Molecular basis of ancestral vertebrate electroreception

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Elasmobranch fishes, including sharks, rays, and skates, use specialized electroreceptive organs to detect extremely small changes in environmental electric fields. Electroreceptors in these organs can discriminate and respond to minute changes in environmental voltage gradients through an unknown mechanism. Here we show that the voltage-gated calcium channel Cav1.3 and the big conductance calcium-activated potassium (BK) channel are preferentially expressed by electroreceptive cells in little skate (Leucoraja erinacea) and functionally couple to mediate electroreceptive cell membrane voltage oscillations, which are important for the detection of specific, weak electrical signals. Both channels exhibit unique properties compared with their mammalian orthologues that support electroreceptive functions: structural adaptations in Cav1.3 mediate a low-voltage threshold for activation, and alterations in BK support specifically tuned voltage oscillations. These findings reveal a molecular basis of electroreception and demonstrate how discrete evolutionary changes in ion channel structure facilitate sensory adaptation.

Cation currents in electroreceptive cells

We obtained whole-cell patch-clamp recordings from dissociated electroreceptive cells (Fig. 1d) using caesium (Cs+) to block potassium (K+) currents and identified a low-threshold, voltage-activated inward current (Fig. 1e). This current (I_Cav) was blocked by nonselective Cav pore blockers, enhanced by the L-type agonist Bay K, and partially inhibited by L-type antagonists (Fig. 1f). I_Cav was not affected by inhibitors of P/Q-, N-, or T-type Cav channels, or by a Nav channel inhibitor (Fig. 1f). The conductance–voltage (G–V) relationship was steep, with a relatively negative half-maximal conductance compared with those of other Cav channels.15 Channel inactivation was slow, contributing to a large ‘window current’ that represents sustained channel activity within a physiologically relevant voltage range (Fig. 1g). Thus, we conclude that I_Cav is mediated by a low-threshold L-type Ca2+ channel with steep voltage dependence.

Previous electrophysiological recordings from little skate ampullary organs have suggested that K+ channels contribute to the detection of weak electrical signals and membrane voltage oscillations, which is required for stimulus selectivity7,8,10. We measured K+ currents directly using a K+-based intracellular solution and detected a large outward current in response to voltage pulses (Fig. 1h) that was blocked by the K+ channel pore blocker tetraethylammonium (TEA+). Furthermore, pharmacological agents that modulated I_Cav also regulated I_K (Fig. 1i), suggesting that a Ca2+-activated K+ channel mediates I_K. Indeed, I_K was blocked by selective inhibitors of BK channels, which are activated by Ca2+ (Fig. 1h, i).

Cav and BK in electroreceptive cells

To identify the ion channel subtypes that mediate I_Cav and I_K, we transcriptionally profiled little skate ampullary organs. The orthologue of cacaLd, which encodes the α1 subunit of Cav1.3, was the predominant Ca2+ channel subtype expressed in ampullae and was greatly enriched (more than 90-fold) in ampullae compared to other tissues examined (Fig. 1j). Several Cav auxiliary subunits were also expressed in the ampullae (Extended Data Fig. 1a). Notably, mammalian Cav1.3 has a relatively low voltage threshold compared to other L-type Cav channels, and plays a critical role in auditory hair cells, which are related to electroreceptors16–20. We also examined the expression profiles of pore-forming α-subunits of K+ channels and, consistent with our functional data, found that kcna1 (which encodes the α-subunit of BK) was the most abundant K+ channel in ampullary organs, expressed at levels substantially higher (more than 35-fold) than other Ca2+-activated K+ channels (Fig. 1j) and Extended Data Fig. 1b). At the cellular level, both Cav1.3 and BK transcripts were robustly expressed in ampullary receptor cells and absent from supporting cells and tubule structures (Fig. 1k). The expression of other Cav and Ca2+-activated K+...
**Figure 1** | CaV1.3 and BK channels mediate the major cation currents in electrosensory cells. 

- **a**: Dorsal profile of little skate (*Leucoraja erinacea*). 
- **b**: Isolated ampullary organs with short lengths of canal and attached afferent nerve fibres (scale bar, 400 μm). 
- **c**: Electrosensory cells in a representative patch-clamp experiment (scale bar, 10 μm). 
- **d**: Representative gating and current traces: green and blue traces show current elicited by −55 and −45 mV pulses, respectively. Right, average current–voltage (I–V) relationship (n = 11). Scale bars, 20 pA pF⁻¹ vertical, 100 ms horizontal. 
- **e**: Pharmacological profile of I_{CaV}. Channel subtype selectivity is indicated above bars. Each circle depicts one experiment; bars represent mean ± s.e.m. measured at peak amplitude; P < 0.05 for L-type channel modulators, one-way ANOVA with post hoc Bonferroni test. 
- **f**: Conductance–voltage (G–V) relationship with half-maximal activation voltage (V_{1/2}) of −52 ± 0.8 mV with slope factor (K_s) = 4.8 ± 0.6 mV. Inactivation–voltage relationship with half-inactivation potential (V_{1/2}) of −55.9 ± 1.7 mV with slope factor (K_i) = −6.3 ± 1.6 mV. Window current was observed between −70 and −30 mV and peaked at about −58 mV with around 40% maximal conductance. Data represented as mean ± s.e.m., n = 10. 
- **g**: Representative K⁺ currents. Scale bars, 20 pA pF⁻¹, 100 ms. 

