$Na_V1.1$ is essential for proprioceptive signaling and motor behaviors

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ABSTRACT

The voltage-gated sodium channel (Na_V), $Na_V1.1$, is well-studied in the central nervous system; conversely, its contribution to peripheral sensory neuron function is more enigmatic. Here, we identify a new role for $Na_V1.1$ in mammalian proprioception. RNAscope analysis and *in vitro* patch clamp recordings in genetically identified mouse proprioceptors show ubiquitously channel expression and significant contributions to intrinsic excitability. Notably, genetic deletion of $Na_V1.1$ in sensory neurons caused profound and visible motor coordination deficits in conditional knockout mice of both sexes, similar to conditional Piezo2-knockout animals, suggesting this channel is a major contributor to sensory proprioceptive transmission. *Ex vivo* muscle afferent recordings conditional knockout mice found that loss of $Na_V1.1$ leads to inconsistent and unreliable proprioceptor firing characterized by action potential failures during static muscle stretch; conversely, afferent responses to dynamic vibrations were unaffected. This suggests that while a combination of Piezo2 and other Na_V isoforms are sufficient to elicit activity in response to transient stimuli, $Na_V1.1$ is required for transmission of receptor potentials generated during sustained muscle stretch. Impressively, recordings from afferents of heterozygous conditional knockout animals were similarly impaired, and heterozygous conditional knockout mice also exhibited motor behavioral deficits. Thus, $Na_V1.1$ haploinsufficiency in sensory neurons impairs both proprioceptor function and motor behaviors. Importantly, human patients harboring $Na_V1.1$ loss-of-function mutations often present with motor delays and ataxia; therefore, our data suggest sensory neuron dysfunction contributes to the clinical manifestations of neurological disorders in which $Na_V1.1$ function is compromised. Collectively, we present the first evidence that $Na_V1.1$ is essential for mammalian proprioceptive signaling and behaviors.

59 **INTRODUCTION**

60 Voltage gated sodium channels (Na_Vs) are critical mediators of neuronal excitability and are
61 responsible for action potential generation and propagation (Ahern et al., 2016; Bean, 2007) . In
62 the mammalian nervous system there are nine isoforms (Na_V1.1-1.9), each with unique
63 biophysical properties, as well as distinguishing cellular expression and subcellular localization
64 patterns (Bennett et al., 2019; Catterall, 2017). Of these different subtypes, Na_V1.1 is notable for
65 its role in brain disease (Escayg and Goldin, 2010; Mulley et al., 2005; Ogiwara et al., 2007).
66 Indeed, *Scn1a*, the gene that encodes Na_V1.1, is referred to as a "super culprit" gene, with over
67 1,000 associated mutations that lead to abnormal brain function, resulting in brain disorders such
68 as epilepsy and migraine, as well as neurodivergent phenotypes, such as autism spectrum
69 disorder (Ding et al., 2021; Lossin, 2009). Homozygous *Scn1a*$^{-/-}$ global knockout mice are ataxic
70 and die by P15, while heterozygous *Scn1a*$^{+/-}$ animals develop seizures and begin to die
71 sporadically starting at P21 (Yu et al., 2006). In addition to the central nervous system, Na_V1.1 is
72 also expressed in the peripheral nervous system (Sharma et al., 2020; Usoskin et al., 2015); yet,
73 the prominent role this channel plays in brain function has left its physiological roles in sensory
74 neuron populations understudied.

75 Peripheral sensory neurons of the dorsal root and trigeminal ganglia (DRG and TG,
76 respectively) are tasked with encoding somatic sensations, such as touch, temperature, pain, and
77 proprioception, and are anatomically and functionally heterogenous (Kupari et al., 2021; Nguyen
78 et al., 2021; Oliver et al., 2021a; Wu et al., 2021). *Scn1a* transcript and protein have been
79 observed primarily in myelinated mechanosensory DRG and TG neurons (Fukuoka et al., 2008;
80 Ho and O'Leary, 2011; Osteen et al., 2016). Indeed, subcutaneous injection of the Na_V1.1
81 activator, Hma1, into mouse hind paw causes non-inflammatory mechanical pain and
82 spontaneous pain behaviors (Osteen et al., 2016). Interestingly, pharmacological inhibition of
83 Na_V1.1 does not affect mechanical thresholds in uninjured mice but does reduce mechanical pain

84 in a spared-nerve injury model (Salvatierra et al., 2018), suggesting $Na_V1.1$ may have a more
85 prominent role in mechanical pain as opposed to normal touch sensing. $Na_V1.1$ in TG neurons
86 has also been reported to mediate mechanical pain in an orbitofacial chronic constriction injury
87 model (Pineda-Farias et al., 2021). In addition to somatosensory neurons, $Na_V1.1$ is found in
88 colon-innervating vagal neurons, where it contributes to firing of colonic mechano-nociceptors
89 and is upregulated in a mouse model of chronic visceral hypersensitivity (Osteen et al., 2016;
90 Salvatierra et al., 2018). Lastly, $Na_V1.1$ contributes to action potential firing in a subset of DRG
91 neurons that express the cold sensitive ion channel, transient receptor potential melastatin 8
92 (TRPM8), suggesting the channel may also contribute to thermosensory transmission (Griffith et
93 al., 2019). While most data support a role for $Na_V1.1$ in pain, the limited number of studies that
94 have investigated $Na_V1.1$ function in sensory neurons has left gaps in our knowledge regarding
95 other potential roles this channel may play in somatosensation.

96 Given the relatively under-explored role of $Na_V1.1$ in the peripheral nervous system, we
97 set out to determine what other somatosensory modalities rely on $Na_V1.1$ expression in sensory
98 neurons. Here, we show that 100% of proprioceptors express *Scn1a* mRNA, where it makes
99 notable contributions to the somal whole-cell sodium current and intrinsic excitability. A functional
100 role for $Na_V1.1$ in proprioceptive signaling was also supported by *ex-vivo* electrophysiological
101 recordings from functionally identified muscle spindle afferents. Importantly, mice lacking $Na_V1.1$
102 in all sensory neurons display visible and profound motor deficits and ataxic-like behavior, which
103 were quantified in rotarod and open field assays. Surprisingly, we found $Na_V1.1$ is haploinsuffcient
104 for normal proprioceptor function and behavior, in *ex vivo* recordings and the open field assay,
105 respectively. Collectively, our data provide the first evidence that peripherally expressed $Na_V1.1$
106 is critical for sensory proprioceptive signaling and motor coordination.
107

108 **RESULTS**

109 Most studies have localized $Na_V1.1$ expression primarily to myelinated sensory neurons
110 that transmit mechanical signals (Fukuoka et al., 2008; Ho and O'Leary, 2011; Osteen et al.,
111 2016b; Wang et al., 2011). In line with prior work, RNAscope analysis of DRG sections from adult
112 mice showed that 93% of myelinated neurons, as determined by neurofilament heavy chain (NFH)
113 labeling, express *Scn1a* transcripts (**Fig 1A**). RNA-sequencing datasets have consistently
114 identified $Na_V1.1$ expression in proprioceptors; thus, we next analyzed $Na_V1.1$ expression in these
115 cells using a Parvalbumin^{Cre};Rosa26^{Ai14} reporter line (*Pvalb*^{Ai14}) and found 100% of genetically
116 identified proprioceptors were positive for *Scn1a* message (**Fig 1B**). This contrasted with low
117 expression of *Scn1a* mRNA in both calcitonin gene related peptide (CGRP) expressing neurons,
118 which represent peptidergic nociceptors, and non-peptidergic polymodal *Mrgprd*-expressing
119 nociceptors (24% and 3%, respectively, **Fig 1C-D**). Frequency and cumulative distribution plots
120 show the spread of integrated fluorescence density measurements obtained for *Scn1a* transcripts
121 in proprioceptors (**Fig 1E-F**).

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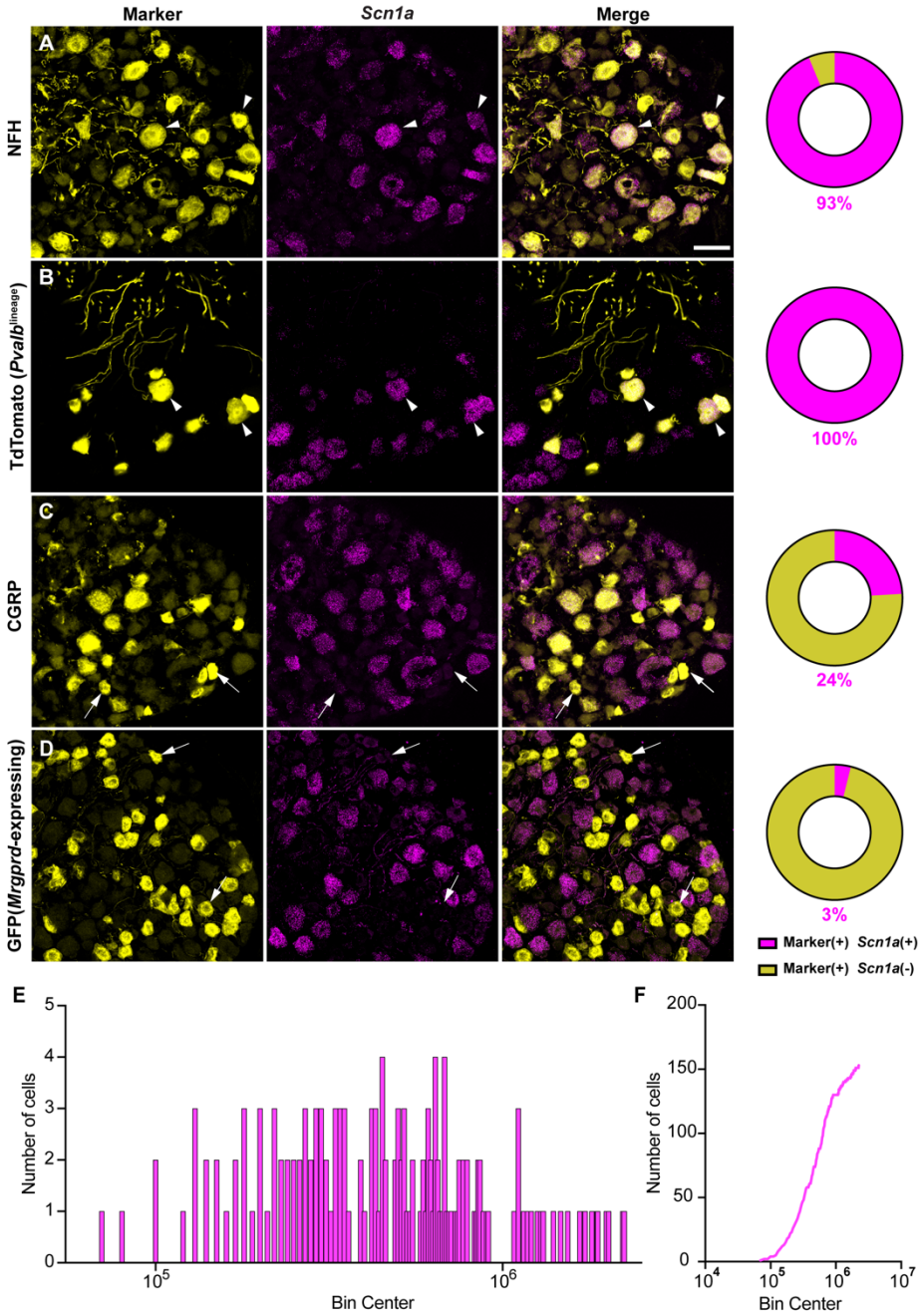

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125 **Figure 1. $Na_V1.1$ is ubiquitously expressed in genetically identified proprioceptors**. **A-D**, Representative confocal
126 images of cryoprotected adult DRG sections (25 μm) with pie chart quantifications indicating the percentage of *Scn1a*+
127 and *Scn1a* - neurons in each subpopulation (magenta and yellow, respectively). Images were acquired with a 40X, 0.9
128 NA water-immersion objective. Sections were hybridized using RNAscope with probes targeting *Scn1a* (*Scn1a*,
129 magenta) and stained with the following antibodies (yellow): (**A**) anti-neurofilament heavy (NFH, n=787) (**B**) anti-DsRed
130 to label TdTomato+ proprioceptors (n=143) (**C**) anti-calcitonin gene-related peptide (CGRP, n=877) and (**D**) anti-GFP
131 to label *Mrgprd*+ neurons. DRG from C57BL/6, *Pvalb*$^{Cre;}$*Rosa*26^{Ai14}, and *Mrgprd*^{GFP} mice of both sexes were used.
132 Scale bar 50μm. White arrowheads indicate *Scn1a* + neurons while white arrows indicate *Scn1a* - neurons. Frequency

133 (**E**) and cumulative (**F**) distribution plots of integrated fluorescence density of the *Scn1a* signal in TdTomato+
134 proprioceptors (n=153). n = cells.

