signaling12–18. In mice, Piezo1 has a critical role in vascular remodeling and red blood cell volume regulation13,15,18, whereas Piezo2 is expressed in sensory neurons and functions as the mechanotransducer for low-threshold mechanoreceptors in murine skin14,16,17. We found that parvalbumin (Pvalb)-expressing sensory neurons, which correspond primarily to proprioceptors19, express nonselective MA cationic currents whose biophysical properties are consistent with Piezo2 channels12,16. Based on these observations, we explored whether Piezo2 is involved in mammalian proprioception.

RESULTS
MA cation channel Piezo2 is expressed in proprioceptors
MA currents in proprioceptive neurons are thought to be mediated by Na+ ions, with Ca2+ ions having a minor role4,10. This assertion is based on stretch-induced extracellular voltage recordings of MS afferents10. The voltage changes recorded in such preparations are a result of the coordinated opening of multiple ion channels. To determine the ion selectivity of the mechanotransducer channel itself, we performed electrophysiological recordings in proprioceptors in vitro16,20.

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To label proprioceptive neurons, we crossed Pvalb-Cre mice, which express Cre recombinase in all proprioceptors and a few rapidly adapting low-threshold cutaneous mechanoreceptors, with Ai9 tdTomato reporter mice (Supplementary Fig. 1a). DRG neurons from Pvalb-Cre; Ai9 mice were isolated, and tdTomato+ neurons were visually selected and subjected to whole-cell patch-clamp recordings in vitro (Fig. 1a). Although physiological mechanotransduction occurs at the nerve terminals, many MA channels are also expressed in, and can be recorded from, DRG cell bodies. Mechanical stimuli were applied to tdTomato+ neurons using a blunt-end glass probe (Fig. 1b). All tdTomato+ neurons responded to this mechanical stimulus: 92% (23 of 25) of the neurons displayed rapidly adapting (RA) currents (inactivation time constant \( \tau = 5.6 \pm 0.33 \) ms) and 8% (2 of 25) exhibited intermediately adapting (IA) currents (\( \tau = 15.56 \) and 31.67 ms) (Fig. 1b). To determine ionic selectivity, we performed current-voltage (I-V) relationship measurements. The RA currents, which form the vast majority of MA responses, had a reversal potential (E_{rev}) of +13.55 ± 0.73 mV (Fig. 1c). These E_{rev} values are inconsistent with Na+-selective channels, as the theoretical E_{rev} for Na+ in our conditions is +64 mV. In addition, we found that application of 100 μM amiloride (an inhibitor of DEG/ENaC Na+ channels) to these cells via bath perfusion had no effect on either current amplitude or I-V relationship of these traces (E_{rev} = +11.72 ± 0.91 mV). These two observations, E_{rev} values close to 0 mV and the lack of inhibition by amiloride, suggest that nonselective, amiloride-insensitive channels mediate the majority of MA currents in Pvalb+ neurons. One of the two cells displaying IA currents reversed at +0.42 mV (Supplementary Fig. 1b). As a result of the low frequency of IA current observations, our conclusions about this cell population are limited, and we cannot determine whether they were expressed in proprioceptors or the aforementioned Pvalb+ cutaneous mechanoreceptors. Nevertheless, the observed E_{rev} value was also inconsistent with this current being mediated by Na+-selective channels.

