**LETTER**

Molecular tuning of electrorception in sharks and skates

Nicholas W. Bellono, Duncan B. Leitch, & David Julius

Ancient cartilaginous vertebrates, such as sharks, skates and rays, possess specialized electroreceptive organs that detect weak electric fields and relay this information to the central nervous system. Sharks exploit this sensory modality for predation, whereas skates may also use it to detect signals from conspecifics. Here we analyse shark and skate electroreceptive cells to determine whether discrete physiological properties could contribute to behaviourally relevant sensory tuning. We show that sharks and skates use a similar low threshold voltage-gated calcium channel to initiate cellular activity but use distinct potassium channels to modulate this activity. Electroreceptive cells from sharks express specially adapted voltage-gated potassium channels that support large, repetitive membrane voltage spikes capable of driving near-maximal vesicular release from elaborate ribbon synapses. By contrast, skates use a calcium-activated potassium channel to produce small, tunable membrane voltage oscillations that elicit stimulus-dependent vesicular release. We propose that these sensory adaptations support amplified indiscriminate signal detection in sharks compared with selective frequency detection in skates, potentially reflecting the electroreceptive requirements of these elasmobranch species. Our findings demonstrate how sensory systems adapt to suit the lifestyle or environmental niche of an animal through discrete molecular and biophysical modifications.

Electroreceptive cells from the little skate (*Leucoraja erinacea*) express specialized low threshold CaV1.3 voltage-gated calcium (CaV) channels and big-conductance potassium (Kv) channels that functionally couple to produce cellular membrane voltage oscillations. In electroreceptive cells from the chain catshark (*Scyliorhinus retifer*), we similarly observed voltage-activated inward calcium currents (I_{CaV}) that were sensitive to L-type voltage-gated CaV^2+^ channels (CaV) modulators, had a low voltage threshold for activation, had steep voltage-dependence, and had a slow inactivation profile. Our results demonstrate how sense organs adapt to suit the lifestyle or environmental niche of an animal through discrete molecular and biophysical modifications.

In skate electroreceptive cells, CaV^2+^ influx activated outward K^+^ currents at relatively negative potentials to occlude inward CaV^2+^ currents. In shark electroreceptive cells, CaV^2+^ influx activated outward K^+^ currents at relatively negative potentials to occlude inward CaV^2+^ currents. Indeed, K^+^ currents were not affected by an I_{CaV} blocker or a BK antagonist. Instead, the voltage-gated K^+^ channel inhibitor 4-aminopyridine (4-AP) blocked outward currents from shark electroreceptive cells. I_{Kv} was selective for K^+^ and exhibited a relatively high voltage-activation threshold. Furthermore, we observed fast activation and deactivation kinetics, whereas voltage-dependent inactivation (similar to desensitization) in response to prolonged voltage pulses was weak and slow, which results in a K^+^ conductance of diminished current amplitude even after repeated activation–deactivation cycles. Among voltage-gated K^+^ channels, transcripts that encode the pore-forming subunit of K_V1.3 predominated in shark ampullary organs (together with several K_V auxiliary subunits) and co-localized with CaV1.3 in electroreceptive cells. Expression of K_V1.3 was not detected in skate ampullary organs, and only shark electroreceptive cells exhibited 4-AP-sensitive voltage-gated K^+^ currents. Furthermore, both shark and skate electroreceptive cells expressed BK transcripts that, when heterologously expressed, produced channels with similar properties; however, functional BK currents were observed only in the latter (Extended Data Fig. 3). As such, BK channels do not contribute appreciably to the major K^+^ conductance in electroreceptive cells, at least not under the developmental or physiological conditions examined here. In summary, shark electroreceptive cells express a specific K_V with voltage-dependent properties that support repetitive stimulation.