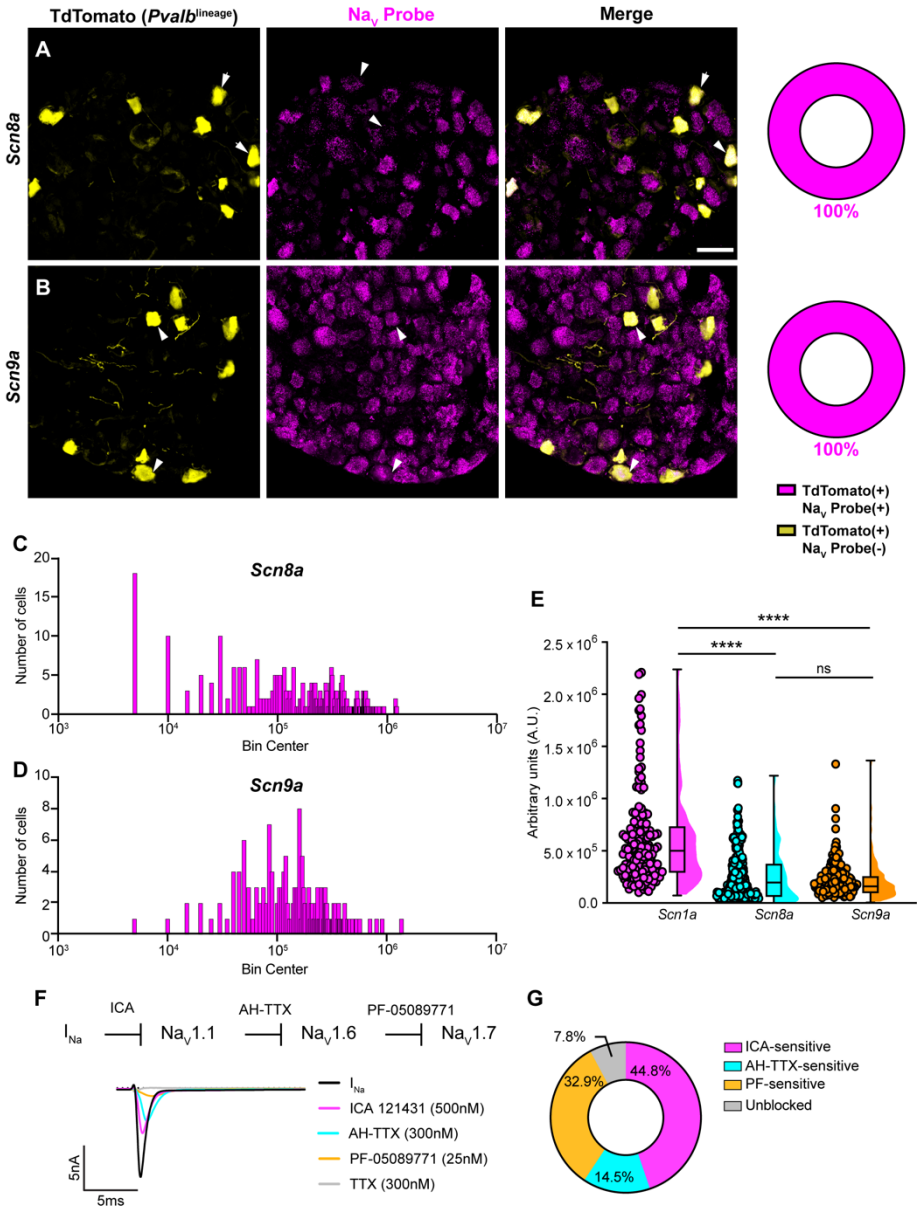
135 In addition to $Na_V1.1$, $Na_V1.6$ and $Na_V1.7$ expression has also been reported in proprioceptors
136 (Carrasco et al., 2017). As with *Scn1a*, mRNA for *Scn8a* and *Scn9a* is also found in 100% of genetically
137 identified proprioceptors (**Fig 2A**-**B**). Cumulative distribution plots of *Scn8a* and *Scn9a* integrated
138 fluorescence density measurements showed higher variability as compared to $Na_V1.1$ (**Fig 2C-D**, **Fig**
139 **1E**). This was quantified using the coefficient of variation, a relative measure of the extent of variations
140 within data. The coefficient of variation for *Scn1a* transcript expression was calculated to be 75.6,
141 whereas this value increased to 97.3 and 88.1 for *Scn8a* and *Scn9a*, respectively. This indicates that
142 while all three isoforms are ubiquitously expressed in proprioceptors, the relative levels differ, with $Na_V1.1$
143 having the most consistent level of expression across neurons analyzed. Furthermore, the average
144 integrated density of the $Na_V1.1$ signal for a given proprioceptive DRG neuron was significantly higher
145 than both $Na_V1.6$ and $Na_V1.7$ (**Fig 2E**, $p < 0.0001$).

146 Due to the ubiquitous expression of $Na_V1.1$, $Na_V1.6$, and $Na_V1.7$ in proprioceptors, we
147 sought to determine the relative contributions of these isoforms to the proprioceptor whole-cell
148 sodium current (I_{Na}). We performed *in vitro* voltage-clamp experiments on TdTomato+ neurons
149 harvested from thoracic spinal levels of adult *Pvalb*^{Ai14} mice (de Nooij et al., 2013) and used serial
150 application of selective Na_V channel antagonists to determine the specific contributions of $Na_V1.1$,
151 $Na_V1.6$, and $Na_V1.7$ (**Fig 2F**). We first applied the selective $Na_V1.1$ antagonist ICA 1214314 (ICA,
152 500 nM), followed by 9-Anhydroustetrodoxin (AH-TTX, 300 nM), which is a selective $Na_V1.6$
153 blocker but also partially blocks $Na_V1.1$ (Denomme et al., 2020; Griffith et al., 2019). We reasoned
154 that by first blocking the $Na_V1.1$ mediated current, the effect of AH-TTX should largely be due to
155 inhibition of $Na_V1.6$. Finally, we blocked $Na_V1.7$ channels using the antagonist PF-05089771 (25
156 nM), followed by tetrodotoxin (TTX, 300 nM) to block any residual current, as proprioceptors do
157 not express TTX-resistant Na_Vs. On average, 7.8% of the current remained unblocked following
158 serial application of these antagonists due to incomplete block by the drugs used. We found that
159 the ICA-sensitive component of I_{Na} was 44.8% of the total current. Conversely, 14.5% and 32.9%

160 of I_{Na} was sensitive to AH-TTX and PF-05089771, respectively (**Fig 2G**). No significant effect of

161 the 0.1% DMSO vehicle solution on I_{Na} amplitude was observed (**Fig 2 – figure supplement 1**).

162 Collectively, these data suggest that in proprioceptors $Na_V1.1$ is a dominant functional Na_V



163 subtype.

164

165 **Figure 2. Transcriptomic and functional expression of sodium channels in proprioceptors. A-B**. Representative
166 confocal images of cryoprotected adult DRG sections (25 μm) with quantifications indicating the percentage of Na_V+
167 and Na_V- neurons in TdTomato+ proprioceptors. Sections were hybridized using RNAscope with probes targeting
168 $Na_V1.6$ or $Na_V1.7$ (*Scn8a* and *Scn9a*, respectively, magenta) and stained with anti-DsRed (yellow). (**A**) *Snc8a*, n=298,
169 (**B**) *Scn9a*, n=166. Scale bar set to 50μm. White arrowheads indicate Na_V+ neurons. **C-D.** Frequency distribution plots
170 of integrated fluorescence density of $Na_V1.6$ and $Na_V1.7$ mRNA in TdTomato+ proprioceptors, respectively. **E**. The

average integrated density of *Scn1a*, *Scn8a*, and *Scn9a* RNAscope probe signal. Dots represent individual cells. Statistical significance was determined using a Kruskal-Wallis test with Dunn's post hoc comparisons. **F.** Top, experimental workflow of serial pharmacological blockade of Na_V channels expressed in proprioceptors. We first elicited a whole-cell sodium current in the absence of drug. We next bath applied 500nM of ICA 121431 to block current carried by $Na_V1.1$. Subsequently, we bath applied AH-TTX (300nM) to block $Na_V1.6$ mediated current, and PF-05089771 (25nM) to block the $Na_V1.7$ mediated current. Finally, TTX (300nM) was used to block residual current and to confirm there was no contribution of TTX-resistant Na_Vs in proprioceptors. Bottom, representative current traces following application of Na_V selective inhibitors. All drugs were applied for 1 minute. (**G**) Quantification of the average percentage of the whole-cell sodium current that was sensitive to the individual drugs used (n=8). n = cells. ****p<0.0001.

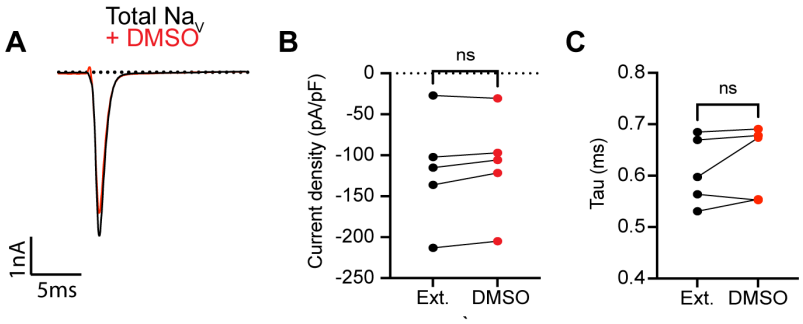


Figure supplement 1. 0.1% DMSO vehicle does not change I_{Na} in proprioceptors. (**A**) Representative current trace before (black trace) and after (red trace) application of 0.1% DMSO to TdTomato+ proprioceptors during *in vitro* electrophysiological experiments. Quantification of current density (**B**) and current decay kinetics (**C**) before (normal external solution) and after DMSO application. A paired t-test was used to determine statistical significance (p = 0.0855, n = 5 cells).

We next determined the biophysical features of the whole-cell sodium current (I_{Na}) in proprioceptors, which has not been previously reported (**Fig 3A-D**). The current-voltage relationship shows the first detectable current appeared at voltages near -50 mV and was maximal at voltages near -30 mV when evoked from a holding potential of -90 mV (**Fig 3B**). Voltage dependence of peak conductance was best fit to a single Boltzmann function and the voltage for half maximal activation was -38.7 mV (**Fig 3C**). The voltage-dependence of inactivation was determined with 40 ms prepulse steps ranging from -120 mV to +10 mV. The midpoint of the inactivation curve was -64.5 mV and was best fit to a single Boltzmann function (**Fig 3D**). To analyze recovery from fast inactivation, TdTomato+ neurons were depolarized to -20 mV, followed by a series of recovery periods ranging from 0.5 ms to 10 ms before a second test step to -20 mV was given to assess sodium channel availability. I_{Na} recovery was rapid (τ = 0.54 ms), with greater than 50% of I_{Na} recovered after 0.5 ms (**Fig 3E**). Finally, entry intro slow inactivation was determined. Cells were held at 0 mV during conditioning voltage steps ranging from 10 ms to 2000 ms, separated by two 2-ms pulses to -20 mV to compare channel availability before and

after the conditioning pulse (**Fig 3F**). The tau for entry into slow inactivation was 928.6 ms, with more than 50% of channels available after a 2000 ms conditioning pulse.

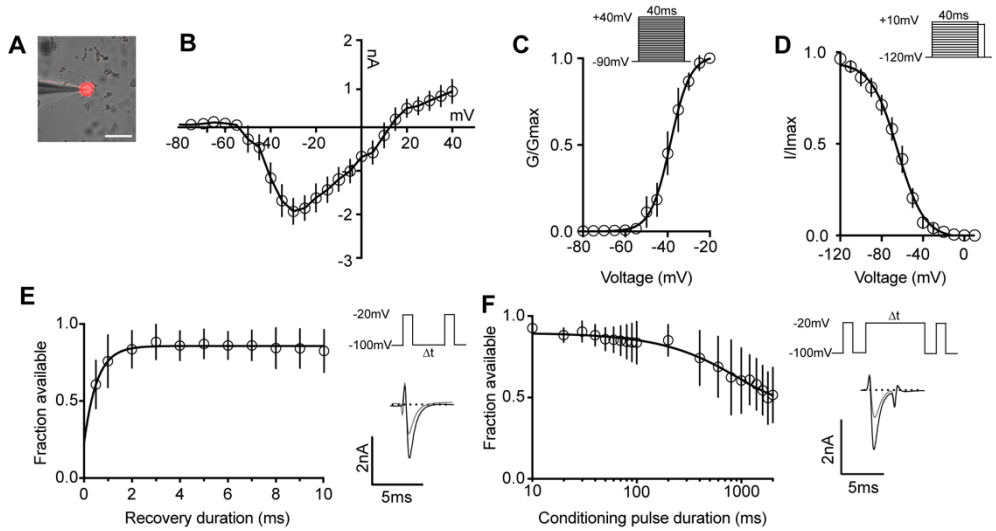


Figure 3. Biophysical analysis of the whole-cell sodium current (I_{Na}) in genetically identified proprioceptors. **(A)** Representative image of a TdTomato+ proprioceptor in culture during electrophysiological recordings (scale bar set to 50μm). **(B)** Current-voltage relationship of I_{Na} from TdTomato+ proprioceptors. Currents were elicited by 40 ms voltage steps from -90 mV to +40 mV in 5 mV increments (n=7-9). (**C**) Top, the voltage protocol used to measure the voltage dependence of for whole-cell sodium current activation in proprioceptors. Currents were elicited using a series of 40 ms voltage steps from -90 mV to 40 mV at 5 mV increments from a holding potential of -90 mV. Data are expressed as conductance over maximum conductance (n=6-9). (**D**) Top, the voltage protocol used to measure the voltage dependence of inactivation. A 40 ms prepulse ranging from -120mV to +10mV was given followed by a test pulse to 0 mV. Data are expressed as current over maximum current (n=8-12). (**E**) Left, quantification of recovery from fast inactivation (n=8). Line shows a monoexponential fit of the data (τ=0.54 ms). Top right, voltage-protocol to measure recovery from fast inactivation. A 20 ms step to -20 mV from -100 mV is followed by varying durations of recovery at -100 mV before a second test step to -20 mV. Bottom right, representative traces of currents elicited before (black trace) and after (grey trace) a 0.5 ms recovery period. (**F**) Left, quantification of entry into slow inactivation (n=6). Line shows a monoexponential fit of the data (τ=928.6 ms). Top right, voltage-protocol to measure entry into slow inactivation. A 3 ms test pulse to -20mV from -100mV was followed by conditioning pulses at 0 mV for varying durations before a third test step to -20mV. 12 ms recovery periods after the first test pulse and before the second were included to remove fast inactivation. Bottom right, representative current trace elicited before and after a 2000 ms conditioning pulse. n = cells.