The inactivation kinetics, reversal potential and voltage dependence of the RA currents that we observed in Pvalb+ neurons resemble those of Piezo2 expressed in heterologous systems (for example, Piezo2 in HEK293T cells, \( \tau = 7.3 \pm 0.73 \) ms, E_{rev} = +8.7 ± 1.5 mV). Piezo2 is a nonselective MA cation channel present in the sensory endings of low-threshold cutaneous mechanoreceptors. To address whether Piezo2 channels could underlie the nonselective RA current that we observed in proprioceptors, we first asked whether Piezo2 is expressed in these sensory neurons. To assess Piezo2 expression in proprioceptors, we used the previously reported Piezo2-GFP reporter line, in which GFP is fused to the last coding exon of Piezo2 and is expressed as a Piezo2-GFP fusion protein under the endogenous Piezo2 promoter. We examined Piezo2-GFP lumbar DRG by immunostaining for GFP and Pvalb and found that 81% of Pvalb+ neurons expressed Piezo2-GFP.
neurons (191 of 236) expressed GFP (Supplementary Fig. 2a). Next, we examined Piezo2 expression in proprioceptor muscle endings, where mechanotransduction initiates. In postnatal day 5 (P5) Piezo2*GFP hind leg muscles, immunofluorescence for GFP and Pvalb colocalized in every MS sensory ending that we observed (13 GFP+ Pvalb* MSs of 13 total; Fig. 1d), but not in MS intrafusal muscle fibers (Fig. 1e). Furthermore, we examined P17 Piezo2*GFP hind leg muscles by immunostaining for GFP and Vglut1 (a marker of MS and GTO afferent endings) and detected GFP and Vglut1 in all of the annulospiral endings of both group Ia and group II MS afferents, as well as in every GTO afferent endings encountered (21 GFP+Vglut1+ MSs of 21 total; 11 GFP+Vglut1+GTOs of 11 total; Fig. 1f and Supplementary Fig. 2b,c). These data suggest that Piezo2 is expressed in all types of proprioceptive peripheral endings.

Piezo2 is required for proprioception in mice
Previously, we found that Piezo2 ablation in sensory neurons of adult mice via the Advlillin-CreERT2 driver caused a severe deficit in cutaneous light-touch sensation16. In addition, these mice displayed an unstable gait, but did not exhibit a complete proprioceptive deficit (for example, severely abnormal limb positioning). We reasoned that the proprioceptive behavioral deficits might be more severe if the mice were to lose proprioception from birth rather than during adulthood, after they have learned to walk. Alternatively, given that Advlillin-CreERT2 marks 87% of spinal sensory neurons18, it is possible that the Advlillin-CreERT2–driven Piezo2 deletion does not represent a complete ablation of Piezo2 in proprioceptors. To test more definitively whether Piezo2 is involved in proprioception, we used two alternative Cre lines that target proprioceptive neurons during development: Pvalb-Cre and HoxB8-Cre23. As described above, Pvalb-Cre targets all proprioceptive neurons and a subset of cutaneous mechanoreceptors19, whereas HoxB8-Cre targets broader neuronal populations in caudal DRG, including the proprioceptors23 (Supplementary Fig. 3).

We generated two tissue-specific Piezo2 conditional knockout (cKO) mouse lines by crossing the Pvalb-Cre and HoxB8-Cre lines to Piezo2+/−/mice (Supplementary Figs. 4a,b and 5a,b). Both Pvalb-Cre; Piezo2cKO and HoxB8-Cre; Piezo2cKO mice were born at the expected Mendelian ratio and were indistinguishable from their control littermates in body size or body movement shortly after birth. Notably, postnatal Piezo2 ckO mice (as early as P7) displayed severely impaired limb coordination compared with their control littermates (Fig. 2a,b and Supplementary Videos 1–4). Typically, wild-type (WT) mice stretch out their limbs when lifted by their tails (Fig. 2a,b). In the case of Pvalb-Cre; Piezo2cKO and HoxB8-Cre; Piezo2cKO mice, they clenched their toes and awkwardly positioned their limbs so that their limbs were either completely folded or pointing to atypical directions (Fig. 2a,b).

Consistent with the aforementioned caudal bias in the HoxB8-Cre activity in DRG, we found that only hind limbs were affected in...
**Figure 3** Characterization of MA currents in proprioceptive neurons of Piezo2-deficient mice. Whole-cell patch-clamp recordings were conducted using mechanical stimulation with a blunt-end glass probe in 1-μm increments. (a) The proportion of tdTomato+ DRG neurons responding with RA (τ_inact < 10 ms) and IA (10 ms < τ_inact < 30 ms) MA currents from 4-6-week-old Pvalb-Cre; Ai9 (WT) and Pvalb-Cre; Ai9; Piezo2cKO mice in voltage-clamp experiments. NR, non-responsive to mechanical displacements. (b) Example traces of mechanically induced currents in WT (black traces) and cKO (red traces) tdTomato+ neurons. Holding potential, −60 mV. (c) Current-clamp recordings of WT (black traces) and cKO tdTomato+ neurons (red traces). Depolarizing currents were injected in 50-pA increments from a holding current of 0 pA. All vertical scale bars represent 200 pA and all horizontal scale bars represent 25 ms. Numbers adjacent to traces indicate the number of neurons represented by the trace of the total number of neurons tested.