**Figure 2** | Skate CaV has a low voltage threshold. 

- **a**: Representative voltage-activated currents recorded in HEK293 cells expressing skate CaV1.3 (sCaV, blue) or the homologous long isoform of rat CaV1.3 (rCaV, red). Scale bars, 200 pA, 50 ms. 
- **b**: Normalized I–V relationship of sCaV (blue) and rCaV (red) (n = 7). 
- **c**: Representative gating and current traces for sCaV (blue) and rCaV (red) G–V (n = 8) and inactivation (n = 7) curves. 
- **d**: Average V_{1/2} for sCaV = −42.68 ± 0.56, n = 8) compared with rCaV = −18.16 ± 0.51, n = 7, P < 0.0001. V_{1/2} was similar, n = 7. 
- **e**: I–V relationship between relative conductance (G/G_{max}, y-axis) and charge movement (Q_{ON}/Q_{ON,max}, x-axis) for sCaV (blue, n = 7) and rCaV (red, n = 8). P < 0.0001 for difference in Q_{ON} required for half-maximal conductance (dashed line). 
- **f**: Inset: representative ON-gating currents and I_{tail} elicited by a voltage step to reversal potential (E_{REV}) from and returning to −100 mV. Scale bars, 500 pA, 10 ms. 

- **sCaV has a low voltage-activation threshold**: The pore-forming subunit of sCaV1.3 is 78% identical to the well-characterized long isoform of rat CaV1.3 (rCaV1.3), and heterologous expression of sCaV1.3 produced voltage-gated currents with ion sensitivities and pharmacological profiles resembling those of rCaV1.3 or the I_{CaV} of native electrosensory cells (Extended Data Figs 2, 3). However, like native I_{CaV}, the voltage threshold of sCaV1.3 was substantially lower than that of rCaV1.3. Currents produced by sCaV1.3 were activated at more negative potentials than those produced by rCaV1.3 and increased steeply to maximal amplitude with increasing voltage (Fig. 2a, b). Although inactivation was similar between sCaV1.3 and rCaV1.3, the G–V curve was notably shifted in the negative direction for sCaV1.3, contributing to a substantially larger window current for the skate channel (Fig. 2c, d). sCaV1.3 also exhibited reduced Ca²⁺-dependent inactivation compared to rCaV1.3 (Extended Data Fig. 2). These functional properties match those of native I_{CaV}, suggesting that sCaV1.3 forms the predominant voltage-gated Ca²⁺ channel in electrosensory cells.

What accounts for the decreased voltage threshold of sCaV1.3? Measuring ionic and gating currents from the same cells allowed us to examine the relationship between relative conductance and voltage sensor movement (represented by ON gating charge, Q_{ON}). Q_{ON} for skate versus rat orthologues. Both gating current kinetics and Q_{ON}–voltage relationships were similar for the two channels (Fig. 2e and Extended Data Fig. 4a, d); however, the G–V relationship was shifted to more negative voltages (Extended Data Fig. 4d), and the Q_{ON}–G relationship was extremely steep for sCaV1.3 compared with rCaV1.3 (Fig. 2f), suggesting that only minimal voltage sensor movement is required.
Figure 3 | Positively charged motif confers skate CaV voltage threshold.

**a.** Predicted topology of CaV1.3, α-subunit. Species alignment reveals a positively charged insert in DIVS2–S3 of the skate orthologue. Charge-neutralized skate CaV1.3 (neutral-sCaV) was generated by replacing charged residues (KKKER) of the skate motif with glutamines.

**b.** Representative currents from HEK293 cells expressing wild-type skate CaV1.3 (WT-sCaV, blue) or neutral-sCaV (red). Scale bars, 100 pA, 50 ms. **c–f.** I–V relationships for WT-sCaV (blue) and neutral-sCaV (red). **g**–**n.** Conductance (dashed line).

**h.** Predicted topology of CaV1.3 human, rat, and zebrafish orthologues reveals a skate-specific insertion that intro-

**i.** Alignments of the skate motif (charged-rCaV) or neutral-rCaV (red) with the charged skate motif (charged-rCaV) or WT-sCaV (blue) and neutral-sCaV (red). n = 7 per condition. V_{1/2} from WT-sCaV (−41.13 ± 0.75 mV) compared with neutral-sCaV (−25.99 ± 0.92 mV), n = 7 per condition, P < 0.0001.

**j.** Q_{ON} relationships of neutral-sCaV (red) and WT-sCaV (blue), n = 7 per condition, P < 0.0001 for difference in Q_{ON} required for half-maximal conductance (dashed line). e. I_{fail} versus Q_{ON,max}. Slopes: 1.92 ± 0.15 for WT-sCaV (blue, n = 9), 0.66 ± 0.16 for neutral-sCaV (red, n = 7). Inset: representative maximal ON gating currents and I_{fail}. Scale bars, 100 pA, 10 ms. f. Representative currents from HEK293 cells expressing rCaV1.3 with the charged skate motif (charged-rCaV) or WT-rCaV1.3 with a neutralized skate motif insert (neutral-rCaV). Scale bars, 100 pA, 50 ms. g. I–V relationships for charged-rCaV (blue) and neutral-rCaV (red). n = 9 per condition. V_{1/2} from charged-rCaV (−37.24 ± 0.32 mV) compared with neutral-rCaV (−19.6 ± 0.32 mV), n = 9 per condition, P < 0.0001.