Shark K_V1.3 is 80% identical to the human orthologue, but its voltage threshold for activation was shifted to more depolarized values compared with human K_V1.3. Shark K_V1.3 was activated at slightly slower rates and deactivated with rapid kinetics, requiring substantially less negative voltage to return to the resting state compared with the human channel. These biophysical properties resemble those of the cloned K_V. Consequently, shark K_V1.3 produced a conductance that could be repetitively stimulated with diminished amplitude, whereas human K_V1.3 quickly inactivated with repetitive voltage pulses. These biophysical properties resemble those of native shark K_V, which also exhibited a comparable pharmacological profile. One notable difference is that the deactivation kinetics of native K_V were faster than those of the cloned channel, particularly at positive voltages. As such, K_V1.3 forms the predominant K^+^ conductance in shark electroreceptive cells, additional regulatory mechanisms may be provided by auxiliary subunits, signalling cascades or structural proteins to further enhance rapid deactivation.

The voltage-dependent properties of shark K_V1.3 probably derive from altered voltage-sensor domain movements, which we verified by comparing gating currents from modified non-conductive shark and human K_V1.3. For these experiments, we analysed a human isoform that...
exhibits increased surface expression and therefore enhanced gating current amplitude.\(^4\) (Extended Data Fig. 4g). Similar to ion (permeating) currents, upward (activating) voltage sensor movements (represented as ON gating charge, \(Q_{\text{ON}}\)) for shark \(K_{V}1.3\) exhibited a higher voltage-activation threshold and slower kinetics compared with human \(K_{V}1.3\) (Extended Data Fig. 5a–c). Moreover, shark \(K_{V}1.3\) gating-current deactivation \((Q_{\text{OFF}})\), return of voltage sensors to a resting state after depolarizing pulses) required less negative voltage and was accelerated (Extended Data Fig. 5d–h). Consequently, ion tail-current deactivation, which represents closure of the channel pore, was faster in shark \(K_{V}1.3\) than in human \(K_{V}1.3\) (Extended Data Fig. 5i). We next asked why shark \(K_{V}1.3\) appears to favour a resting state. When we recorded \(Q_{\text{OFF}}\) after a series of depolarizing voltage pulses of varying lengths, human \(K_{V}1.3\) deactivation kinetics markedly slowed after pulses longer than 1 ms, whereas shark channel deactivation rates remained relatively fast and constant with increasing pulse lengths (Extended Data Fig. 6a–d). This slowing in deactivation is characteristic of voltage-sensor domain ‘relaxation’, which has been proposed to slow the closure of \(K_{V}\) channels.\(^6\) We therefore propose that reduced voltage-sensor relaxation in shark \(K_{V}1.3\) results in decreased stability of the open state; as such, much less negative voltage is required to return channels to a resting state and mediate fast channel closure (depicted in our model, Extended Data Fig. 6e).

We analysed shark–human \(K_{V}1.3\) chimaeras to see if specific domains specify relevant biophysical attributes. Replacement of the S1–S6 region of human \(K_{V}1.3\) with that of shark recapitulated the high voltage-activation threshold, rapid deactivation kinetics and weak inactivation of wild-type shark \(K_{V}1.3\) channels, and the converse chimera also altered channel properties (Extended Data Fig. 7a, b). We next substituted just the voltage-sensor-domain region (S1–S4) and found that activation threshold and deactivation kinetics were greatly affected, whereas inactivation remained similar (Extended Data Fig. 7a, b). By contrast, only inactivation was affected by replacing S5–S6 (Extended Data Fig. 7c–e), consistent with a role for the outer pore in C-type inactivation.\(^3\) Therefore, specific structural adaptations in the \(K_{V}1.3\) transmembrane core specify physiologically relevant biophysical properties.