*HoxB8-Cre; Piezo2cKO* mice, whereas all four limbs showed impaired coordination in Pvalb-Cre; Piezo2cKO mice (Supplementary Fig. 3a,b). Pvalb-Cre; Piezo2cKO mice stumbled while walking, and their limbs appeared to be stiff in general (Supplementary Videos 1 and 2). These phenotypes markedly resembled the walking pattern of Pvalb-Cre; Piezo2cKO mice (Supplementary Videos 3 and 4). This increased severity in phenotype could potentially be a result of broader Piezo2 deletion in DRG neurons compared with the Pvalb+ neurons (Supplementary Figs. 1a and 3a).16 Despite their impaired body coordination, both lines of Piezo2 cKO mice were able to eat, drink and groom similar to WT littermates, and we observed no substantial difference in their body weight (data not shown). Piezo2 expression was not observed in cerebellum, motor neurons and interneurons, or skeletal muscle (Fig. 1d–g, Supplementary Fig. 2c and data not shown); thus, Piezo2 cKO behavioral phenotypes are likely to be caused by Piezo2 ablation in proprioceptive sensory neurons.

We next tested whether Piezo2 ablation affected the number of proprioceptive neurons to ensure that impaired proprioception in the two Piezo2 cKO lines was not a result of anatomical deficits. We labeled and quantified the number of proprioceptors by immunostaining (Fig. 3a) tdTomato+ neurons with mechanical recordings of WT (black traces) and cKO (red traces) tdTomato+ neurons. Proprioceptive neurons to ensure that impaired proprioception in Pvalb-Cre; Ai9; Piezo2cKO mice in voltage-clamp experiments. NR, non-responsive to mechanical displacements. (b) Example traces of mechanically induced currents in WT (black traces) and cKO (red traces) tdTomato+ neurons. Holding potential, −60 mV. (c) Current-clamp recordings of WT (black traces) and cKO tdTomato+ neurons (red traces). Depolarizing currents were injected in 50-pA increments from a holding current of 0 pA. All vertical scale bars represent 200 pA and all horizontal scale bars represent 25 ms. Numbers adjacent to traces indicate the number of neurons represented by the trace of the total number of neurons tested.

**Piezo2 mediates RA MA currents in proprioceptors in vitro**

Next, we asked whether Piezo2 is required for proprioceptor mechanotransduction. To address this, we performed whole-cell patch-clamp recordings in tdTomato+ neurons isolated from Pvalb-Cre; Ai9; Piezo2cKO and Pvalb-Cre; Ai9 WT DRG (Supplementary Table 1). Consistent with our earlier experiments (Fig. 1b), all WT neurons responded to mechanical stimulation, with the majority of the responses (89.5%) displaying RA characteristics (τ = 5.89 ± 0.47). In contrast, RA currents were observed in only 8.3% (2 of 24) of Piezo2-deficient tdTomato+ neurons (Fig. 3a,b). One of the two residual RA currents exhibited currents comparable to those of WT neurons (Supplementary Fig. 6a), whereas the other RA response was small in amplitude (Fig. 3b and Supplementary Fig. 6a). These remaining RA currents in tdTomato+ cKO neurons might be a result of incomplete Piezo2 deletion, as we observed a similar percentage (11.2%) of Pvalb+ neurons with intact Piezo2 in Pvalb-Cre; Piezo2cKO DRG (Supplementary Fig. 4b). In contrast, the percentage of IA current responses increased slightly in the Pvalb-Cre; Ai9; Piezo2cKO neurons (Fig. 3a,b and Supplementary Fig. 6b). This suggests that the IA currents are not mediated by Piezo2 and might indicate a possible compensatory increase in other MA channels in the absence of Piezo2.