**h.** Q_{ON} relationships of charged-rCaV (blue, n = 9) and neutral-rCaV (red, n = 8). P < 0.0001 for difference in Q_{ON} required for half-maximal conductance (dashed line). I_{fail} versus Q_{ON,max}. Slopes: 2.45 ± 0.19 for charged-rCaV (blue), 1.19 ± 0.04 for neutral-rCaV (red). n = 9 per condition. Inset: representative maximal ON gating currents and I_{fail}. Scale bars, 100 pA, 10 ms. All data represented as mean ± s.e.m. All P values from two-tailed Student’s t-test.

to elicit maximal channel opening for the skate channel. As another index of coupling efficiency, we measured maximal Q_{ON} in the absence of pore blockers by applying voltage pulses to the channel’s reversal potential (E_{REV}) and then stepping to −100 mV to induce large tail currents (I_{tail})^{21}. A similar Q_{ON} induced a considerably larger I_{tail} for sCaV1.3 than for rCaV1.3 (Fig 2g), suggesting that sCaV1.3 exhibits a greater channel open probability or open-state stability in response to equal voltage sensor movement. Collectively, these data indicate that the low voltage threshold of the skate channel originates from increased coupling between voltage sensors and channel opening.

alignment of the α-subunit of sCaV1.3 with its human, rat, and zebrafish orthologues revealed a skate-specific insertion that introduces four positively charged residues (KKKER) into an intracellular loop of domain IV (DIVS2–S3) (Fig 3a). A charge-neutralized mutant (neutral-sCaV) required much greater depolarization for maximal activation and exhibited decreased current density compared with wild-type (WT)-sCaV1.3 (Fig 3b, c). Gating current properties were not affected by charge neutralization (Extended Data Fig 4b, e), but consistent with the increased voltage threshold of neutral-sCaV1.3, more relative Q_{ON} was required for maximal conductance compared with WT-sCaV1.3 (Fig 3d). Furthermore, maximal Q_{ON} elicited by voltage pulses to E_{REV} resulted in smaller I_{fail} amplitude in neutral-sCaV1.3 than in WT-sCaV1.3 (Fig. 3e). These results suggest that coupling between voltage sensor movement and channel opening is decreased in neutral-sCaV1.3 and that the low voltage threshold of sCaV1.3 is determined by the charged insertion in DIVS2–S3. Indeed, the charged motif from sCaV1.3 (but not a neutralized control) was sufficient to confer skate-like voltage sensitivity to rCaV1.3 (Fig. 3f, g). The gating current properties of ‘charged-rCaV1.3’ and ‘neutral-rCaV1.3’ were similar (Extended Data Fig 4c, f), but comparatively less relative Q_{ON} was required for maximal conductance of charged-rCaV1.3 and maximal Q_{ON} elicited a larger I_{fail} than in neutral-rCaV1.3 (Fig 3h, i), indicating enhanced coupling between voltage sensor movement and pore opening in charged-rCaV1.3.

According to recent structural models of a related mammalian CaV channel^{22,23}, the charged skate motif within the intracellular loop of DIVS2–S3 could be relatively close to the bottom of the charged voltage sensor (DIVS4) such that electrostatic interactions could repel DIVS4 into a partially activated or primed state and thereby decrease the voltage threshold. To test this hypothesis, we examined voltage-dependent channel activation kinetics and found that activation occurred more rapidly in cells expressing charged-rCaV1.3 than in those expressing neutral-rCaV1.3 or WT-rCaV1.3 (Extended Data Fig 5a). If charge interactions between the skate motif and DIVS4 position the voltage sensor in a primed state, then extremely negative voltages might force the voltage sensor into a resting state, resulting in activation kinetics similar to those of wild-type channels. Indeed, following a long negative prepulse (1 s, −170 mV), the activation kinetics of charged-rCaV1.3, neutral-rCaV1.3 and WT-rCaV1.3 were identical. As we increased the prepulse voltage to more positive values, the activation rate of charged-rCaV1.3 increased, whereas those of neutral-rCaV1.3 and WT-rCaV1.3 did not change (Extended Data Fig 5b, c). These Cole–Moore shifts^{23} demonstrate that an additional voltage-dependent step in channel activation occurs at very negative potentials in the presence of the
charged skate motif, supporting our hypothesis that charge repulsion regulates the domain IV voltage sensor to decrease voltage threshold and enhance open-state stability at physiological membrane potentials (Extended Data Fig. 5d). Gating currents measure the movement of all voltage sensors (domains I–IV) irrespective of heterogeneity, so a small difference, such as a primed voltage sensor, could be missed. While Cole–Moore effects support our model, further structural insights are required to confirm this hypothesis.

**sBK has small conductance**

We wondered whether skate BK (sBK) is also specially adapted for electro-oscillation. Single-channel recordings from HEK293 cells expressing the α-subunit (kcnma1) of skate or mouse BK (mBK) showed that sBK had drastically reduced current amplitude at all voltages compared with mBK, resulting in a markedly decreased slope conductance (Fig. 4a). Both channels were similarly sensitive to intracellular Ca$^{2+}$ (Extended Data Fig. 6a), but sBK single-channel currents were smaller and had a shorter open-state dwell time (Fig. 4b), so that sBK passes less current than mBK.

Considering the unique conductance profile of sBK, we aligned its pore region with those of its canonical mouse, rat, human, and zebrafish orthologues to reveal high conservation (sBK is 87% identical to mBK), with a few notable alterations within an exon encoding an intracellular region near the pore that is alternatively spliced in mammals and affects channel conductance through electrostatic interactions with K$^{+}$ (Fig. 5a). To determine whether the altered amino acids affect the properties of sBK, we converted arginine and/or alanine of sBK to match the cognate residues of mBK. The sR340S channel showed appreciably altered conductance and open-state dwell time, whereas the sA347E channel showed smaller differences (Fig. 5b–d). Remarkably, substitution of both amino acids (sBK-SE) produced a single-channel conductance nearly identical to that of mBK, with an open-dwell time akin to that of the mouse channel (Fig. 5b–d and Extended Data Fig. 6b). Conversely, these two amino acids from sBK were sufficient to convert the conductance and open-time of mBK to those of sBK (mBK-RA; Fig. 5b–d and Extended Data Fig. 6b).