We next asked how blocking $Na_V1.1$ channels affects proprioceptor function *in vitro*. Similar to serial pharmacological experiments, I_{Na} density in proprioceptors was significantly reduced from ~-140 pA/pF to ~-75 pA/pF following ICA application (**Fig 4B,** p = 0.0003). The proprioceptor I_{Na} had an average tau of 0.6 ms, which was significantly slowed to 1.0 ms following application of ICA (**Fig 4C,** p = 0.0007), in line with loss of a fast gating channel. Blocking $Na_V1.1$ did not change I_{Na} rise time (**Fig 4D,** p = 0.1611). Quantification of the ICA-sensitive component found an average tau of 0.4 ms and an average current density of -96 pA/pF (**Fig 4E**). Of note, there was a wide distribution of current densities for the ICA-sensitive component, ranging from

231 ~-28 pA/pF to ~-263 pA/pF, suggesting some variability in the contribution of $Na_V1.1$ to
232 proprioceptor excitability that may be proprioceptor subtype dependent. We next used current
233 clamp experiments to determine the effect of ICA on proprioceptor intrinsic excitability (**Fig 4F**).
234 Pharmacological inhibition of $Na_V1.1$ significantly reduced the number of evoked action potentials
235 in most genetically identified proprioceptors (**Fig 4G**); however, 5 of the cells recorded had low
236 firing rates that were not inhibited by ICA. This further suggests $Na_V1.1$ is important for repetitive
237 firing in most proprioceptors, but some subtypes with lower intrinsic excitability instead rely on a
238 combination of $Na_V1.6$ and $Na_V1.7$. Action potential amplitude (**Fig 4H**, p = 0.0420) and action
239 potential threshold (**Fig 4I**, p = 0.0186) were also significantly reduced following ICA application,
240 and action potential full-width half-max was significantly increased following ICA application (**Fig**
241 **4K**, p = 0.0068), in line with loss of the fast $Na_V1.1$-mediated current.
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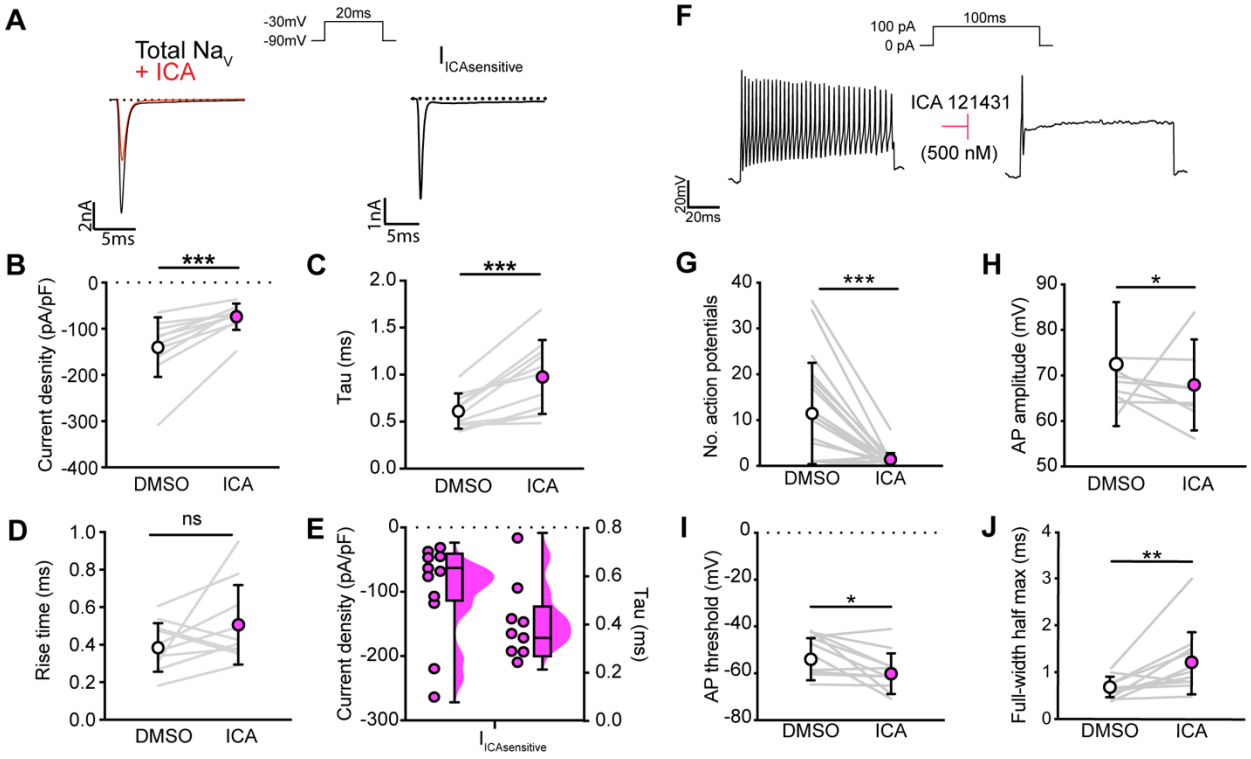

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244 **Figure 4. $Na_V1.1$ significantly contributes to the whole-cell sodium current and intrinsic excitability in**
245 **genetically identified proprioceptors. A.** Left, representative whole-cell voltage-clamp traces elicited before (black)
246 and after (red) application of ICA121431 (500nM). Right, the subtracted ICA-sensitive current shown in black. **B.**

Quantification of the reduction in whole-cell current density before (white) and after (magenta) ICA, p = 0.0003, n=11 cells. **C.** Quantification of rate of current decay before and after ICA, p = 0.0007, n=11 cells. **D.** Quantification of whole-cell current rise time before and after ICA, p = 0.1611, n=10 cells. **E.** Left, current densities of ICA-sensitive sodium currents (n=11), right, current decay taus of ICA-sensitive sodium currents (n=9 cells). **F.** Representative whole-cell current clamp traces before (left) and after (right) application of ICA. **G-J,** Quantification of number of action potentials in response to current injection (**G,** p = 0.0002, n=20 cells), action potential amplitude (**H,** p = 0.0420, n=20 cells), action potential threshold (**I,** p = 0.0186, n=20 cells), and full-width half max (**J,** p = 0.0068, n=20 cells). Grey lines represent paired observations, circles and lines represent means and standard deviations. White circles, before ICA application. The Wilcoxon matched-pairs signed rank test was used to determine statistical significance.

It is important to note that ICA 121431 also blocks $Na_V1.3$ channels, which could be upregulated in our cultured DRG neuron preparations (Wangzhou et al., 2020). Indeed, a small but significant decrease in I_{Na} was observed in recordings from large-diameter DRG neurons harvested from *Pirt*^{Cre};Scn1a-floxed mice (Scn1a-cKO), which lack $Na_V1.1$ in all sensory neurons. Thus, inhibition of upregulated $Na_V1.3$ channels could contribute to the effect of on ICA on the proprioceptor I_{Na} **(Fig 4 - figure supplement 2)**.

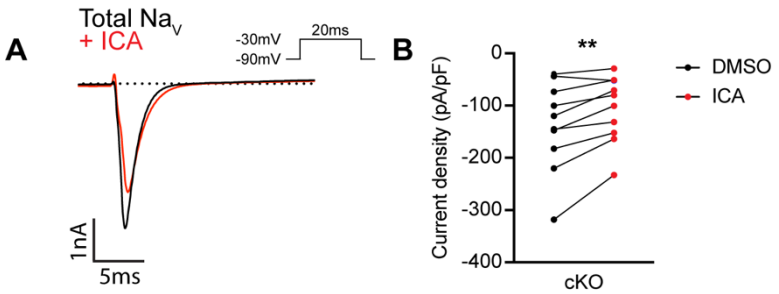


Figure supplement 2. ICA121431 may inhibit upregulated Nav1.3 channels in cultured DRG neurons. (**A**) and Representative whole-cell current trace from a large diameter DRG neurons (>60pF) from a Scn1a-cKO mouse. The black trace represents the I_{Na} recorded in external solution and the red trace represents the current following application of 500nM of ICA121431 for 1 minute. (**C**) Quantification of the current density before and after ICA (n=9). A significant effect of ICA was found (Paired student's t test, p = 0.0041). n = cells.

To clarify the importance of $Na_V1.1$ to proprioceptor function and avoid the caveats associated with *in vitro* pharmacological studies, we took an *in vivo* approach and analyzed motor behaviors in Scn1a-cKO mice of both sexes. We were precluded from using a *Pvalb*^{cre} driver line to directly interrogate a role for $Na_V1.1$ in proprioceptors, as loss of $Na_V1.1$ in Pvalb-expressing brain interneurons produces an epilepsy phenotype that prevents behavioral analyses in adult animals (Ogiwara et al., 2007). Consistent with *in vitro* data, Scn1a-cKO animals of both sexes displayed profound and visible motor abnormalities. These abnormalities include ataxic-like

276 tremors when suspended in the air (**Fig 5 - video 1**), abnormal limb positioning (**Fig 5 -videos 2-**
277 **3**), and paw clasping, which are absent in Scn1a-floxed littermate controls and heterozygous
278 animals (*Pirt*^{Cre};*Scn1a*$^{fl/+}$, Scn1a-Het, respectively, **Fig 5A**). We first ran animals in the open field
279 test for ten minutes each to quantify spontaneous locomotor behaviors (**Fig 5B**). We found that
280 Scn1a-cKO animals traveled significantly less (**Fig 5C**) and slower (**Fig 5D**) than Scn1a-floxed
281 littermate controls ($p = 0.0077$ and 0.0057, respectively). Surprisingly, Scn1a-Het mice also
282 displayed motor abnormalities in the open field test, performing similarly to Scn1a-cKO animals
283 (**Fig 5B-D**), demonstrating $Na_V1.1$ haploinsufficiency in sensory neurons for motor behaviors. No
284 genotype-dependent differences were observed in the amount of time spent moving, suggesting
285 gross motor function was intact (**Fig 5E**). Additionally, the amount of time spent in the center of
286 the open field chamber was also independent of genotype (**Fig 5F**). We next used the rotarod
287 assay to investigate differences in motor coordination. Mice were assayed on three consecutive
288 days and latency-to-fall and RPM were quantified. Unlike in the open field assay, both Scn1a-
289 floxed and Scn1a-Het mice performed at similar levels during the three-day period (**Fig 5G-H**).
290 Conversely, Scn1a-cKO animals performed significantly worse. By day 3, on average they were
291 only able to maintain their position on the rotarod for 41 s, falling over 50% faster Scn1a-floxed
292 and Scn1a-Het mice. We did not observe any sex dependent differences in performance in the
293 open field or rotarod tests (**Fig 5 - figure supplement 3**). We confirmed that our mouse model
294 selectively targeted sensory neurons by crossing a *Pirt*^{Cre} driver with a fluorescent reporter line
295 (*Pirt*^{Cre};*Rosa*26^{Ai14}). We observed little-to-no neuronal expression of TdTomato in both dorsal and
296 ventral spinal cord (**Fig 5 - figure supplement 4**). In contrast, DRG somata and axons showed
297 strong labeling. Collectively, our behavioral data provide evidence for a new *in vivo* role of $Na_V1.1$
298 in sensory neurons in mammalian proprioception.

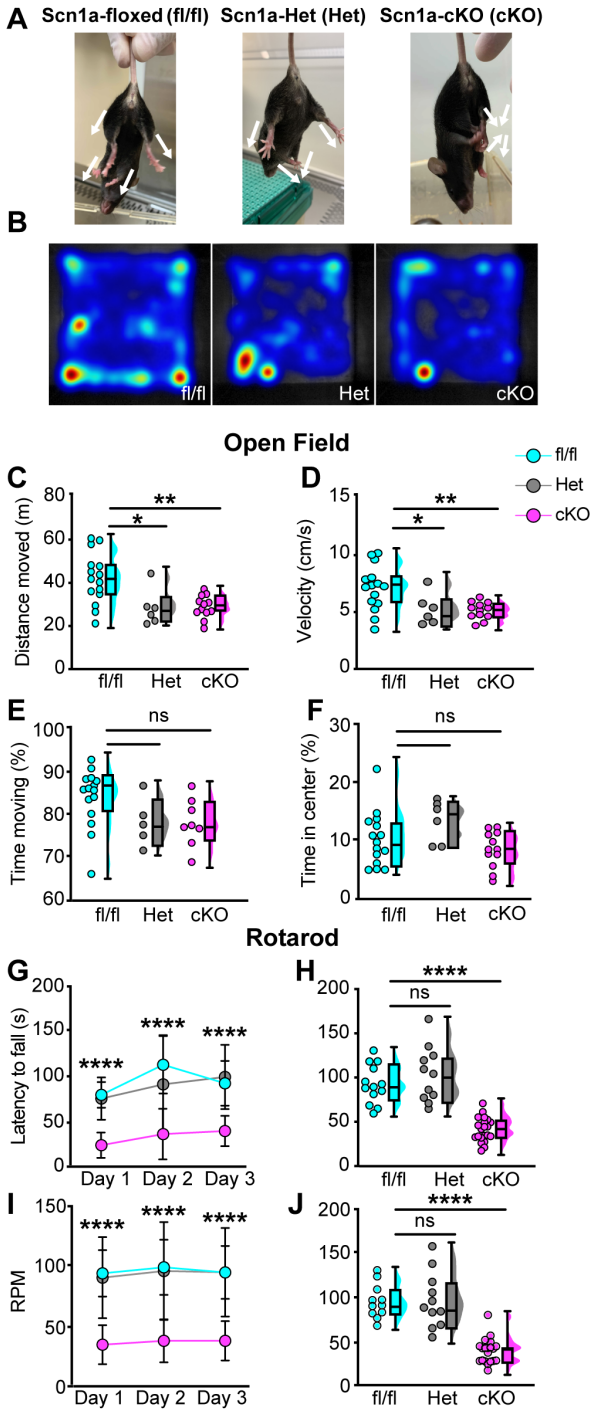


Figure 5. Loss of $Na_V1.1$ in peripheral sensory causes deficits in motor behaviors. A. Representative images showing limb positions of adult Scn1a-floxed (left), Scn1a-Het (middle), and Scn1a-cKO (right) mice. White arrows represent the direction of limbs. **B.** Representative heat maps from open field experiments between Scn1a-floxed (left), Scn1a-Het (middle), and Scn1a-cKO (right). Open Field (**C-F**), Quantification of total distance traveled during a 10-minute open-field test between Scn1a-floxed (cyan), Scn1a-Het (grey), and Scn1a-cKO (magenta) mice (**C,** Scn1a-Het p = 0.0255, Scn1a-cKO, p = 0.0077, compared to Scn1a-floxed,), average animal velocity (**D,** Scn1a-Het p = 0.00311, Scn1a cKO, p = 0.0057, compared to Scn1a-floxed), percent time moving (**E,** Scn1a-Het p = 0.1362, , p = 0.0730, compared to Scn1a-floxed), and percent time spent in center (**F,** Scn1a-Het p = 0.2297, Scn1a-cKO, p = 0.2494,

compared to Scn1a-floxed) during the test. Rotarod (**G-I**), Quantification of the latency to fall across three consecutive training days (**G**) and the day-three (**H**). Quantification of revolutions per minute (RPM) at the moment of animal fall (**I**) and the day-three average (**J**). Each dot represents one animal. ****p < 0.0001. A one-way ANOVA (Dunnett's post hoc comparison) was used to determine statistical significance in **C-F** and **I** and **J**. A two-way mixed-design ANOVA (Dunnett's post hoc comparison) was used to determine statistical significance in **G** and **H.** Open field: Scn1a-floxed N=15, Scn1a-Het N=6, Scn1a-cKO N=12. Rotarod: Scn1a-floxed N=11, Scn1a-Het N=11, Scn1a-cKO N=20.