The presence of MA currents detectable in a voltage-clamp setting does not address the issue of biological relevance of these currents to neuronal excitation. We therefore used current-clamp recordings to address whether mechanical stimulation of the soma could elicit
action potentials in WT and Piezo2-deficient tdTomato+ neurons (Fig. 3c). In WT neurons, action potentials were evoked by mechanical stimulation in 94.7% of the cases, as compared with only 20.8% in Piezo2 cKO neurons. The difference in mechanically induced action potential firing was not a result of a deficiency of excitability in Piezo2 cKO neurons, as action potentials could be elicited in all WT and Piezo2 cKO neurons by current injection (Fig. 3d). Notably, although the resting potential and voltage threshold for action potentials were

Figure 4  Ex vivo recordings of stretch-sensitive muscle afferent activities in two Piezo2 conditional knockout mice.  
(a,b) Percentage of muscles with stretch-sensitive MS afferent activity in adult Piezo2fl/+ (WT) and Pvalb-Cre; Piezo2cKO mice (a: Pearson chi-square, $\chi^2 = 9.479$, df = 1, $P < 0.05$) and in Piezo2fl/+ (WT) and HoxB8-Cre; Piezo2cKO mice (b: Pearson chi-square, $\chi^2 = 10.811$, df = 1, $P < 0.05$). (c,d) Average baseline instantaneous firing rate (Hz, top) and stretch response (bottom, firing rate during static phase of stretch – baseline firing rate; sample area labeled on g) from WT and Pvalb-Cre; Piezo2cKO mice (c) and WT and HoxB8-Cre; Piezo2cKO mice (d). (e,f) Calculated stretch responses from four identified MS afferents from WT (black) and one MS afferent from Pvalb-Cre; Piezo2cKO mice (red) and from 11 identified MS afferents from WT (black) and two MS afferents from HoxB8-Cre; Piezo2cKO mice (red) (f). Stretch responses were calculated by subtracting baseline instantaneous firing frequency (Hz) from instantaneous firing frequency during static phase of stretch. (g) Two representative responses to stretch from WT muscles (control littermates of Pvalb-Cre; Piezo2cKO mice). Top trace includes a single identified MS afferent. Middle trace is an example of multiple units firing during baseline and stretch. Individual unit responses could not be determined from this sample, although all units paused during twitch contraction. The length of the muscle is shown on bottom. (h) Top trace, only stretch-responsive sample recorded in Pvalb-Cre; Piezo2cKO muscle. Middle trace, representative of a non-responsive sample site. (i) Two representative responses to stretch from WT muscles (control littermates of HoxB8-Cre; Piezo2cKO mice) similar to those shown in g. (j) Two stretch-responsive samples recorded in HoxB8-Cre; Piezo2cKO muscle. For all panels, Pvalb-Cre; Piezo2cKO mice = 4, WT mice = 3; HoxB8-Cre; Piezo2cKO mice = 4, WT mice = 5; both EDL muscles were used for analysis except for 1 WT animal for Pvalb-Cre.
similar between WT and Piezo2 cKO neurons, the input resistance of Piezo2-deficient neurons was higher. Thus, less depolarizing current was required to elicit an action potential in cKO neurons (Supplementary Table 1). Taken together, our in vitro DRG recording data suggests that Piezo2 is the major mechanotransduction channel of proprioceptive neurons in vitro.

Piezo2 is required for MS afferent activity
To analyze proprioceptor mechanotransduction in a more physiologically relevant setting, we assayed stretch-evoked neuronal activity in WT and Piezo2 cKO mice using an ex vivo muscle-nerve preparation\(^\text{26}\). In this analysis, the extensor digitorum longus (EDL) muscle was stretched while the innervating sciatic nerve was sampled in at least two places to find stretch-induced nerve activity\(^\text{26}\). Muscles from all groups generated maximal tetanic forces in the healthy range\(^\text{27}\), and no group differences in age, baseline muscle length (Lo) or contractile properties were observed between Piezo2 cKO mice and their WT controls in both strains (Supplementary Table 2).

Stretch-sensitive muscle afferent activity was observed in most (5 of 5 control muscles for Pvalb-Cre, 9 of 10 control muscles for HoxB8-Cre) WT muscles, whereas only one of the eight muscles for each Piezo2 cKO line exhibited any increase in afferent firing frequency during stretch (Fig. 4a,b). In WT mice, we detected at least two sample sites with stretch-sensitive activity on the nerve per muscle (5 of 5 control muscles for Pvalb-Cre, 7 of 10 control muscles for HoxB8-Cre). In Piezo2 cKO mice, we only detected one sample site from one Pvalb-Cre; Piezo2\(^{\text{cKO}}\) muscle and two sample sites from one HoxB8-Cre; Piezo2\(^{\text{cKO}}\) muscle that exhibited stretch-sensitive activity.