We next investigated whether an altered K$^{+}$ concentration near the pore accounts for the reduced conductance of sBK. When patches expressing wild-type and mutant BK channels were exposed to various concentrations of intracellular K$^{+}$ (140 mM, 640 mM, or saturating 3.14 M), single-channel amplitude increased for all BK channels with increasing K$^{+}$ concentration, and sBK and mBK-RA exhibited the smallest current amplitude at 140 mM and 640 mM (Fig. 5e). Current amplitude was the same for all channels when exposed to a saturating
K⁺ concentration of 3.14 M, indicating that the pore is intrinsically capable of passing the same current (Fig. 5e). Notably, in the presence of 640 mM K⁺, sBK channels passed nearly as much current as sBK-SE or wild-type mBK in 140 mM (Fig. 5e and Extended Data Fig. 6c). Thus, adaptations in sBK alter intracellular electrotactics near the pore to decrease the apparent conductance by reducing local K⁺ concentration by over 500 mM.

**Voltage oscillations in electroreception**

Membrane voltage (V_m) oscillations, which have been described in ampullary epithelial current-clamp experiments, control neurotransmitter release from electrosensory cells onto postsynaptic nerve fibres. Under our conditions, electrosensory cells had a resting V_m of around −55 mV and exhibited small, low-frequency voltage oscillations. Injecting current to bring the V_m to various potentials modified oscillatory behaviour (Fig. 6a). Because oscillations occur over voltages at which sCaV1.3-mediated ICa is activated, we plotted oscillation amplitude against membrane voltage and overlaid the normalized window current of ICa (Fig. 6b). Interestingly, the average oscillation amplitude increased with window current, suggesting that tonic ICa activity underlies the depolarization phase of V_m oscillations in electrosensory cells. In the presence of TEA⁺, current injection elicited prolonged depolarization (Fig. 6c), suggesting that sBK-mediated IK contributes to V_m oscillations, potentially by restoring cells to a hyperpolarized state after the initial depolarization. Furthermore, spontaneous oscillatory behaviour was significantly reduced by TEA⁺ or nifedipine (Fig. 6d). Together, our data suggest that ICa and IK couple to mediate V_m oscillations.

How might the properties of sBK affect functional coupling of the two channels? As expected from the reduced conductance and open time of sBK, intracellular Ca²⁺ elicited smaller whole-cell currents from HEK293 cells expressing sBK compared to sBK-SE or mBK (Extended Data Fig. 7a, b). Voltage pulses in cells coexpressing Cav1.3 and sBK also elicited smaller K⁺ currents and decreased K⁺ permeability compared with sBK-SE or mBK. Thus, sBK allows relatively more CaV-mediated Ca²⁺ current, while K⁺ currents mediated by sBK-SE or mBK quickly occlude measurable Ca²⁺ current (Extended Data Fig. 7c, d).

To determine whether sBK-specific properties are important in native electrosensory cells, we used the selective BK agonist NS11021 (NS) to pharmacologically increase average open probability (P_o) and open-state dwell time, producing a BK channel that more closely resembled mBK (Extended Data Fig. 8a). In recordings from cells coexpressing Cav1.3 and sBK, or from native electrosensory cells, NS increased outward current amplitude and shifted reversal potentials in the negative direction, indicating increased BK activity and K⁺ permeability (Extended Data Fig. 8b–d). In current-clamp experiments with electrosensory cells, treatment with NS greatly reduced the amplitude of voltage oscillations and increased their frequency (Fig. 6f, g). The addition of iberiotoxin blocked oscillations, consistent with a requirement for BK channels in spontaneous electrosensory cell V_m oscillations (Fig. 6f). Thus, evolutionary tuning of BK decreases conductance and its activity controls V_m oscillation amplitude and frequency. We hypothesize that because CaV-mediated Ca²⁺ influx immediately activates a BK current to limit the CaV-mediated depolarization, a smaller BK current will more slowly return V_m to rest, thus supporting large-amplitude, low-frequency oscillation events.

Electrosensory cells probably contain a mechanism to dampen BK-mediated hyperpolarization, thereby maintaining a membrane voltage at which CaV could initiate another oscillation event. Indeed, the most highly expressed transcript in ampullary organs is that of parvalbumin 8, a Ca²⁺-binding protein implicated in V_m oscillations that could chelate CaV-mediated Ca²⁺ influx to produce only brief BK activation (Extended Data Fig. 9a). Consistent with this hypothesis, a plasma membrane Ca²⁺-ATPase is also highly enriched in ampullary organs, presumably to support persistent oscillations (Extended Data Fig. 9b, c).

To examine the relative contributions of sCaV1.3 and sBK at an organismal level, we preincubated behaving skates with vehicle or with nifedipine to inhibit Cav1.3, NS to stimulate BK, or mibebradil, a T-type Cav antagonist that does not affect ICa. We then tested whether they favoured a zone in which a submerged dipole electrical stimulus was buried under a sand-covered surface (Fig. 6h and Extended Data Fig. 10a). For each treatment condition, a startle response was subsequently measured to confirm that the drug did not generally affect mobility (Extended Data Fig. 10b, c). Although both untreated and mibebradil-treated skates spent the majority of their time in the vicinity of the hidden electrical stimulus, skates treated with nifedipine or NS spent considerably less time near the active electrode (Fig. 6i). These results are consistent with the notion that Cav1.3 and low-level BK activity are important for electroreception-related behaviours.