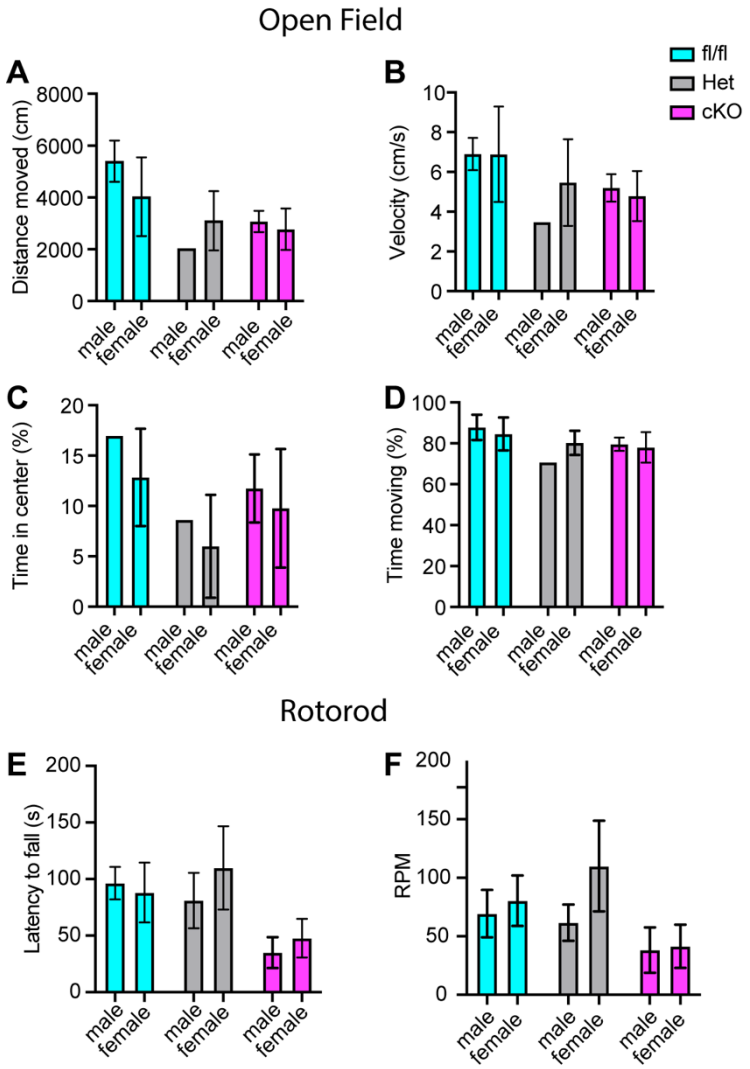


Figure supplement 3. Motor deficits in Scn1a-Het **and** Scn1a-cKO **animals are not sex dependent. A-D**. Quantification of sex-dependent parameters during a 10-minute open field trial. Total distance moved (**A**). Velocity (**B**). Percent time spent in center of box (**C**). Total percent time moving (**D**). Quantification of the sex-dependent differences in rotarod day-three trial latency to fall (**E**) and RPM (**F**).

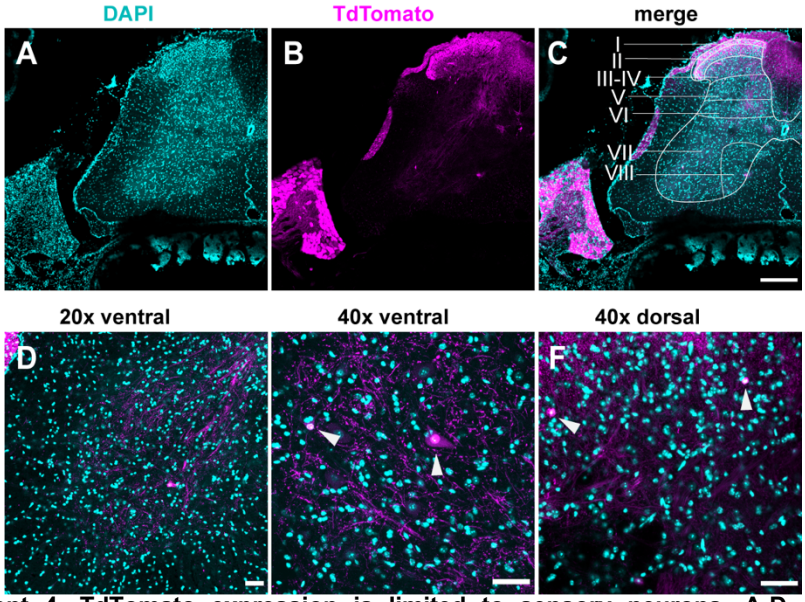
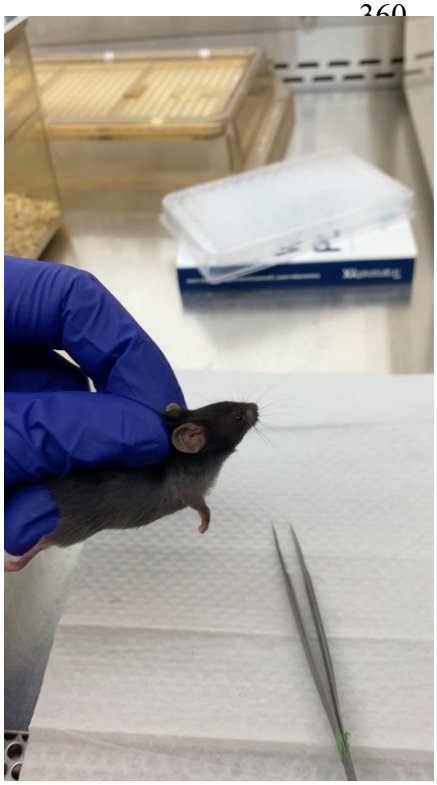


Figure supplement 4. TdTomato expression is limited to sensory neurons. A-D, Images stained using immunohistochemistry with anti-DsRed (TdTomato) (**A-C**) Representative confocal images of spinal cord sections at 10X, 0.45 NA dry objective. Scale bar 200μm. (**D**) Image was taken at 20X, 0.8 NA dry objective of the ventral horn. (**E**) Image was taken at 20X, 0.8 NA dry objective of the dorsal horn. Scale bar 50μm. (**F**) Image was taken at 40X, 1.3 NA oil-immersion objective. Arrows indicate TdTomato+ puncta. Scale bar 50μm. (N=3, n=30 sections).

Video 1. Uncoordinated movements in Scn1a-cKO **animals.** A Scn1a-cKO mouse (left) shows abnormal and spastic movements when suspended in the air. These movements are absent in Scn1a-floxed mice (right).

Video 2. Abnormal limb position in Scn1a-cKO **animals.** A Scn1a-cKO mouse has uncoordinated leg movements and makes an abnormal rotation of its hind paw to grasp its tail while suspended in the air.



Video 3. Abnormal paw position in Scn1a-cKO **animals.** A Scn1a-cKO mouse is scruffed and places hind paws with foot pads facing down. This contrasts with the normal paw positioning seen in the forepaws, in which foot pads are in the outward facing position.

367 Could the motor deficits observed in Scn1a-cKO mice be due to abnormal proprioceptor
368 development? To address this question, we performed RNAscope analysis of DRG sections from
369 Scn1a-floxed, Scn1a-Het, and Scn1a-cKO mice. We quantified the number of neurons per DRG
370 section that were positive for both *Runx3* and *Pvalb* transcript, the molecular signature of mature
371 proprioceptors (**Fig 6**, Oliver et al., 2021). We found no significant genotype-dependent
372 differences in the number of proprioceptors in Scn1a-Het and Scn1a-cKO mice compared to
373 Scn1a-floxed controls ($p = 0.3824$ and $p = 0.1665$, respectively), indicating that the behavioral
374 deficits observed in Scn1a-cKO mice are not the result of a developmental loss of proprioceptors.
375 We also analyzed muscle spindle morphology to determine if aberrant sensory end organ
376 development may contribute to the observed motor abnormalities. Similar to conditional Piezo2-
377 knockout animals (Woo et al., 2015), no qualitative differences were observed between genotypes
378 (**Fig 6 - figure supplement 5**). Thus, abnormal proprioceptor development does not contribute
379 to the overall phenotype of Scn1a-cKO mice.

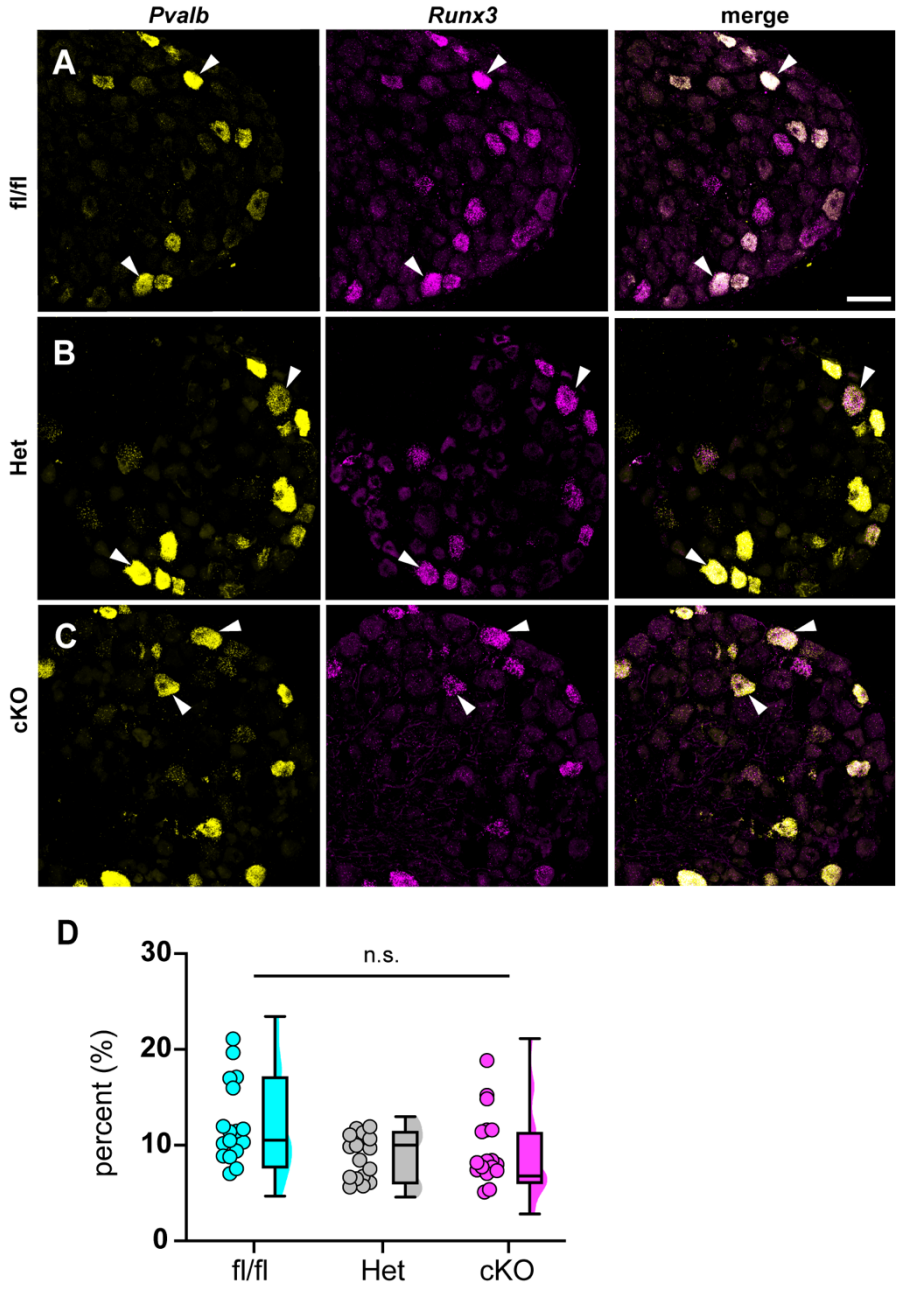
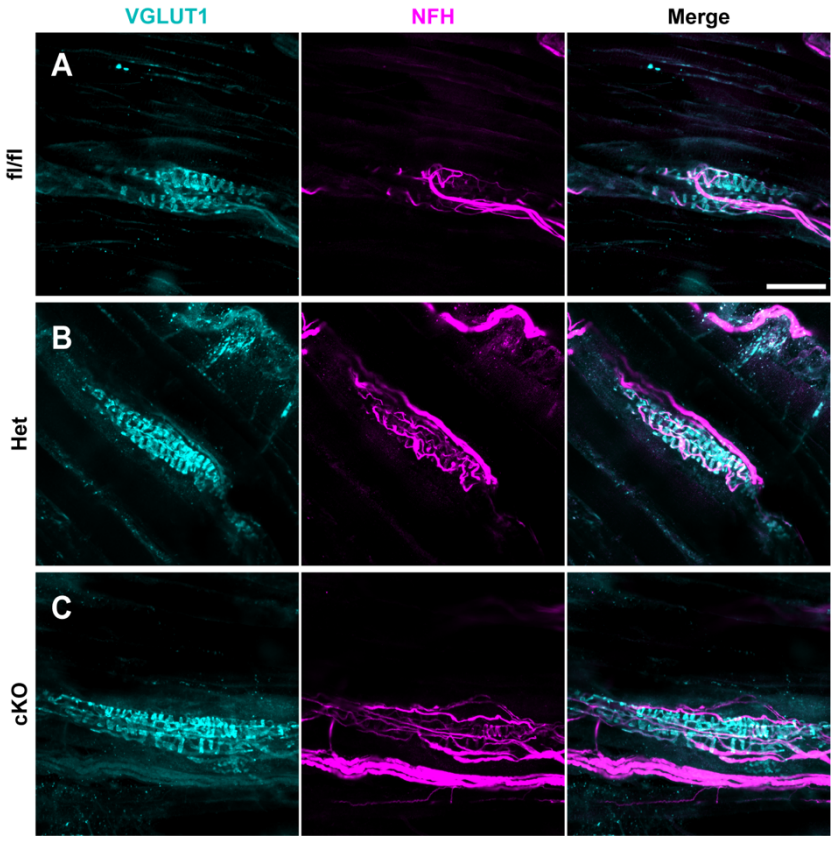


Figure 6. Loss of $Na_V1.1$ in sensory neurons does not affect proprioceptor development. Representative images of Scn1a-floxed (**A**), Scn1a-Het (**B**), and Scn1a-cKO (**C**) adult DRG neuron sections (25 μm). Images were acquired with a 40X, 0.9 NA water-immersion objective. Sections were hybridized with probes targeting parvalbumin (*Pvalb*, yellow) and *Runx3* (magenta). (**D**) Quantification of the percentage of Pvalb+/*Runx3*+ neurons in each genotype. Each dot represents one DRG section. A Kruskal-Wallis test with Dunn's post hoc comparison was used to determine statistical significance ($p = 0.1971$, Scn1a-floxed n=17, Scn1a-Het n=17, Scn1a-cKO n=18)). Scale bar 50μm.