In all WT sample sites, average firing rate following stretch increased (Fig. 4c,d), suggesting the presence of MS afferents. In contrast, no change in firing frequency during stretch occurred in most Piezo2 cKO sample sites (Fig. 4c,d). In addition, although baseline firing activity was observed in all WT sample sites (Fig. 4c,d), there was sporadic or no baseline firing activity in most Piezo2 cKO sample sites (Fig. 4c,d). This is consistent with the observation that most baseline activity in control muscles can be identified as MS afferent activity\(^\text{26}\). To confirm that the stretch-sensitive activity that we observed was from MS afferents, we next identified and characterized the responses of individual afferents when possible (examples of sample sites where similarities in spike shape made individual unit discrimination impossible are shown in Fig. 4e,i). All identified WT afferents displayed frequency adaptation during the hold phase of stretch, increased firing frequency in response to increasing stretch lengths (Fig. 4e,f) and paused during twitch contraction, behaviors characteristic of MS afferents\(^\text{26}\). The three Piezo2 cKO afferents exhibited a very small response to stretch (Fig. 4e,f,h,j), and the single Pvalb-Cre; Piezo2\(^{\text{cKO}}\) afferent only increased firing rate at the highest stretch length (Fig. 4e,h). In all of the muscles tested, we were able to record a group III/IV afferent response to lactic acid pH 6 administration, suggesting that the sensory deficit in Piezo2 cKO mice was limited to decreased stretch sensation (Supplementary Fig. 7). Collectively, our data demonstrate that the lack of Piezo2 MA channels results in a marked decrease in stretch-sensitive neuronal activity in proprioceptive muscle afferents ex vivo.

**DISCUSSION**
Mammalian proprioceptors have been characterized for nearly 50 years\(^\text{10,28}\), yet the identity of the mechanotransduction channel underlying stretch activation in these neurons has remained unknown\(^\text{4,29}\). Our data show that Piezo2 is the principal MA ion channel of proprioceptors in mice. First, we found strong Piezo2 expression in MS and GTO proprioceptive sensory endings. Second, independent Piezo2-deficient mouse models revealed that proprioceptor mechanotransduction was dependent on Piezo2, as assayed by in vitro electrophysiological recordings of DRG neurons and an ex vivo muscle-nerve preparation. Finally, we observed severe proprioceptive behavioral deficits in both Piezo2-deficient strains.

Piezo2 appears to be the primary mechanosensor of proprioceptors; however, our data raise the possibility that other mechanosensor(s) exist in these neurons. Our in vitro DRG recording data show that some RA and IA MA currents were still present in Piezo2-deficient Pvalb\(^{+}\) neurons. The residual RA currents can be explained by intact Piezo2 proteins from incomplete deletion by Pvalb-Cre (11.2% Pvalb\(^{+}\) DRG neurons still retained Piezo2 in Pvalb-Cre; Piezo2\(^{\text{cKO}}\) DRG). In contrast, IA MA currents were slightly upregulated in Piezo2-deficient neurons compared with WT neurons. This observation suggests that IA MA currents are mediated by Piezo2-independent mechanosensor(s), and that its upregulation might indicate a potential compensatory mechanism caused by Piezo2 ablation. It is, however, also possible that IA currents are more abundantly observed in Piezo2-deficient neurons because robust RA Piezo2 currents mask them in WT neurons. Notably, given that the Pvalb-Cre activity is not completely specific to proprioceptors, we do not currently know whether these Piezo2-independent IA currents are generated in Pvalb\(^{+}\) proprioceptors or Pvalb\(^{+}\) cutaneous mechanoreceptors\(^\text{19}\).

There was less evidence for a second proprioceptive mechanosensitive channel from our ex vivo muscle-nerve recordings and behavioral analyses. In ex vivo muscle-nerve recordings, approximately 12% of Piezo2 cKO muscles (one of eight total muscles for both Piezo2 cKO lines) still showed stretch-activated MS-afferent firing; however, the observed stretch-sensitive responses were mostly atypical, and this could reflect the presence of residual Piezo2 (see above). At the behavioral level, there was no clear evidence for an additional mechanosensor in proprioceptors as the two lines of Piezo2 cKO mice showed full coordination defects. Taken together, our data raise the possibility that a secondary mechanotransducer channel might exist in proprioceptors; however, the identity of the channel(s) responsible for the remaining MA currents is currently unknown. Piezo1, a related mechanosensitive ion channel, is not expressed at high enough levels in sensory neurons to be a likely candidate\(^\text{16}\), but this cannot be ruled out.