**Discussion**

Electroreception is an ancient sensory modality that has evolved independently multiple times to facilitate the detection of environmental electrical signals. Our results demonstrate that the skate electroreceptive system uses low-threshold Cav1.3 coupled to BK to produce cellular V_m oscillations. This is reminiscent of electrical resonance in auditory hair cells, which also contain ribbon synapses and use Cav and BK orthologues to produce V_m oscillations that regulate vesicle release dynamics, allowing the coding of stimulus strength and frequency. In some animals, alterations in Cav and BK properties regulate electrical resonance to tune the frequency selectivity for incoming auditory signals. Indeed, ampullary organs are developmentally derived from the lateral line mechanosensory system (itself an elaboration of the auditory system), and some mechanosensory hair cells similarly express Cav or BK sequence variations. Thus, electroreceptive cells may have evolved from specially adapted lateral line hair cells. Considering the simplicity and flexibility of this tuning mechanism, modifications in electroreceptive cell transducers may provide a means to alter V_m oscillations for selective electrical frequency detection of salient signals according to developmental maturation, reproductive state, or nutritional condition. Oscillating tuberous electroreceptors in weakly electric fishes (for example, Gymnotiformes and Mormyriformes) are functionally tuned to detect distortions of self-generated electric organ discharges; whether these systems use similar molecular mechanisms remains to be determined.
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METHODS

Animals and cells. Male and female little skates (Leucoraja erinacea) were provided by the Marine Biological Laboratory (Woods Hole, Massachusetts) and their use approved by the UCSC Animal Care and Use Committee. Animals used for cellular physiology experiments were euthanized with triacane methanesulfonate (MS222, 1 g/l). Hidroy capsules were removed on ice, and individual ampullae were dissected by cutting the canals and afferent nerve fibres. Ampullae were treated with papain for 2–3 min and then electrore sensory cells were mechanically dissociated over the recording chamber. Isolated electrore sensory cells were identified by the presence of their large single kinocilium. HEK297T cells (ATCC) were grown in DMEM, 10% fetal calf serum, and 1% penicillin/streptomycin at 37 °C, 5% CO2. Cells were transfected using lipofectamine 2000 (Invitrogen/Life Technologies) according to the manufacturer’s protocol. One micrometer skate or rat cacna1d was co-expressed with 1 μg rat cacnb3, 1 μg rat cacna2d1, and 0.3 μg GFP. Mock transfection experiments (1 μg rat cacnb3, 1 μg rat cacna2d1, and 0.3 μg GFP, but no cacna1d) were performed as controls, in which no voltage-activated inward currents were observed. For BK experiments, 1 μg skate or mouse kcnma1 was co-expressed with 0.3 μg GFP. Mock transfection experiments with 0.3 μg GFP were performed as controls. To enhance expression of wild-type and charge-neutralized skate CaV1.3, cells were transfected for 6–8 h and then incubated at 28 °C for 3–4 days, plated on poly-L-lysine-coated coverslips, incubated for an additional 3–4 days at 28 °C, and then used for experiments40.

Molecular biology. Cacna1d and kcnma1 from little skate ampullary organs were synthesized via Genscript. Rat cacna1d, cacnb3, and cacna2d1 were gifts from D. Lipscombe (Addgene plasmids 49332, 26574, 26575) and mouse kcnma1 was from L. Salkoff (Addgene plasmid 16195). Cacna1d mutagenesis was carried out and verified by Genscript. BK point mutations were induced using QuikChange Lightning site-directed mutagenesis kit (Agilent Genomics).

Electrophysiology. Recordings were carried out at room temperature using a MultiClamp 700B amplifier (Axon Instruments) and digitized using a Digidata 1322A (Axon Instruments) interface and Pclamp software (Axon Instruments). Whole-cell recording data were filtered at 1 kHz and sampled at 10 kHz. Data were leak subtracted online using a P/4 protocol, and membrane potentials were corrected for liquid junction potentials. Single-channel data were filtered at 5 kHz and sampled at 50 kHz. Electrosensory cell recordings were made using borosilicate glass pipettes polished to 3–4 MΩ.

Voltage pulses following a series of 1-s prepulses ranging from a −115 mV holding potential to 10 mV increments. Voltage-dependent inactivation was quantified as

\[ Q_{100} = \frac{1}{\cosh(\frac{100}{mV}) - \cosh(\frac{115}{mV})} \]

was derived from I–V curves by calculating

\[ G = \frac{I_{Ca}(V)}{V_{rev}} - E_{rev} \]

and fit with a Boltzmann equation. Voltage-dependent inactivation was measured during −20 mV (electrore sensory cells) or −10 mV (heterologously expressed channels) voltage pulses following a series of 1-s prepulses ranging from −115 to 65 mV in 10 mV increments. Voltage-dependent inactivation was quantified as

\[ I_{Ca}(V)_{max} - \frac{I_{Ca}(V)}{I_{Ca}(V)_{max}} \]

occurring at the voltage pulse following a −115 mV prepulse. Q100 represents the integral of nonlinear ON-gating current measured during voltage pulses from a holding potential of −110 mV. Q100 was quantified only from cells with no tonic current. I–V–J_{elast} relationships were examined by applying short pulses to predetermined E_{rest} for each cell from −100 mV and stepping back to −100 mV to induce a large J_{elast}.

Transcriptome sequencing and analysis. Poly-A+ RNA was extracted from the ampullae, ampullary tubules or canals, non-electroreceptor-covered skin, and liver of an adult L. erinacea and then was reverse-transcribed using the SuperScript III kit (Invitrogen/Life Sciences). Sequencing libraries were prepared using the Illumina TruSeq Stranded mRNA Library Prep Kit according to the manufacturer’s instructions. Libraries were sequenced on the Illumina Hi-Seq 4000 (V. C. Genomics Sequencing Laboratory, University of California, Berkeley) using 150 cycles of paired end reads, producing 20–30 million inserts for each sample.