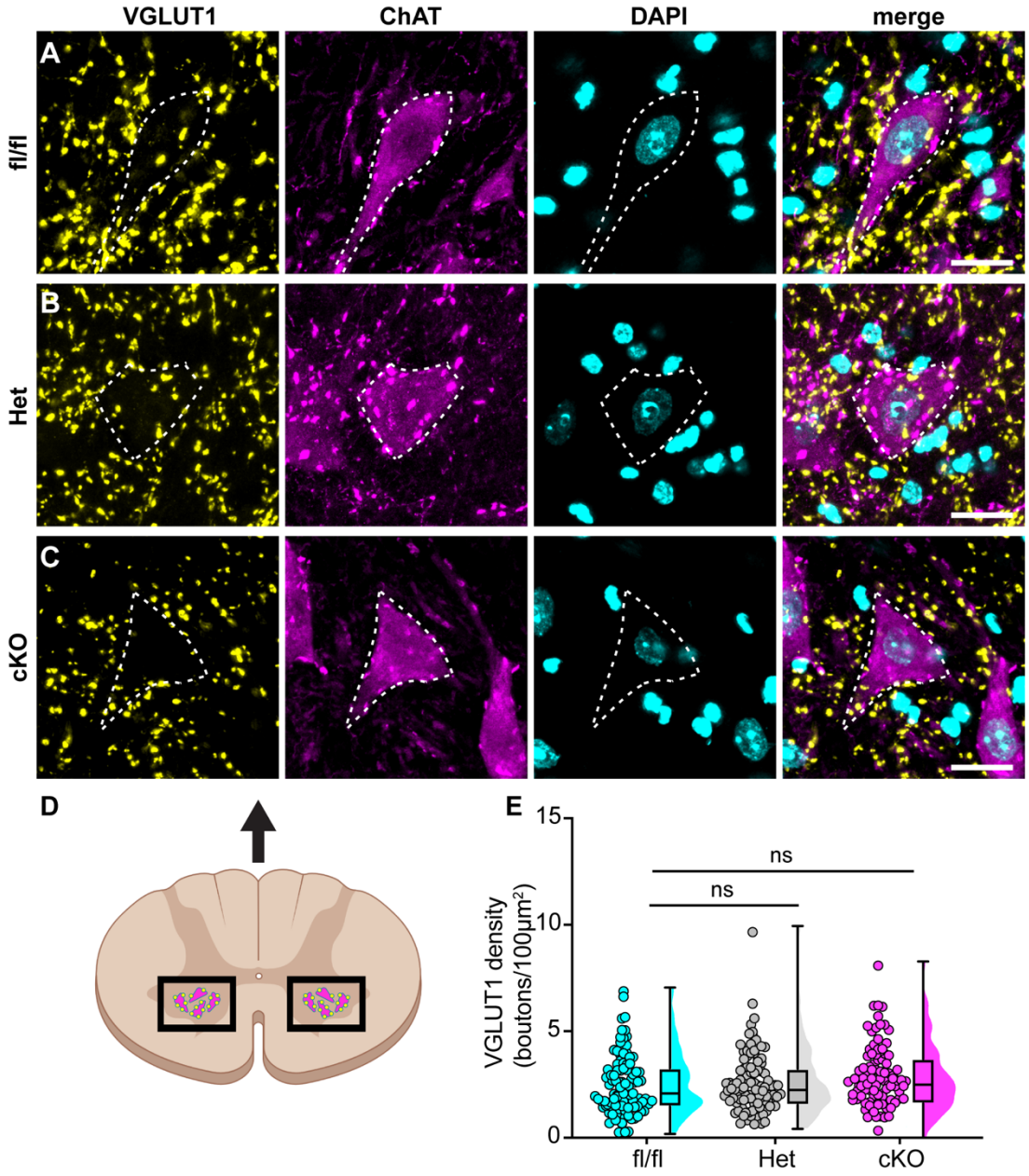


388

389 **Figure supplement 5. Muscle spindle development is normal in** Scn1a-Het **and** Scn1a-cKO **animals**. **A-C**,
390 Representative confocal images of muscle spindle whole mounts. Images were acquired with a 40x, 1.3 NA oil-
391 immersion objective. Sections were stained using immunohistochemistry with VGLUT1 (cyan) and neurofilament heavy
392 (NFH, magenta). (**A**) Representative images from Scn1a-floxed (**B**) Scn1a-Het and (**C**) Scn1a-cKO mice. Scn1a-floxed,
393 n=7; Scn1a-Het, n=8; Scn1a-cKO, n=9. Scale bar 50μm. n = muscle spindles.
394

395 We next asked whether the motor deficits of Scn1a-cKO mice are due to altered synaptic
396 connectivity between proprioceptive axons and motor neurons in the ventral spinal cord. Spinal
397 cord sections were harvested from Scn1a-floxed, Scn1a-Het, and Scn1a-cKO mice and stained
398 with antibodies against vesicular glutamate transporter 1 (VGLUT1) to label proprioceptor axons,
399 and choline acetyltransferase (ChAT). ChAT primarily labels α- and γ-motoneurons in the ventral
400 horn, which can be distinguished based on size. We analyzed the number of VGLUT1 puncta on
401 the somata and proximal dendrites of individual cholinergic neurons greater than 400 μm^2 to bias
402 our quantification towards α-motorneurons. We found no significant decrease in VGLUT puncta
403 density per ChAT+ neuron between Scn1a-Het or Scn1a-cKO when compared to Scn1a-floxed

404 littermate controls (**Fig 7,** Scn1a-Het, p>0.9999, Scn1a-cKO, p=0.4573). This suggests that
405 general deficits in proprioceptor innervation of motor neurons do not contribute to the phenotype
406 of Scn1a-cKO mice.
407



408

409 **Figure 7. Loss of $Na_V1.1$ in sensory neurons does not change proprioceptor innervation of α-motoneurons**. **A-**
410 **C**, Representative images of Scn1a-floxed(**A**), Scn1a-Het (**B**), and Scn1a-cKO (**C**) adult spinal cord sections (30 μm).
411 Images were acquired with a 63X, 1.4 NA water-immersion objective. Sections were stained using immunochemistry

with VGLUT1 (yellow) and ChAT (magenta). Nuclei (cyan) were labeled with DAPI. (**D**) Schematic of spinal cord regions of interest. (**E**) Quantification of the average density of VGLUT1+ puncta per 100μm^2 onto ChAT+ neurons that were larger than 400μm^2. A Kruskal-Wallis test with Dunn's post hoc comparison was used to determine statistical significance Each dot represents a motor neuron. Scn1a-floxed, n=101; Scn1a-Het, n=102; Scn1a-cKO, n=92. Scale bar 20μm. n = cells.

We next asked whether proprioceptor electrical signaling is altered in Scn1a-cKO mice. While *in vitro* patch-clamp electrophysiology can assess Na_V function at DRG somata and provide insight as to how they contribute to intrinsic excitability, the physiological contributions of ion channels in DRG soma to somatosensory transmission *in vivo* are not well understood. Thus, to directly investigate how $Na_V1.1$ shapes action potential propagation down proprioceptor axons, we used an *ex vivo* preparation to recording muscle afferent activity during ramp-and-hold stretch and sinusoidal vibration. Afferents from both Scn1a-Het and Scn1a-cKO mice exhibited impaired static stretch sensitivity as evidenced by a decreased likelihood of firing during rest as compared to Scn1a-floxed mice, as well as an inability to maintain firing throughout the entire 4s stretch. Almost all afferents from Scn1a-floxed mice could fire consistently throughout the entire 4s hold phase (**Fig 8A**), but loss of one or both copies of $Na_V1.1$ led to either firing only near the beginning of stretch or inconsistent firing in a high percentage of afferents lacking $Na_V1.1$ (**Fig 8B,C**). We quantified this inconsistent firing by determining the coefficient of variation (CV) of the interspike interval (ISI) during the plateau phase of stretch (1.5-3.5 s into the hold phase) across different stretch lengths and found a significant effect of genotype, with the knockout afferents both having higher ISI CV than the Scn1a-floxed afferents (**Fig 8D**; 0.074 $\pm$ 0.06, 0.313 $\pm$ 0.456, .497 $\pm$ .831, at 7.5% Lo, Scn1a-floxed, Scn1a-Het, and Scn1a-cKO afferents, respectively, Two-way ANOVA, main effect of genotype, $p = 0.015$). In contrast to the clear deficits in static sensitivity in afferents lacking $Na_V1.1$, dynamic sensitivity was not significantly impaired. The maximum firing frequency during the ramp up phase (Dynamic Peak) was independent of genotype, and even trended slightly higher in afferents lacking $Na_V1.1$ (**Fig 8E**; Two-way ANOVA, effect of genotype $p=0.0633$).

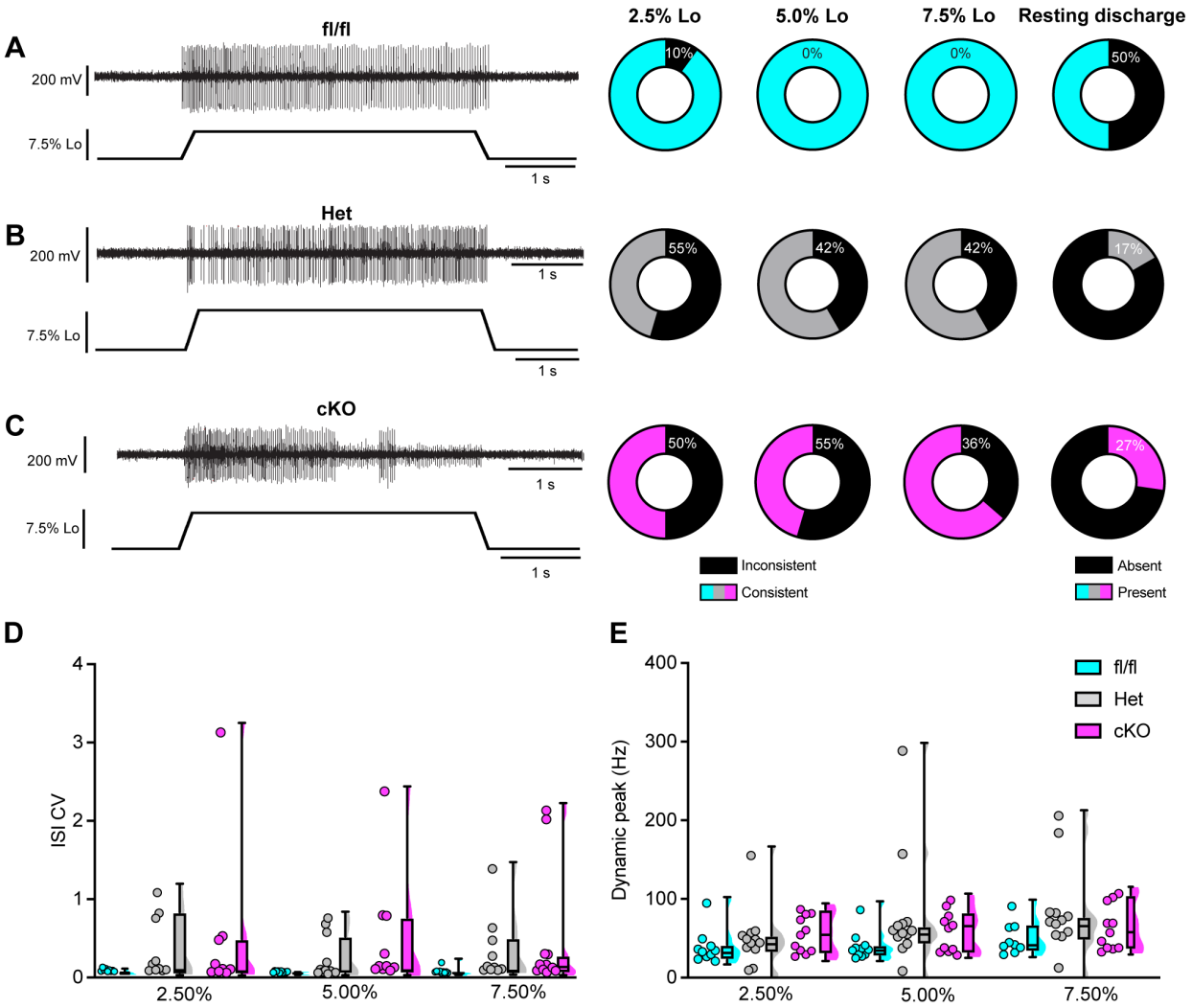


Figure 8. Loss of $Na_V1.1$ reduces static muscle stretch sensitivity and reliability. A-C. Representative responses to ramp-and-hold muscle stretch at 7.5% of optimal length (Lo) from Scn1a-floxed (**A**), Scn1a-Het (**B**) and Scn1a-cKO (**C**) afferents. Afferents from Scn1a-Het and Scn1a-cKO mice were more likely to show inconsistent firing during the hold phase of stretch. The percentage of afferents from each genotype that were able to fire consistently for the entire duration of stretch at 2.5%, 5.0%, and 7.5% of Lo are shown in the pie charts next to the representative trace from their genotype (black indicates percentage with inconsistent firing). The final pie charts represent the proportion of afferents that exhibited resting discharge at Lo for every stretch for each genotype (black indicates absence of resting discharge). **D.** Inconsistency in firing was quantified as the interspike interval coefficient of variation (ISI CV) during the plateau stage of the hold phase of stretch (1.5-3.5s into stretch) for the 3 different genotypes. A significant effect of genotype was observed (two-way mixed-design ANOVA, p=0.015) **E.** The highest firing rate during the ramp up phase of stretch (dynamic peak) is a measure of dynamic sensitivity. No significant effect of genotype on dynamic peak was observed (two-way mixed-design ANOVA, p=0.0633). Each dot represents one afferent in **D** & **E** (Scn1a-floxed, n=10; Scn1a-Het, n=12; Scn1a-cKO, n=11).