Regardless of the possibility of a second MA channel, our data suggest that Piezo2-deficient proprioceptive neurons are mostly mechanically insensitive. What happens to silenced proprioceptors with Piezo2 ablation? Anatomically, although MS-afferent endings in Piezo2 cKO muscles overall appeared to be healthy, less organized MS endings were sporadically observed, suggesting that the maintenance of MS endings might depend on the activity of proprioceptors. In addition, our in vitro DRG recordings revealed that the input resistance was increased in Piezo2-deficient Pvalb\(^{+}\) neurons, whereas the resting potential and voltage threshold for action potentials were similar between WT and Piezo2-deficient neurons. Increased input resistance caused Piezo2-deficient neurons to become more excitable (that is, smaller depolarizing receptor current was needed for these neurons to fire). It is possible that a lack of mechanotransduction activity in these neurons caused the observed compensatory increase in excitability via modulating expression or function of other ion channels. A similar homeostatic change has been observed in hippocampal pyramidal neurons after complete genetic ablation of glutamatergic synaptic transmission\(^\text{30}\). This might represent a mechanism for the neuron to increase its gain in response to a lack of input signal.

Our in vitro DRG recordings showed that Piezo2-deficient proprioceptive neurons, although mechanically insensitive, were still
able to generate action potentials. These studies also revealed that MA currents mediated by proprioceptors were mainly RA. Given that MS afferents produced slowly adapting (SA) responses in response to stretch \textit{ex vivo}, this phenomenon is reminiscent of mechanotransduction of Merkel cell-neurite complexes in the skin, where the presence of Piezo2 in both Merkel cells and sensory afferents may explain how RA transduction currents can be used to generate SA firing in sensory afferents\(^{14,17}\). How RA currents generated in proprioceptors \textit{in vitro} convert to SA responses \textit{in vivo} is currently unknown. It is possible that the main discrepancy is a result of the \textit{in vitro} experimental setting, as measurements on the soma of isolated neurons may not fully recapitulate channel properties in the receptive terminals. For example, these RA currents might be modulated by accessory proteins only present in the nerve endings\(^{29}\). It is also possible that, similar to the case of Merkel cell-neurite complexes, intrafusal muscle cells express an unknown mechanosensor that modulates Piezo2 activity in MS afferents\(^{31}\).

Overall, our findings elucidate a long-standing puzzle regarding the identity of the mechanotransducer of proprioceptors in mammals. Proprioception, the elusive ‘sixth’ sense, is crucial for coordinating the activity of motor neurons and skeletal muscles to achieve basic tasks such as standing and walking. Indeed, various diseases that affect the function of skeletal muscles, motor neurons or sensory neurons lead to profound pathological consequences. Notably, gain-of-function mutations in human PIEZO2 are associated with distal arthrogryposis type 5 (DA5)\(^{32}\). These patients develop neuromuscular and connective tissue disorders, and it has been suggested that PIEZO2 is involved in the development of joints and the neuromuscular system\(^{32}\). Although the expression of human PIEZO2 in proprioceptors and the skeletal system has not been determined, mouse Piezo2 is selectively expressed in proprioceptors and not in skeletal muscle cells. Our findings raise the possibility that DA5 could arise from dysregulated mechanotransduction in proprioceptors.

**METHODS**

Methods and any associated references are available in the online version of the paper.

**Note:** Any Supplementary Information and Source Data files are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

V.L. performed whole-cell electrophysiology in isolated DRG neurons. J.C.d.N. characterized Piezo2-cKO muscles in the laboratory of T.M.J.D.Z., C.R.C. and K.A.W. contributed to data collection and analyses for \textit{ex vivo} muscle-nerve recordings in the laboratory of K.A.W. A.F. isolated DRG neurons for whole-cell electrophysiology. S.-H.W. contributed to all of the other experiments. S.-H.W., V.L., K.A.W. and A.F. wrote the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. All animal procedures were approved by The Scripps Research Institute and San Jose State University Institutional Animal Care and Use Committees. Animals were maintained on a 12-h light/dark cycle (lights on from 6 a.m. to 6 p.m.). Five adult animals or less were housed in one cage. Both sexes were used for all experiments except for ex vivo muscle-nerve recordings in which only male mice were used.