Transcriptomes for each sample were assembled de novo using the Trinity suite (version 2.1.0). Sequences were aligned to the zebrafish protein database (NCBI assembly GRCz10) using the blastsx tool from NCBI blast (version 2.2.31) using a maximum E value of 1 × 10−7. Reciprocal blasts alignments (using zebrafish protein sequences that aligned to L. erinacea sequences) were performed to the human protein database. Estimates of relative abundance for differential expression comparisons were performed using the RSEM software package within Trinity. These values are reported as fragments per kilo base of exon per million fragments mapped (FPKM).

Whole mount preparations. L. erinacea embryos were removed from egg cases, euthanized with an overdose of MS-222 in artificial seawater, and fixed in 4% paraformaldehyde for at least 24 h. The cartilage matrix and electroreceptor tubules were stained using Acan blue (20 mg Acan blue 8GX in 30 ml glacial acetic acid and 70 ml 100% ethanol) following previously published methods41.

In situ hybridization histochemistry. Adult skates were euthanized with an overdose of MS-222 in artificial seawater and transcardially perfused with PBS followed by 4% PFA. The hydoid capsule, which contained the aleuropiles of the ampullary organs, was dissected and cryo-protected in 30% sucrose in PBS overnight. Cryostat sections (15 μm thick) were probed with digoxigenin-labelled cRNA for skate CaV1.3 and fluorescein-labelled cRNA for skate BK receptors. Probes were generated by T7/T3 in vitro transcription reactions using a 510-nucleotide fragment of CaV1.3 cDNA (nucleotides 4501–5011) and a 510-nucleotide fragment of BK cDNA (nucleotides 2934–3444). Hybridization was developed using anti-digoxigenin and anti-fluorescein Fab fragments, followed by incubation with fast red and streptavidin-conjugated Dylight 488 (to probe for BK) according to published methods42. Following hybridization and detection, sections were Coverslipped and co-stained with DAPI as a nuclear marker (Prolong Gold Antifade Mountant with DAPI; Invitrogen).

Behavioural analysis. In an isolated location and under normal lighting conditions, male and female little skates were placed in 250 ml of seawater or seawater with 5 mM nifedipine, 10 μM NS-1102, or 5 μM mibefradil for 30 min. Following incubation, skates were allowed to habituate for 10 min in an acrylic cylindrical tank (diameter 28 cm) and were surrounded by a barrier to block external visual cues. A DC dipole stimulus (18 μA over 5 mm), generated by threading positive and negative ends of tin-plated copper wire (300 VH, 22 gauge, NTE Electronics, Inc.) into seawater-filled Tygon tubing, was randomly positioned and obscured by the sand substrate in one of four circles (diameter 5.5 cm), all equally spaced from the centre of the tank (see Extended Data Fig. 10A). All skates were exposed to a plume of Myss shrimp odorant originating in the centre of the arena in order to attract them. A digital video (manuscript) was positioned above the tank was used to record skate activity for 30 min. Trials in which the skate executed more than three large movements and remained visible above the sand substrate for the majority of the time were quantified. Time spent with the majority of the pectoral disc within the outlined circle containing the electrical stimulus was compared to time spent in all other outlined circular areas. Following 30 min of undisturbed observation, tactile startle responses were observed from skates in response to gentle taps of the lateral pectoral fins to verify normal movement capabilities. Startle responses were quantified as the distance moved following a straight line from the dorsal side center between the eyes in still water and after the elicited startle response.

Statistical analysis. Data were analysed with Clampfit (Axon Instruments) or Prism (Graphpad). Data are represented as mean ± s.e.m. and n represents the number of cells for electrophysiological experiments and trials for behavioural experiments. Data were considered significant if P < 0.05 using paired or unpaired
two-tailed Student’s t-tests or one- or two-way ANOVAs. All significance tests were justified considering the experimental design and we assumed normal distribution and variance, as is common for similar experiments. Sample sizes were chosen based on the number of independent experiments required for statistical significance and technical feasibility. Behavioural trials were randomized, and the experimenter was blinded to each trial condition.

Data availability. Deep sequencing data that support the findings of this study have been archived in the Gene Expression Omnibus (GEO) database repository with accession code GSE93582. GenBank accession numbers for skate Cav1.3 α-subunit and skate BK α-subunit are KY355736 and KY355737, respectively. All other data are available from the authors upon reasonable request.
Extended Data Figure 1 | \( \text{Ca}_V \) and \( \text{K}^+ \) channel expression in little skate.

a. \( \text{Ca}_V \) auxiliary subunit mRNA expression in skate ampullary organs, ampullary canals, skin, and liver. Bars represent fragments per kilobase of exon per million fragments mapped (FPKM).