We next examined the requirement of $Na_V1.1$ for proprioceptor afferent responses to sinusoidal vibration, which is a measure of dynamic sensitivity, and found no differences with loss

457 of $Na_V1.1$ (**Fig 9A-C, Tables 2-4**). We characterized a unit as having entrained to vibration if it
458 fired at approximately the same time every cycle of the 9s vibration. In most cases, afferents
459 lacking $Na_V1.1$ were equally likely to entrain to vibration than Scn1a-floxed afferents (**Fig 9D-F**).
460 Indeed, Scn1a-cKO afferents were able to maintain firing during the entire 9 s sinusoidal vibration,
461 in contrast to their inability to maintain consistent firing during 4 s of static stretch. There were no
462 significant differences in firing rate during vibration between Scn1a-floxed, Scn1a-Het, and
463 Scn1a-cKO afferents (**Fig 9D-F**). Taken together, our *ex vivo* recordings suggest that behavioral
464 deficits in Scn1a-cKO result from abnormal proprioceptor responses to static muscle movement,
465 whereas afferent responsiveness to dynamic stimuli is $Na_V1.1$-independent. Furthermore,
466 recordings from Scn1a-Het animals support the notion that $Na_V1.1$ is haploinsufficient for
467 proprioceptor function at the cellular level.

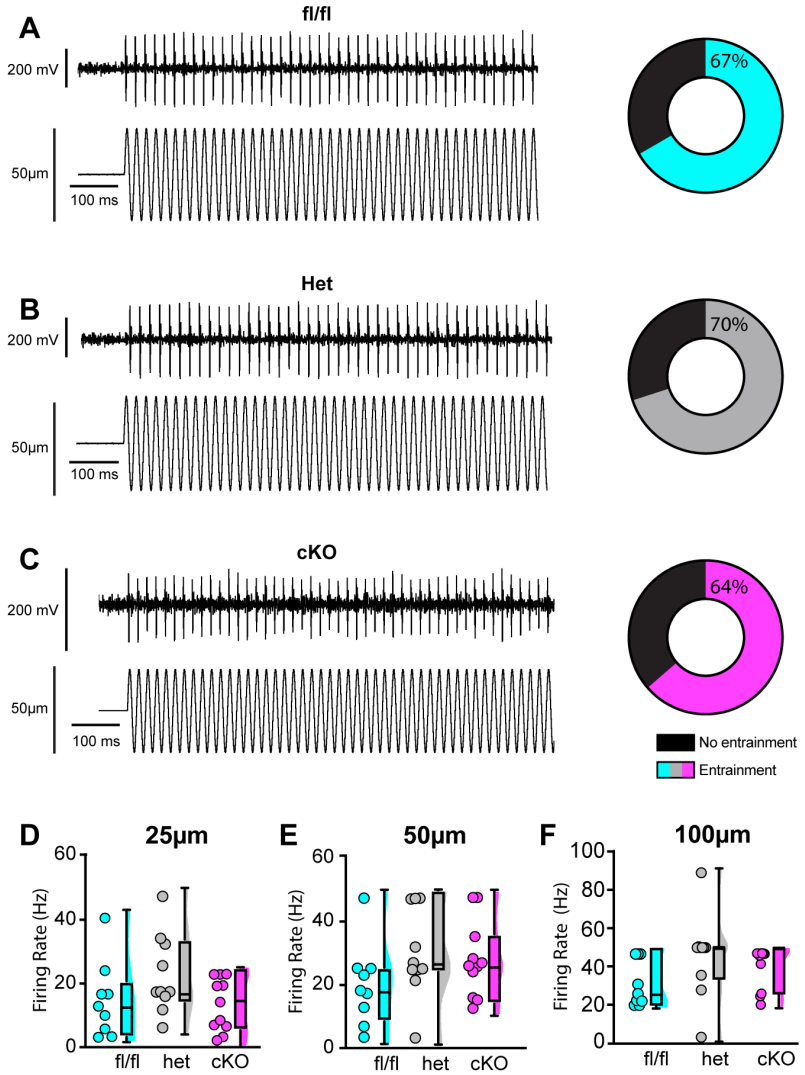


Figure 9. Loss of $Na_V1.1$ does not alter muscle spindle afferent response to vibratory muscle stretch. A-C. Representative traces from afferents that were able to entrain to a 50Hz, 100µm vibration as well as graphs with the percentage of all Scn1a-floxed (cyan; **A**), Scn1a-Het (gray; **B**), and Scn1a-cKO (magenta; **C**) afferents that could entrain to the vibration shown in **A-C**. Average firing frequency during a 9 s 50 Hz vibration shown for a 25 µm (**D**), 50 µm (**E**), and 100 µm (**F**) amplitude vibration. There was no significant effect of genotype on the firing frequency during vibration (25 µm, p = 0.2398, 50 µm, p = 0.2413, 100 µm, p = 0.1276). A one-way ANOVA was used to determine statistical significance in **D** and **E**. A Kruskal-Wallis test was used to determine statistical significance in **F**. Each dot represents one afferent (Scn1a-floxed, n=9; Scn1a-Het, n=10; Scn1a-cKO,n=11).

Table 2. Afferent entrainment to 25 µm amplitude vibration

Genotype	10 Hz	25 Hz	50 Hz	100 Hz
Scn1a-floxed	33.33%	11.11%	0.00%	0.00%
Scn1a-Het	50.00%	40.00%	10.00%	10.00%
Scn1a-cKO	27.27%	9.09%	0.00%	0.00%

Table 2. The percentage of muscle spindle afferents that entrained to a 25µm amplitude sinusoidal vibration.

480
481
482 **Table 3. Afferent entrainment to 50 μm amplitude vibration**

Genotype	10 Hz	25 Hz	50 Hz	100 Hz
Scn1a-floxed	88.89%	22.22%	11.11%	11.11%
Scn1a-Het	80.00%	70.00%	30.00%	10.00%
Scn1a-cKO	45.45%	54.55%	18.18%	0.00%

483 **Table 3.** The percentage of muscle spindle afferents that entrained to a 50μm amplitude sinusoidal vibration.
484
485
486 **Table 4. Afferent entrainment to 100 μm amplitude vibration**

Genotype	10 Hz	25 Hz	50 Hz	100 Hz
Scn1a-floxed	100.00%	44.44%	33.33%	22.22%
Scn1a-Het	90.00%	90.00%	70.00%	40.00%
Scn1a-cKO	63.64%	72.73%	63.64%	18.18%

487 **Table 4.** The percentage of muscle spindle afferents that entrained to a 100μm amplitude sinusoidal vibration.
488

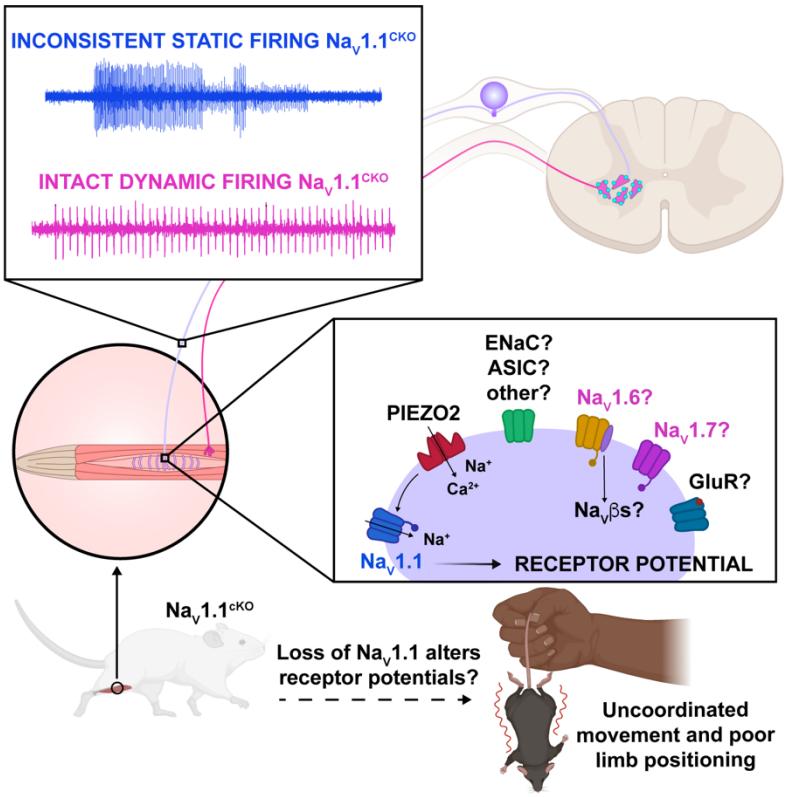
489 **DISCUSSION**

490 The critical role for $Na_V1.1$ in various brain disorders has overshadowed the potential
491 contributions of this channel in peripheral signaling. The results presented in this study are the
492 first to provide functional evidence that $Na_V1.1$ in peripheral sensory neurons is required for
493 normal proprioception. We found that mice lacking $Na_V1.1$ in sensory neurons exhibit visible motor
494 deficits and ataxic-like behaviors, which we propose is largely attributed to loss of $Na_V1.1$ in
495 proprioceptors. Indeed, RNAscope analysis showed expression of $Na_V1.1$ mRNA in 100% of
496 genetically identified proprioceptors. While $Na_V1.6$ and $Na_V1.7$ were also ubiquitously expressed
497 in proprioceptors, $Na_V1.1$ displayed higher expression levels, consistent with a previous
498 RNAsequencing study (Zheng et al., 2019b). There are anywhere from 5 to 8 different
499 proprioceptor molecular subclasses (Oliver et al., 2021b; Wu et al., 2021), however, and it is
500 possible that these distinct classes rely on different combinations of these channels for function.
501 Nevertheless, our functional *in vitro* patch-clamp experiments found $Na_V1.1$ to be the dominant
502 subtype across recorded neurons, comprising nearly half of the proprioceptor I_{Na}. In line with this,
503 pharmacological inhibition of $Na_V1.1$ is sufficient to significantly attenuate action potential firing is

504 most proprioceptors; yet, it should be noted that 25% of the neurons we recorded fired action
505 potentials that were insensitive to ICA application. This suggests that some proprioceptor
506 subtypes rely more heavily on $Na_V1.6$ and $Na_V1.7$ for electrical activity. Interestingly, the
507 proprioceptors that were insensitive to ICA application also were less intrinsic excitable, firing only
508 1-2 action potentials in response to current injection, as opposed to the other 75% on neurons
509 recorded, which fired repetitively during the 100 ms injection protocol. This further supports a role
510 for $Na_V1.1$ in maintaining action potential firing in response to sustained stimulation. These results,
511 however, should be interpreted with the caveat that in these experiments ICA may also be acting
512 on $Na_V1.3$ channels that are upregulated during DRG neuron culturing (Wangzhou et al., 2020).
513 Nevertheless, at the afferent level Scn1a-cKO and Scn1a-Het animals show clear deficits in static
514 stretch sensitivity, but not dynamic sensitivity, and could even entrain to vibrations as fast as 100
515 Hz, suggesting a specific role for $Na_V1.1$ in proprioceptors responses to static muscle movement.
516 Finally, we found that loss of $Na_V1.1$ in sensory neurons had no effect on proprioceptor
517 development, muscle spindle morphology, or proprioceptive afferent innervation of ChAT+ motor
518 neurons in the spinal cord. Thus, the observed motor behavioral deficits are likely due to reduced
519 static sensitivity of proprioceptor afferents.

520 Our model proposes that $Na_V1.1$ is tasked with maintaining consistent firing in spindle
521 afferents during static muscle stretch for normal motor behaviors, whereby activation of the
522 mechanotransduction channel Piezo2 initiates electrical signaling, which in turn activates a
523 complement of tetrodotoxin-sensitive Na_V channels (Carrasco et al., 2017; Florez-Paz et al., 2016;
524 Woo et al., 2015, **Fig 10**). During dynamic or vibratory stimuli, Piezo2, and likely a combination of
525 other molecular mediators, including $Na_V1.6$ and $Na_V1.7$, are sufficient to elicit normal electrical
526 activity. Conversely, during prolonged muscle stretch when Piezo2 channels presumably
527 inactivate, $Na_V1.1$ is required for regular and reliable firing. While other signaling molecules and
528 channels, such as vesicle-released glutamate (Bewick et al., 2005; Than et al., 2021), and

529 mechanosensitive ASIC channels (Lin et al., 2016) and ENaC channels (Bewick and Banks,
530 2015), also contribute to mammalian proprioception, our data suggests $Na_V1.1$ is a critical for
531 muscle spindle afferent mechanotransduction, given the overt behavioral deficits observed in
532 Scn1a-cKO mice. Due to the ubiquitous expression of $Na_V1.1$ in all proprioceptors and the
533 importance of Golgi Tendon Organ (GTO) feedback to motor control, alterations in function in
534 those proprioceptors likely contribute to the behavioral deficits we observed; however, we did not
535 directly measure their function.



536

537 **Figure 10. Proposed model of the role of $Na_V1.1$ in proprioception.** Upon muscle static stretch, various channels
538 activate, including Piezo2 (red) which results in an influx of calcium and sodium ions, causing a depolarization that
539 activates $Na_V1.1$ (dark blue). $Na_V1.1$ activation drives reliable repetitive firing of proprioceptors during static stretch for
540 normal motor behavior. Loss of $Na_V1.1$ in sensory neurons results in inconsistent static firing at the afferent level while
541 maintaining dynamic firing, resulting in uncoordinated movements and abnormal limb positioning. It is possible that a
542 combination of Piezo2, $Na_V1.6$ (yellow), $Na_V1.7$ (pink), and/or other channels, such as glutamate receptors (dark blue),
543 ASIC and ENaC channels (green) mediate dynamic firing.
544

545 To date, our knowledge of the functional contributions of $Na_V1.1$ in the PNS is limited.
546 Most studies have identified roles for this channel in mechanical pain signaling in DRG, TG, and
547 vagal sensory neurons. Intraplantar pharmacological activation of $Na_V1.1$ induces spontaneous
548 pain behaviors and mechanical pain, which is absent in mice lacking $Na_V1.1$ in small- and
549 medium-diameter sensory neurons (Osteen et al., 2016b). Inhibition of $Na_V1.1$ prevented the
550 development of mechanical pain in several preclinical models, including spared nerve injury
551 (Salvatierra et al., 2018b), an irritable bowel syndrome mouse model (Salvatierra et al., 2018),
552 and infraorbital nerve chronic constriction injury (Pineda-Farias et al., 2021). Additionally, blocking
553 $Na_V1.1$ channels inhibited firing in TRPM8-expressing neurons *in vitro*, suggesting a potential role
554 for this channel in thermosensation (Griffith et al., 2019). No prior studies, however, have reported
555 a functional role for $Na_V1.1$, or Na_Vs in general, in proprioception.