Mouse lines. Piezo2GFP, IRES-Cre (Piezo2GFP); Pvlab-Cre (The Jackson Laboratory, stock #6069); Piezo2GFP; HoxB8Cre;2; A9 (The Jackson Laboratory, stock # 7909). Note that Pvlab-Cre; Piezo2KO and HoxB8-Cre; Piezo2KO lines refer to Pvlab-Cre; Piezo2GFP and HoxB8-Cre; Piezo2GFP mice. For these Piezo2 cKO lines, Piezo2GFP+ mice were used as WT controls. For Figures 2c and 3 and Supplementary Figures 4c and 6, Pvlab-Cre; A9; Piezo2KO and Pvlab-Cre; A9 (WT control) mice were used to label proprioceptive neurons.

Immunofluorescence. Skeletal muscles (from hind limbs), lumbar DRG, spinal cord from the lumbar region, and dorsal skin were isolated from postnatal and adult mice, following perfusion in 4% paraformaldehyde (PFA)/phosphate-buffered saline (PBS) (wt/vol). All tissues were post-fixed in 4% PFA for 30 min to 2 h on ice and then incubated in 30% sucrose/PBS (wt/vol) overnight at 4 °C. Next day, fixed tissues were cryo- preserved in OCT and cryo-sectioned at 8 µm (for DRG and spinal cord), 20 µm (for skin) and 50–80 µm (for skeletal muscles) for immunofluorescence (IF) as previously described. For IF staining, frozen sections were blocked in 10% normal goat serum (vol/vol), 1% bovine serum albumin (BSA), wt/vol and PBS for 30 min at 25 °C. All primary antibodies were incubated in 1% BSA/PBS overnight at 4 °C. Next day, sections were incubated with secondary antibodies for 30 min at 25 °C temperature.

Antibodies. GFP (1:500, Life Technologies, A10262), Pvlab (rabbit, 1:500, Swant, PV 2; chicken, 1:10,000, ref. 19), Vglut1 (1:16,000)33, Runx3 (1:50,000)34, α-Bungarotoxin (1:500, Life Technologies, B13423), Piezo2 (1:1,000)17, Nef (1:1,000, Aves Labs, NFFH).

In situ hybridization. Lumbar DRG were isolated from adult mice and fixed in 4% PFA/PBS for 4 h on ice. They were then incubated in 30% sucrose/PBS (wt/vol) overnight at 4 °C. Next day, fixed tissues were cryo- preserved in OCT and cryo- sectioned at 8 µm. Sections were subjected to in situ hybridization according to manufacturer’s protocols (Advanced Cell Diagnostics). Bright puncta (instead of a putative diffuse staining pattern) represent true RNA signals.

Quantitative RT-PCR (qRT-PCR). Lumbar DRG and cervical/upper thoracic DRG from adult mice (n = 3 mice from 3 independent litters) were isolated and placed directly in TRIzol. Total RNA was isolated and subjected to qRT-PCR as previously described.16,17

DRG cultures and whole-cell electrophysiology. DRG from 4–6-week-old Pvlab-Cre; A9 (WT control) and Pvlab-Cre; A9; Piezo2KO mice were isolated and dissociated for neuronal cultures as previously described.12 All DRG neurons including both tdTomato+ and tdTomato− neurons were plated together onto poly-n-lysine– and laminin-coated coverslips (day 0). DRG neurons were cultured at least overnight and were used for patch-clamp recording experiments from days 1–3.