b. Ten most highly expressed \( \text{K}^+ \) channel \( \alpha \)-subunit transcripts in ampullary organs.
Extended Data Figure 2 | Skate Cav ion selectivity and Ca$^{2+}$-dependent inactivation. a–c. Representative currents measured from electrosensory cells (native $I_{CaV}$, a), HEK293 cells expressing skate Cav 1.3 (sCaV, b), or HEK293 cells expressing rat Cav 1.3 (rCaV, c) in the presence of 5 mM extracellular Ca$^{2+}$, Ba$^{2+}$, or Sr$^{2+}$. At the end of a 200-ms voltage pulse eliciting maximal current, approximately 50% of current remained in native electrosensory cell $I_{CaV}$ or HEK293 cells heterologously expressing sCaV 1.3, whereas cells expressing rCaV 1.3 had only about 20% of current remaining. In electrosensory cells or cells expressing heterologous sCaV 1.3 or rCaV 1.3, the percentage of remaining current was significantly increased by replacing extracellular Ca$^{2+}$ with Ba$^{2+}$ or Sr$^{2+}$ ($P < 0.05$, one-way ANOVA with post hoc Bonferroni test). Data represented as mean relative current remaining at the end of the 200-ms voltage pulse that elicited maximal currents (± s.e.m., $n = 5$ per condition).
Extended Data Figure 3 | Skate CaV pharmacology. a, b, Pharmacology of skate CaV1.3 (sCav). Representative currents recorded in response to voltage pulses in the presence of vehicle (control, <0.1% DMSO) or 10 μM nifedipine or nimodipine. Currents were incompletely inhibited, similar to native electrosensory cell I_Cav (Fig. 1e). Dose–response relationships of current amplitudes measured at voltages that elicited maximal currents. Data are represented as mean ± s.e.m., n = 6 per treatment. c, d, Pharmacology of rat CaV1.3 (rCav). Representative currents in the presence of vehicle or 10 μM nifedipine or nimodipine and associated dose–response relationships. n = 6 per treatment.
Extended Data Figure 4 | Skate CaV gating current properties.

**Extended Data Figure 4** shows gating current properties including peak amplitude (peak $I$), time-to-peak (TTP), exponential decay time constant ($\tau$ decay), and peak width at 50% of maximal gating current (width) for skate CaV1.3 (sCaV) versus rat CaV1.3 (rCaV), wild-type skate CaV1.3 (WT) versus charge-neutralized skate CaV1.3 (neutral), and rat CaV1.3 with charged skate motif (charged) versus rat CaV1.3 with neutralized skate motif (neutral). All values were similar except for peak $I$ for sCaV versus rCaV, which is likely to represent increased expression of rCaV compared with sCaV.

Data are presented as mean ± s.e.m., $n$ listed above bars. d, Wild-type skate CaV1.3 (sCaV, blue, $n = 7$) and wild-type rat CaV (rCaV, red, $n = 8$) relative $G$-V and $Q_{ON}$-V relationships. Data represented as mean ± s.e.m. e, G-V and $Q_{ON}$-V relationships for wild-type sCaV1.3 (WT, blue) and charge-neutralized sCaV1.3 (neutral, red). Data represented as mean ± s.e.m., $n = 7$ per condition. f, G-V and $Q_{ON}$-V relationships for rCaV1.3 with charged skate motif (charged, blue) and rCaV1.3 with neutral skate motif (neutral, red). Data represented as mean ± s.e.m., $n = 8$ per condition.
Extended Data Figure 5 | Charged skate motif modulates voltage-dependent activation kinetics. a, Activation kinetics were faster in charged-rCaV (blue, n = 6) than in wild-type rCaV1.3 (WT-rCaV, grey, n = 7) or neutral-rCaV (red, n = 8). Data represent mean ± s.e.m., P < 0.05 at all voltages for charged-rCaV versus WT-rCaV1.3 or neutral-rCaV, two-way ANOVA with post hoc Bonferroni test. b, Representative currents recorded in response to 1-s voltage pulses between −170 and −90 mV followed by a pulse to −10 mV for 20 ms. Cole–Moore effects, indicated by increased current activation rate at −170 mV (purple) versus −170 mV (green), were observed in currents recorded from charged-rCaV, but not neutral-rCaV. Scale bars, 50 pA, 10 ms. c, Cole–Moore effects quantified as the time to reach half-maximal current (t₁/₂). Charged-rCaV (blue, n = 9) reached maximal current amplitude faster with increasing prepulse voltage, whereas WT-rCaV (grey, n = 6) and neutral-rCaV (red, n = 8) were unchanged. All data represented as mean ± s.e.m., n ≥ 7, P < 0.05 for charged-rCaV t₁/₂ comparing −170 with −130, −110, or −90 mV, two-way ANOVA with post hoc Bonferroni test. d, Hypothetical model depicting the intracellular charged motif in the domain IV voltage sensor of sCaV1.3 destabilizing the inactive state of the channel, pushing it into a partially activated or primed state (gold oval) before full activation (green ovals). Because sCaV1.3 is primed for activation, channel activation requires a smaller increase in voltage compared with rCaV1.3.
Extended Data Figure 6 | Skate BK properties. a, Currents measured in response to 0, 1, or 10 μM intracellular Ca\(^{2+}\) at 80 mV from inside-out patches expressing sBK or mBK. Scale bars, 10 pA, 50 ms. \(P_o\) for sBK compared with mBK was similar for all concentrations tested. Data represented as mean ± s.e.m., \(n=5\). b, Representative single-channel records at various voltages from patches expressing indicated BK channels. Scale bars, 25 pA, 20 ms. c, Representative currents recorded at 80 mV from patches expressing indicated BK channels. The same patch was exposed to local intracellular K\(^+\) concentrations of 140 mM, 640 mM, or 3.14 M. Dashed lines indicate single-channel current amplitude for sBK at 140 mM (green), 640 mM (orange), or 3.14 M (maroon). Scale bars, 50 pA, 20 ms.
Extended Data Figure 7 | Adaptations in skate BK promote increased relative $I_{\text{CaV}}$ current during channel coupling. 