556 The loss of consistent firing we observed during static stretch in Scn1a-cKO and Scn1a-
557 Het animals is functionally similar to deletion of $Na_V1.1$ in other brain cell types. Indeed, loss of a
558 single copy of $Na_V1.1$ is sufficient to attenuate sustained action potential firing in parvalbumin-
559 positive hippocampal interneurons (Ogiwara et al., 2007; Yu et al., 2006) and cerebellar Purkinje
560 neurons (Yu et al., 2006). $Na_V1.1$ has been associated with persistent sodium current ($I_{Na}P$) and
561 resurgent sodium current ($I_{Na}R$), both of which promote repetitive firing in a wide variety of cell
562 types in the CNS and PNS (Barbosa et al., 2015; Kalume et al., 2007; Khaliq et al., 2003). $Na_V1.1$
563 also recovers rapidly from fast inactivation compared to other channel subtypes (Herzog et al.,
564 2003; Patel et al., 2015) and has been shown to be refractory to entry into slow inactivation in
565 TRPM8-expressing DRG neurons (Griffith et al., 2019). We observed similar characteristics when
566 analyzing the proprioceptor I_{Na}. Future studies will determine whether proprioceptors rely on these
567 features of $Na_V1.1$ for reliable and consistent encoding of static muscle stretch.

568 Loss of $Na_V1.1$ notably impacted proprioceptor afferent static sensitivity during ramp-and-
569 hold stretch, but not dynamic sensitivity as measured by entrainment to sinusoidal vibrations using
570 *ex vivo* muscle-nerve recordings. Afferents from Scn1a-cKO animals were more likely to have

571 action potential failures and thus were largely unable to fire consistently throughout the 4 s of
572 stretch, which was accompanied by a higher coefficient of variability in the ISI. This indicates that
573 $Na_V1.1$ has a critical role in transmitting static stretch information to the central nervous system.
574 Interestingly, however, dynamic sensitivity in these afferents appears to be unimpaired. Both
575 Scn1a-cKO and Scn1a-Het afferents were able to entrain throughout the entire 9 s vibration;
576 therefore, $Na_V1.1$ does appear to have a generalized role in maintaining high frequency firing, but
577 a more specific deficit in static sensitivity. $Na_V1.1$ has been localized to muscle spindle afferent
578 endings and has been hypothesized to help amplify receptor current (Carrasco et al., 2017) . Our
579 results support a model whereby current from Piezo2 and potentially other mechanically sensitive
580 ion channels at the start of stretch produces a sufficient receptor potential to generate firing at the
581 heminode, but that amplification of the receptor potential by $Na_V1.1$ is necessary to maintain firing
582 during held stretch.

583 A similar deficit in static but not dynamic sensitivity was seen following loss of synaptic-
584 like vesicle released glutamate from afferent endings (Than et al., 2021); however in those
585 afferents firing only occurred at the beginning of stretch and patchy firing was never observed.
586 This may indicate that glutamate plays a more general role maintaining excitability, whereas
587 $Na_V1.1$ is required for reliable action potential generation at heminodes during static stimuli.
588 Alternatively, or in addition, $Na_V1.1$ expressed along the axon could be essential for sustained
589 static firing. A detailed examination of $Na_V1.1$ subcellular localization along proprioceptor afferents
590 could shed light on how this channel contributes to signal propagation. The lack of an effect on
591 dynamic sensitivity could suggest the upregulation of other Na_V subtypes or other molecules as a
592 compensatory mechanism to counteract the loss of $Na_V1.1$. Indeed, our *in vitro*
593 electrophysiological experiments found a more pronounced effect of acute $Na_V1.1$ inhibition on
594 proprioceptor excitability. This could be due, however, to artificially upregulated $Na_V1.3$ channel
595 activity in culturing conditions, or conversely, a higher density of $Na_V1.1$ expression in

596 proprioceptor somata. Future studies using temporally controlled deletion of $Na_V1.1$ in sensory
597 neurons could tease this apart. Nevertheless, as static sensitivity is still very much impaired in
598 both Scn1a-cKO and Scn1a-Het afferents, $Na_V1.1$ may play a potentially unique role in
599 maintaining afferent firing during the sustained stretch.

600 Loss of $Na_V1.1$ in sensory neurons did not impact proprioceptor development, as the
601 number of proprioceptors in DRG sections was unchanged between genotypes, and muscle
602 spindles developed normally in our model. While we did not directly examine GTOs, we do not
603 anticipate that abnormal end organ development would be restricted to that proprioceptor
604 subtype. Additionally, we did not observe a general decrease in α-motor neuron innervation,
605 which is consistent with the findings of Mendelsohn et al., 2015, who reported loss of proprioceptor
606 activity does not generally reduce proprioceptive input into the spinal cord. Interestingly, however,
607 the authors did observe changes in heteronymous sensory-motor connectivity when proprioceptor
608 transmission was blocked, whereby proprioceptor innervation of motor neurons that project to
609 antagonistic muscles was increased. Whether loss of $Na_V1.1$ in proprioceptive afferents causes
610 them to "mis-wire" in our model to make connections with inappropriate motor neuron pools is
611 unclear. Future studies will determine if this contributes to the observed behavioral deficits.

612 We found effects of both pharmacological inhibition and genetic deletion of $Na_V1.1$ in *in*
613 *vitro* and *ex vivo* electrophysiological experiments, respectively (**Figs 2-3, 8-9**); however, in our
614 mouse model $Na_V1.1$ is deleted in all sensory neurons. Thus, we cannot rule out that loss of
615 $Na_V1.1$ in other mechanosenory neuron populations, such as touch receptors, contributes to the
616 motor deficits observed. Deletion of $Na_V1.1$ in small- and medium-diameter DRG neurons using
617 a peripherin-Cre driver did not produce visible motor deficits (Osteen et al., 2016b), indicating
618 sensory neuron populations in those categories are not involved. We did observe $Na_V1.1$
619 transcripts in the vast majority of myelinated DRG neurons (a combination of large- and medium-
620 diameter DRG neurons), consistent with its presence in different subclasses of tactile sensory

621 neurons (Zheng et al., 2019). However, the severe motor phenotype of Scn1a-cKO mice
622 precludes mechanical threshold analysis using von Frey or tactile sensitivity using tape test.
623 Notably, baseline mechanical thresholds were unchanged following intraplantar injection of a
624 selective $Na_V1.1$ inhibitor (Salvatierra et al., 2018). This suggests that while $Na_V1.1$ mRNA is
625 expressed in most tactile sensory neurons, functional protein may only be upregulated in these
626 populations during pathological states.

627 Despite this limitation, one noteworthy and intriguing finding from our study was the
628 haploinsufficiency of $Na_V1.1$ in sensory neurons for proprioceptor function and normal motor
629 behavior in the open field test. At the afferent level, heterozygous and homozygous loss of $Na_V1.1$
630 produced similar deficits in static firing, suggesting that loss of less than a quarter of the
631 proprioceptor I_{Na} is sufficient to impair proprioceptor responsiveness to muscle stretch. $Na_V1.1$ is
632 haploinsufficeint in several brain neuron cell-types for normal excitability and function (Ogiwara
633 et al., 2007; Yu et al., 2006), suggesting the contributions of $Na_V1.1$ to neuronal function are highly
634 sensitive to genetic perturbations. At the behavioral level, Scn1a-Het mice had an identical
635 phenotype to Scn1a-cKO mice, moving more slowly and less than controls, despite not having
636 the more severe and visible motor coordination deficits. Indeed, their performance on the rotarod
637 was identical to that of Scn1a-floxed controls (**Fig 5**). How these behavioral differences arise given
638 the similar transmission deficits in Scn1a-cKO and Scn1a-Het afferents is unclear. One possibility
639 is a presynaptic role for $Na_V1.1$ in proprioceptive terminals that is unveiled when both copies of
640 $Na_V1.1$ are lost. For example, loss of presynaptic $Na_V1.7$ channels in the spinal cord reduced
641 glutamate release from nociceptive afferents onto dorsal horn neurons (MacDonald et al., 2021).
642 If a similar mechanism is at play for $Na_V1.1$ in proprioceptors, reduced neurotransmitter release
643 from Scn1a-Het afferent terminals could be sufficient to produce quantifiable, albeit more subtle,
644 motor deficits. Future studies are required to test this possibility.

645 Notably, *Scn1a* is a super culprit gene with over one thousand associated disease-causing
646 mutations, most of which are linked to different forms of epilepsy. Many epilepsy patients with

647 hemizygous $Na_V1.1$ loss-of-function display ataxia and motor delays and deficiencies (Claes et
648 al., 2001; Fujiwara et al., 2003), which has traditionally been attributed to loss of $Na_V1.1$ function
649 in the brain, namely the cerebellum (Kalume et al., 2007). Our findings suggest that some of the
650 clinical manifestations associated with epilepsy are not solely due to $Na_V1.1$ loss-of-function in
651 the brain, but also may manifest in part as a result from unreliable coding by peripheral
652 proprioceptors.

653 Data presented in this study provide new evidence of a role for peripherally expressed
654 $Na_V1.1$ in motor coordination. We show that $Na_V1.1$ is ubiquitously and strongly expressed in
655 proprioceptors, contributes to proprioceptor excitability *in vitro* and *ex vivo,* and is haploinsufficient
656 in sensory neurons for normal motor behaviors. Collectively, this works identifies a new role for
657 $Na_V1.1$ in mammalian proprioception.

658 **MATERIALS AND METHODS**

659 ***Key resources***. **Table 1** contains a list of key resources and supplies used for this study.

Key Resources Table				
Reagent type or resource	**Designation**	**Source or Reference**	**Identifiers**	**Additional information**
Antibody	Rabbit polyclonal anti-DsRed	Takara Bio	Catalogue #632496	1:3000
Antibody	Chicken polyclonal GFP	Abcam	Catalogue #ab13970	1:3000
Antibody	Chicken polyclonal NFH	Abcam	Catalogue #ab4680	In muscle spindles: (1:300) In DRG: (1:3000)
Antibody	Rabbit polyclonal CGRP	Immunostar	Catalogue #24112	1:1000
Antibody	Guinea pig polyclonal VGLUT1	Zuckerman institute (Columbia University)	Catalogue #CU1706, RRID:AB_2665455	In spinal cord: (1:8000) In muscle spindles: (1:800)

Antibody	Chicken polyclonal β3-tubulin	Abcam	Catalogue #ab41489	1:500
Antibody	Rabbit polyclonal β3-tubulin	Abcam	Catalogue #ab18207	1:3000
Antibody	Rabbit polyclonal ChAT	Zuckerman institute (Columbia University)	Catalog #CU1574	1:10,000
Chemical compound, drug	VECTASHIELD ® Antifade Mounting Media with DAPI	Vector Laboratories	Catalogue #H-2000	
Chemical compound, drug	Tissue-Tek OCT compound	Sakura	Catalogue #4583	
Chemical compound, drug	Laminin	Sigma-Aldrich	Catalogue #L2020-1MG	
Chemical compound, drug	Collagenase type P	Sigma-Aldrich	Catalogue #11213865001	
Chemical compound, drug	TrypLE Express	Thermofisher	Catalogue #12605-010	
Chemical compound, drug	MEM	Thermofisher	Catalogue #11095-080	
Chemical compound, drug	Penicillin-streptomycin	Thermofisher	Catalogue #15140-122	
Chemical compound, drug	MEM vitamin solution	Thermofisher	Catalogue #11120-052	
Chemical compound, drug	B-27 supplement	Thermofisher	Catalogue #17504-044	
Chemical compound, drug	Horse serum, heat inactivated	Thermofisher	Catalogue #26050-070	
Chemical compound, drug	ICA 121431	Tocris Bioscience	Catalogue #5066/10	
Chemical compound, drug	4,9 – anhydrous tetrodotoxin	Tocris Bioscience	Catalogue #6159	

Chemical compound, drug	PF- 05089771	Tocris Bioscience	Catalogue #5931	
Chemical compound, drug	Tetrodotoxin	Abcam	Catalogue ab120054	
Assay Kit	RNAscope Fluorescence Multiplex Kit	Advanced Cell Diagnostics	Catalogue #320851	
Oligonucleotide	*Pvalb* probe channel 1	Advanced Cell Diagnostics	Catalogue #421931	
Oligonucleotide	*Scn1a* probe channel 2	Advanced Cell Diagnostics	Catalogue #556181-C2	
Oligonucleotide	*Scn8a* probe channel 2	Advanced Cell Diagnostics	Catalogue #313341-C2	
Oligonucleotide	*Scn9a* probe channel 2	Advanced Cell Diagnostics	Catalogue #434191-C2	
Oligonucleotide	*Runx3* probe channel 3	Advanced Cell Diagnostics	Catalogue #451271-C3	
Mouse Strain	*Pirt*^{cre}	Dr. Xinzhong Dong		
Mouse Strain	*Rosa26*^{Ai14}	Jackson Laboratories	Stock #007914	
Mouse Strain	*Pvalb*^{cre}	Jackson Laboratories	Stock #008069	
Mouse Strain	Scn1a-floxed	UC Davis MMRRC	Stock # 041829-UCD	
Mouse Strain	*Mrgprd*^{GFP}	Zheng et al., 2019		
Software/ Algorithms	pClamp 11.2 Software Suite	Molecular Devices	https://www.moleculardevices.com	
Software/ Algorithms	Image J	Schneider et al. (2012)	https://imagej.nih.gov	
Software/ Algorithms	Prism 9	Graphpad	https://www.graphpad.com	
Software/ Algorithms	LabChart	ADInstruments	https://www.adinstruments.com/products/labchart	

Software/ Algorithms	MATLAB	MathWorks	https://www.mathworks.com	
Software/ Algorithms	Software, algorithm, custom (MATLAB)	This study	https://github.com/doctheagrif/Current-Clamp-Matlab-Code_O-Neil-DA	Current clamp experiments were analyzed with a custom written Matlab Script that is available on Github

660

661 ***Animals***. *Pirt*^{cre} mice were a kind gift from Dr. Xinzhong Dong (Johns Hopkins University).
662 *Rosa26*^{Ai14} (stock #007914; (Madisen et al., 2010)) and *Pvalb*^{cre} (stock # 008069) were obtained
663 from Jackson Laboratories. Scn1a-floxed (stock # 041829-UCD) mice were purchased from the
664 UC Davis MMRRC. *Mrgprd* mice were originally published in Zheng et al., 2019. All mice used
665 were on a mixed C57BL/6 background (non-congenic) Genotyping was outsourced to Transnetyx.
666 Animal use was conducted according to guidelines from the National Institutes of Health's Guide
667 for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care
668 and Use Committee of Rutgers University-Newark (PROTO201900161), UC Davis (#21947 and
669 #22438) and San José State University (#990, ex vivo muscle recordings). Mice were maintained
670 on a 12 h light/dark cycle, and food and water was provided *ad libitum*.