Patch-clamp experiments were performed in standard whole-cell configuration using an Axopatch 200B amplifier (Axon Instruments). Patch pipettes were pulled to a resistance of 2–4 MΩ using a Flaming-Brown P97 pipette puller (Sutter Instruments). Pipette solutions for I-V measurements consisted of (in mM): 137 NaCl, 4 KCl, 1.25 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose (pH adjusted to 7.4 with NaOH). All currents were recorded using a pipette solution consisting of (in mM): 125 potassium glutonate, 7 KCl, 1 CaCl2, 5 MgCl2, 10 HEPES, 4 Na2ATP, and 0.4 Na2GTP (pH adjusted to 7.3 with CsOH). The extracellular solution for I-V measurements consisted of (in mM): 40 TEA-Cl, 100 NaCl, 4 KC1, 1.25 CaCl2, 1 MgCl2, 10 HEPES, 4 Na2ATP, and 0.4 Na2GTP (pH adjusted to 7.3 with CsOH) and an extracellular solution consisting of (in mM): 137 NaCl, 4 KC1, 1.25 CaCl2, 1 MgCl2, 10 HEPES, 10 glucose (pH adjusted to 7.4 with NaOH). All experiments were conducted at 25 °C. Currents were sampled at 5 kHz and filtered at 2 kHz. Mechanical stimulation of DRG cell bodies was achieved as described previously.13 For I-V recordings, neurons were held at 0 mV and stepped to test voltages for duration of 4 s to allow for a steady state in residual voltage-activated currents. The mechanical stimulus was applied at the end of the 4 s interval. Background currents before mechanical stimuli were subtracted offline from recorded traces.

Muscle spindle afferent recordings from isolated muscle nerve preparation. Muscle sensory neuron response to physiological stretch was assayed ex vivo using an isolated muscle-nerve preparation as previously described. Briefly, the extensor digitorum longus (EDL) muscle and deep peroneal branch of the sciatic nerve were placed in an oxygenated tissue bath. The tendons were sutured to a tissue post and a lever arm connected to a dual force and length controller and transducer (300C-LR, Aurora Scientific). Baseline muscle length was set at the length at which maximal twitch contractile force was generated (Lo). A suction electrode (tip diameter 50–100 µm) connected to an extracellular amplifier (Model 1800, A&M Systems) was used to sample sensory activity. The nerve was sampled to find stretch sensitive sensory activity and a series of 9 ramp and hold stretches were given (3 stretches each to Lo plus 2.5%, 5% and 7.5% Lo; ramp speed 40%/s; 1 stretch; held 4 s with 1 min in between each stretch). Sensory response to a series of 60 twitch contractions given at 1-Hz frequency was then sampled to look for a characteristic pause in muscle spindle afferent firing during contraction. If no stretch sensitive activity was found, the response to stretch at 2–3 unique electrode placements on the nerve was still recorded to ensure that stretch sensitive activity did not appear following repeated stretch. Sensory response to a 10-min exposure to a 15 mM lactic acid (pH 6) solution was also measured to ensure that the sensory deficit was specific to stretch. Muscle health was determined by comparing the maximal contractile force during a tetanic stimulation (500-ms train, 120-Hz frequency, 0.5–ms pulse width, supramaximal voltage) to previously reported values. The number of muscles in which stretch sensitive activity could be recorded was determined for each group. For each electrode placement, we quantified the average baseline firing rate and the static stretch response (firing rate during the plateau phase of stretch (3.25–3.75 s into the stretch; see Fig. 4g) – baseline firing rate). Individual sensory neurons were identified by spike shape when possible by using the Spike Histogram feature of Lab Chart (AD Instruments; Fig. 4g). An individual neuron was classified as a putative muscle spindle afferent if it displayed a characteristic instantaneous frequency response to stretch as well as a pause during twitch contraction. Spindle afferent baseline firing rate and static stretch response were then determined as described above.

Sample size and randomization. For ex vivo muscle spindle afferent recordings: Pvlab-Cre; Piezo2KO mice = 4 from 2 independent litters and WT mice = 3 from 3 litters; HoxB8-Cre; Piezo2KO mice = 4 from 3 litters and WT mice = 5 from 3 litters. For all other experiments (except in Supplementary Fig. 5: 3 mice from 3 independent litters were used for qRT-PCR and in situ hybridization), at least 4 mice from at least 4 independent litters per genotype were used. Immunofluorescent staining yielded consistent results for every experiment, so our current sample size is sufficient. In vitro DRG recordings and ex vivo muscle-nerve recordings mostly yielded all or none responses for all recording experiments; therefore, our current sample size is justified. All experiments were repeated at least three times. All animals were randomly selected for experiments. Immunofluorescent studies were performed in a blind manner. Conditional knockout mouse characterization could not be performed in a blind manner due to behavioral conditions observed in Piezo2 conditional knockout mice.

A Supplementary Methods Checklist is available.

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