**a**, Whole-cell currents in response to 200-ms voltage pulses from $-80\text{ mV}$ to $+80\text{ mV}$ from HEK293 cells expressing sBK, sBK-SE, or mBK in the presence of 0 or 20μM intracellular Ca$^{2+}$. Scale bars, 5 nA, 50 ms. **b**, Average $I$–$V$ relationships for sBK (blue), sBK-SE (green) and mBK (red) in the presence of 0 or 20μM intracellular Ca$^{2+}$. $n = 7$. **c**, Whole-cell currents from HEK293 cells expressing charged-rCaV1.3 coexpressed with sBK, sBK-SE, or mBK. Scale bars, 500 pA, 50 ms. $t$, transient current evoked by voltage pulse; $s$, sustained current. In the presence of CaV1.3, average transient and sustained $I$–$V$ relationships showed a negative shifted reversal potential ($E_{\text{REV}}$) for sBK-SE (green) or mBK (red) compared with sBK (blue), indicating increased relative K$^+$ permeability. 

**d**, Reversal potentials for transient and sustained currents evoked in cells coexpressing charged-rCaV1.3 and BK were affected by BK identity. Inset: transient currents mediated by coupling of CaV1.3 and BK (scale bars, 100 pA, 5 ms). Transient $E_{\text{REV}}$: sBK = 32.96 ± 2.17, mBK = 8.43 ± 2.76, sBK-s.e. = 3.42 ± 2.38, $P < 0.0001$ for sBK versus mBK or sBK-SE. Sustained $E_{\text{REV}}$: sBK = −17.00 ± 2.48, mBK = −50.95 ± 4.16, sBK-SE = −45.13 ± 4.59, $P < 0.0001$, $n = 10$. All data represented as mean ± s.e.m. and $P$ values from two-tailed Student’s $t$-test.
Extended Data Figure 8 | BK agonist NS11021 modulates skate BK channels. a, In representative records from outside-out patches expressing sBK, the BK agonist NS11021 (NS, 10 μM) increased the Po and open-state dwell time of sBK channels and this effect was blocked by iberotoxin (IbTx, 100 nM). Scale bars, 5 pA, 100 ms. Associated all-points histograms demonstrate the increase in open time. Po: basal, 0.0024 ± 0.00068; NS, 0.16 ± 0.041; NS + IbTx, 0.00036 ± 0.00025. P < 0.0001 for NS versus basal or NS + IbTx. Open dwell time: basal, 0.62 ± 0.32; NS, 4.59 ± 0.34; NS + IbTx, 0.30 ± 0.010. P < 0.0001, n = 5. b, Whole-cell currents and average transient and sustained I–V relationships from HEK293 cells expressing charged-rCaV1.3 and sBK (scale bars, 500 pA, 50 ms). Transient and sustained I–V relationships made from normalizing currents in the presence of NS to basal currents show an increase in CaV1.3-activated sBK current amplitude and negative-shifted E_{REV} in response to 10 μM NS. Transient E_{REV} basal = 20.71 ± 3.46, NS = −0.72 ± 0.94, P < 0.01. Sustained E_{REV} basal = −24.62 ± 0.61, NS = −47.21 ± 5.37, P < 0.05. n = 5. c, Representative currents recorded from an electrosensory cell show that 10 μM NS increases I_{Cav}-activated I_{K} amplitude resulting in a decrease in relative I_{Cav} (scale bars, 100 pA, 50 ms). d, Transient and sustained I–V relationships from normalizing currents in the presence of NS to basal currents. I–V relationships demonstrate an NS-mediated negative shift in E_{REV} indicating increased K⁺ permeability. Transient E_{REV} basal = −6.15 ± 5.95, NS = −24.9 ± 8.23, P < 0.01. Sustained E_{REV} basal = −7.59 ± 6.02, NS = −25.65 ± 1.06, P < 0.05. n = 4. All data represented as mean ± s.e.m. and P values from two-tailed Student’s t-test.
Extended Data Figure 9 | Ca\textsuperscript{2+}-handling proteins are enriched in ampullae of Lorenzini. a, Four highest expressed transcripts in ampullae. The Ca\textsuperscript{2+}-binding protein parvalbumin 8 is the most highly expressed and is enriched in ampullae compared with other examined tissues. Bars represent fragments per kilobase of exon per million fragments mapped (FPKM). b, Four highest expressed ATPase transcripts in ampullae. Notably, the plasma membrane Ca\textsuperscript{2+} ATPase 1a is highly expressed and is enriched in ampullae. c, Proposed mechanism for electrosensory cell V_m oscillations. sCav1.3 is activated by electrical signals to depolarize the cell and mediate Ca\textsuperscript{2+} influx. Ca\textsuperscript{2+} stimulates sBK-mediated K\textsuperscript{+} current to hyperpolarize the cell. Ca\textsuperscript{2+}-binding proteins (CBP) bind incoming Ca\textsuperscript{2+} to inhibit BK-mediated hyperpolarization and continue sCav1.3-driven oscillations.
Extended Data Figure 10 | Behavioural paradigm for pharmacologically treated skates and startle response-related control. **a**, Schematic drawing of electrical stimulus. A 9-V battery was used to generate a dipole DC stimulus through two independent leads placed into Tygon rubber tubing filled with seawater (left). The ends of these tubes were threaded through an acrylic plate to four different equally spaced locations on the base of the behavioural observation tank, which were then obscured by sand (right). **b**, Following 30 min of free exploration, control and pharmacologically treated skates were gently tapped upon the pectoral fin. The average distance moved during the startle response is represented as mean ± s.e.m.; n = 10. Differences were not significant according to a two-way ANOVA with post hoc Tukey's test. **c**, Schematic drawing traced from typical example of skate startle response following pectoral fin stimulation (red arrow). The distance covered during the startle response was measured from the initial location (left) to the final location where the body axis became straight again (right), and the distance from the centre between the eyes from each respective position was recorded (dotted yellow line).