671

672 **Rotarod.** To assess motor coordination, a rotarod machine (IITC Life Sciences, Woodland Hills,
673 CA) that has an accelerating rotating cylinder was used. 8-10 week old mice of both sexes were
674 acclimated to the behavior room for 2 h prior to testing. Mice were assayed on the rotarod for 3
675 consecutive days, with 3 trials per day and an intertrial interval of at least 15 min. The average of
676 the three trials per day was used. The experimenter was blind to genotype.

677

678 **Open field test.** 8-10 week old mice of both sexes were acclimated to the behavior room for 2 h
679 prior to testing. The open field apparatus consisted of a black square sound attenuating box of

680 dimensions 40.6 cm × 40.6 cm. A camera suspended above the arena was connected to a
681 computer running Ethovision XT software, which tracked animal movement and velocity. An
682 animal was placed in the center of the arena and allowed to freely explore for a 10 min trial. The
683 experimenter was blind to genotype.
684
685 ***Tissue processing***. For spinal cord immunolabeling experiments, whole spinal columns from
686 adult (8-15 weeks) *Pirt*^{Cre};*Rosa26*^{Ai14} and *Pirt*^{Cre};Scn1a-floxed animals of both sexes were
687 harvested on ice. For Tdtomato IHC, spinal columns were fixed overnight at 4°C in 4%
688 paraformaldehyde. For vesicular glutamate transporter 1 (VGLUT1) and choline acetyltransferase
689 (ChAT) co-labeling experiments, spinal columns were fixed in 4% paraformaldehyde for 1 h on
690 ice. Tissue was then placed in 30% sucrose solution overnight at 4°C. Following cryoprotection,
691 tissue was embedded in optimal cutting temperature compound (OCT, Tissue-Tek® Sakura) and
692 stored at -80ºC until sectioning. DRG from adult (8-15 weeks) animals of both sexes were
693 harvested from thoracic spinal levels and fixed in 4% formaldehyde for 15 min at 4°C and were
694 then incubated in 30% sucrose for 2-4 h at 4°C. DRG were embedded in OCT and stored at -
695 80°C until sectioning.
696
697 ***Immunohistochemistry***. Immunohistochemistry of spinal cord cryostat sections (30μm) was
698 performed using the following primary antibodies: Rabbit anti-DsRed (1:3000, Takara Bio,
699 632496), guinea pig anti-VGLUT1 (1:8000, Zuckerman Institute, 1705), and rabbit anti-ChAT
700 (1:10,000, Zuckerman Institute, 1574). Secondary antibodies used were as follows: anti-rabbit
701 594 (1:1000, ThermoFisher, A32740), anti-guinea pig 488 (1:1000, ThermoFisher, A11073), and
702 anti-chicken 647 (ThermoFisher, A32733). Specimens were mounted with Fluoromount-G with
703 DAPI (SouthernBiotech, 0100-20). EDL muscles used in *ex vivo* muscle afferent recordings were
704 placed in ice cold 4% paraformaldehyde for 1 h followed by ice cold methanol for 15 min. Muscles

705 were incubated in blocking solution (0.3% PBS-T and 1% BSA) followed by incubation in primary
706 antibodies (guinea pig anti-VGLUT1 1:800 and chicken anti-NFH 1:300, ThermoFisher ab4680)
707 for 3-6 days at 4ºC. After primary antibody treatment, tissue was washed in blocking solution and
708 treated with secondary antibody (anti-guinea pig 488 1:50 and anti-chicken 594 1:300, Invitrogen,
709 WA316328) for 2-3 days. Specimens were mounted with VECTASHIELD® with DAPI (H-2000,
710 Vector Laboratories). All specimens were imaged in three dimensions on a on a Zeiss LSM880
711 Airyscan confocal microscope. Images were analyzed using ImageJ software.
712
713 ***Multiplex* in situ *hybridization*.** Fixed-frozen DRG tissue from 8-15 week old mice of both sexes
714 was cut in 25μm sections and placed on electrostatically coated slides. Sections were processed
715 for RNA *in situ* detection using a modified version of the manufacturer's protocol ((Griffith et al.,
716 2019), Advanced Cell Diagnostics) and the following probes: *Pvalb* (421931- C1, mouse), *Runx3*
717 (451271-C3, mouse), *Scn1a* (556181-C2, mouse), *Scn8a* (313341-C2, mouse), and *Scn9a*
718 (434191-C2, mouse). Following *in situ* hybridization, sections were incubated in blocking solution
719 (5% normal goat serum, 0.1% PBS-T) for 1 h at RT. Tissue was then incubated in primary
720 antibodies overnight at 4°C. The following antibodies were used: rabbit DsRed (1:3000, Takara
721 Bio, 632496), rabbit β3-tubulin (1:3000, Abcam, ab18207), chicken β3-tubulin (1:500, Abcam,
722 ab41489), rabbit CGRP (1:1000, Immunostar, 24112), chicken GFP (1:3000, Abcam, ab13970),
723 and chicken NFH (1:3000, Abcam, ab4680). Tissue was treated with the following secondary
724 antibodies for 45 min at RT: anti-rabbit 448 (1:1000, Invitrogen, A32731), 594 (1:1000, Invitrogen,
725 A11037) and 647 (1:1000, Invitrogen, A32733), anti-chicken 488 (1:1000, Invitrogen, A32931)
726 and 594 (1:1000, Invitrogen, A32740). Sections were washed and mounted with Fluoromount-G
727 with DAPI and imaged in three dimensions (2μm axial steps) on an Olympus confocal (LV3000)
728 using a 40X 0.90 NA water objective lens. Images were auto-thresholded and the probe signal
729 integrated density for individual neurons was analyzed using ImageJ software. The coefficients

730 of variation for *Scn1a*, *Scn8a*, and *Scn9a* integrated densities were calculated in Prism 9.0
731 (GraphPad Software) using the following formula: CV = μ/σ *100.
732
733 ***DRG culture preparation.*** DRG were harvested from thoracic spinal levels of adult
734 *Pvalb*^{cre};*Rosa*26^{Ai14} and *Pirt*^{cre};Scn1a-floxed (6-16 weeks) mice of both sexes and transferred to
735 Ca^{2+}-free and Mg^{2+}-free HBSS solution (Invitrogen, 14170-112). Upon isolation, processes were
736 trimmed, and ganglia were transferred into collagenase (1.5 mg/mL; Type P, Sigma-Aldrich,
737 11213865001) in HBSS for 20 min at 37°C followed by TrypLE Express (ThermoFisher, 12605-
738 010) for 3 min with gentle rotation. TrypLE was neutralized with 10% horse serum (heat-
739 inactivated; Invitrogen, 26050-070) and supplemented with culture media (MEM with L-glutamine,
740 Phenol Red, without sodium pyruvate, ThermoFisher, 11095-080), containing 10,000 U/mL
741 Penicillin-streptomycin (ThermoFisher, 15140-122), MEM Vitamin Solution (Invitrogen, 11120-
742 052), and B-27 supplement (ThermoFisher, 17504-044). Serum containing media was decanted
743 and cells were triturated using a fire-polished Pasteur pipette in the MEM culture media described
744 above. Cells were resuspended and triturated using a plastic pipette tip. Cells were plated on
745 glass coverslips that had been washed in 2M NaOH for at least 4 h, rinsed with 70% ethanol, UV-
746 sterilized, and treated with laminin (0.05 mg/mL, Sigma-Aldrich, L2020-1MG) for 1 hour prior to
747 plating. Cells were then incubated at 37°C in 5% CO_2. Cells were used for electrophysiology
748 experiments 14-36 h post-plating.
749
750 **In Vitro *Electrophysiology.*** Whole-cell voltage-clamp recordings were made from dissociated
751 DRG neurons using patch pipettes pulled from Model P-1000 (Sutter Instruments). Patch pipettes
752 had a resistance of 3-5MΩ when filled with an internal solution containing the following (in mM):
753 140 CsF, 10 NaCl, 1.1 EGTA, .1 $CaCl_2$, 10 HEPES, and 2.5 MgATP, pH with CsOH to 7.2. Seals
754 and whole-cell configuration were obtained in an external solution containing the following (in

755 mM): 145 NaCl, 5 KCl, 10 HEPES, 10 Glucose, 2 $CaCl_2$, 2 $MgCl_2$, pH 7.3 with NaOH, osmolarity
756 ~320mOsm. Series resistance ranged from 6-11MΩ and was compensated by 70-80%. To isolate
757 whole-cell sodium currents during voltage clamp experiments, a modified external solution was
758 applied containing the following (in mM): 15 NaCl, 130 TEA-Cl, 10 HEPES, 2 $BaCl_2$, 13 glucose,
759 0.03 $CdCl_2$, pH 7.3 with NaOH, Osmolarity ~320mOsm. Voltage clamp recordings were performed
760 at room temperature and current clamp recordings were conducted at 37ºC. Bath temperature
761 was controlled and monitored using CL-100 (Warner Instruments).
762
763 **Ex vivo *electrophysiology*.** The effect of the loss of $Na_V1.1$ on muscle spindle afferent firing
764 rates during muscle stretch and sinusoidal vibration was determined using an isolated muscle
765 nerve preparation. The extensor digitorum longus muscle and innervating peroneal branch of the
766 sciatic nerve were dissected from adult (2-4 month old) mice of both sexes. Muscles were held at
767 optimal length (Lo), or the length of the muscle that maximal force of twitch contraction occurred.
768 A series of nine 4 s ramp-and-hold stretches were given to 3 different stretch lengths repeated 3
769 times each (2.5%, 5%, and 7.5% Lo; ramp speed 40% Lo/s). A series of twelve 9 s sinusoidal
770 vibrations were given (25, 50, and 100 μm amplitude; 10, 25, 50, and 100 Hz frequency). A one-
771 minute rest was given between each length change. Firing rates during a 10 s baseline before
772 stretch (resting discharge or RD) and the maximal firing rate during the ramp up phase of stretch
773 (dynamic peak or DP) were calculated for all animals. We determined whether the response to
774 static stretch was maintained consistently throughout the 4s stretch, as well as the coefficient of
775 variability of the interspike interval (ISI) during the plateau phase of stretch (CV = Std Dev/Mean
776 of ISI over the time period of 1.5-3.5s after end of ramp up). Average firing rate during the 9 s of
777 vibration and whether the unit could entrain in a 1:1 fashion to vibration was also determined.
778 Detailed methods can be found in Wilkinson et al., 2012.
779

780 ***Pharmacology.*** ICA 121431 (#5066), 4,9-Anhydrotetrodotoxin (AH-TTX, #6159), and PF-
781 05089771 (#5931) was purchased from Tocris Bioscience. Tetrodotoxin (TTX, ab120054) was
782 purchased from abcam. All other chemicals were from Sigma-Aldrich and Fisher Chemical.
783
784 ***Data acquisition and analysis***. Currents and voltages were acquired using pClamp software
785 v11.2 (Molecular Devices). Recordings were obtained using an AxoPatch 200b patch-clamp
786 amplifier and a Digidata 1550B and filtered at 5 kHz and digitized at 10 kHz. For biophysical
787 analysis of whole-cell sodium currents, conductance (G) was calculated as $G = I / (V - E_{Na})$, in
788 which *I* is the peak current, *V* is the voltage step, and E_{Na} is the reversal potential for sodium
789 calculated from Nernst equation based on the intracellular and extracellular sodium
790 concentrations in our recording solutions (10.38mV). Conductance data were normalized by the
791 maximum conductance value, G_{max}, and data was fit with the Boltzmann equation: Fraction
792 available = Minimum + ([Maximum-Minimum]/[1+exp(V50 Vm)/*k*]), where V_{50} denotes the
793 membrane potential at which half the channels are inactivated and *k* denotes the Boltzmann
794 constant/slope factor. For voltage dependence of steady state inactivation, peak current data
795 were normalized based on the maximum current, I_{max}. Analysis of action potential amplitude, full-
796 width half max, and threshold were performed on the first action potential elicited in response to
797 a 100 pA current injection (100 ms). Action potential threshold was calculated as the membrane
798 potential at which the first derivative of the somatic membrane potential (dV/dT) reached 10 mV
799 ms^{-1} (Griffith et al., 2019; Kress et al., 2008). Tau values were calculated from 20 ms voltage
800 steps from -90 mV to -30 mV and analyzed with single exponential curve fits. Voltage-clamp and
801 current-clamp experiments were analyzed with Clampfit software v11.2 (Molecular Devices) and
802 custom MatLab Scripts. *Ex vivo* recordings were obtained using an A-M Systems Model 1800
803 extracellular amplifier with headstage and digitized using an ADInstruments PowerLab. Data was
804 analyzed using ADInstruments LabChart software using the Spike Histogram function.
805

806 ***Experimental design and statistical analysis.*** Summary data are presented as mean ± SEM,
807 from *n* cells or afferents, or *N* animals. For quantitative analysis of *in situ* hybridization data, at
808 least 3 biological replicates per condition were used and the investigator was blinded to genotype
809 for analysis. Behavioral experiments and analysis were also performed genotype-blind. Statistical
810 differences were determine using parametric tests for normally distributed data and non-
811 parametric tests for data that did not conform to Gaussian distributions or had different variances.
812 Statistical tests are listed in *Results* and/or figure legends. Statistical significance in each case is
813 denoted as follows: *p <0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. Statistical tests and
814 curve fits were performed using Prism 9.0 (GraphPad Software). All data generated or analyzed
815 during this study are included in the manuscript and supporting file; Source Data files have been
816 uploaded to Mendeley for all figures. Code has been uploaded to GitHub. A key resources table
817 with specific organism and reagent information has been included in the method section.
818

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