Membrane voltage \((V_{m})\) oscillations within ampullary control neurotransmitter release from electrocytes onto afferent nerves, thereby shaping signals to the central nervous system.\(^2\) In skate electrocyte cells, we found that functional coupling of Cav1.3 and BK mediates \(V_{m}\) oscillations that are tuned to low frequencies, such as those detected by behaving animals.\(^6\) Consistent with our previous results, skate electrocytes cells had a resting \(V_{m}\) of \(-54\) mV, near the peak of the \(I_{\text{CaV}}\) window current, at which cells exhibited spontaneous voltage oscillations (Fig. 3a, b). Injecting current to bring the skate cell \(V_{m}\) to various potentials modulated oscillatory behaviour, markedly changing both frequency and amplitude across physiological membrane potentials (Fig. 3a, b). By contrast, shark electrocyte cells had an average resting \(V_{m}\) of \(-66\) mV, which is on the cusp of the \(I_{\text{CaV}}\) window current at which cells were relatively quiet (Fig. 3a, b). Injecting current to bring \(V_{m}\) to within the range of the \(I_{\text{CaV}}\) threshold and window current elicited robust, repetitive \(V_{m}\) spiking that lasted for the duration of the recordings. Spiking only slightly decreased in amplitude and frequency at more positive voltages (Fig. 3a, b), because spike amplitude is probably determined by the voltage threshold of \(I_{\text{CaV}}\) in addition to \(I_{\text{CaV}}\) window current. Indeed, the \(I_{\text{CaV}}\) inhibitor nifedipine blocked evoked depolarization and 4-AP prevented or greatly slowed repolarization (Fig. 3c).

As such, in both shark and skate, activity is limited to membrane voltages in which \(I_{\text{CaV}}\) window current is observed, but the dynamics in the two species are markedly different: the system behaves as an all-or-none ON/OFF switch in sharks, but it is more tunable in skates.
implicates $I_{KV}$—while also revealing a resurgent $I_{CaV}$ upon hyperpolarization that could contribute to the initiation of the following voltage spike (Fig. 3d). Therefore, the low voltage threshold and inactivation properties of $I_{CaV}$, coupled with the high voltage threshold, rapid deactivation and weak inactivation of $I_{KV}$, are suited to cooperatively mediate $V_m$ spiking in shark electrosensory cells. Notably, transcripts for $Ca^{2+}$-binding proteins and pumps were enriched in shark ampullary organs, which may help to facilitate repetitive $Ca^{2+}$ influx (Extended Data Fig. 8a). Together, these cellular properties could support robust repetitive activity in shark electrosensory cells to amplify responses to incoming electrical signals. By contrast, skate cells exhibit low-level tonic activity at rest that could be reitmed or modulated in response to particular incoming electrical frequencies to alter neurotransmitter release.

To determine how synaptic vesicle release is affected by differences in $V_m$ activity, we monitored membrane capacitance ($C_m$) to measure vesicle fusion in response to electrical stimuli. Depolarization of shark or skate electrosensory cells elicited inward currents and capacitance changes that were blocked by Cd$^{2+}$, which indicates that $I_{CaV}$ is required for vesicular release (Fig. 4a). Increasing the stimulus duration increased changes in $C_m$ (Fig. 4b, Extended Data Fig. 9a), but with distinct dynamics in shark compared with skate electrosensory cells: brief voltage stimuli induced larger changes in shark-cell $C_m$ that saturated in response to longer stimuli, whereas skate cells exhibited $C_m$ changes that increased linearly with the duration of the stimulus (Fig. 4b).

Differences in the number or distribution of synaptic vesicles could account for these distinct vesicle release dynamics. Skate electrosensory cells contain large synaptic ribbons that tether numerous vesicles for release onto postsynaptic afferents$^{11-12}$. Indeed, shark and skate ampullae expressed transcripts associated with ribbon synapses, and ultrastructural analysis of electrosensory cells revealed that both contain remarkably large synaptic ribbons compared to those from mammalian hair cells$^{11}$ (Fig. 4c, d, Extended Data Fig. 8b, c). Moreover, shark and skate ribbons were similarly shaped and tethered an equivalent number of vesicles of comparable diameter. However, shark electrosensory cells had a larger ‘readily releasable’ vesicle pool (Extended Data Fig. 8d), which facilitates rapid and efficient exocytosis in other systems$^{13-15}$ and might account for our observation that short voltage stimuli induce larger changes in $C_m$ in electrosensory cells from the shark. Conversely, skate electrosensory cells contained more free cytosolic vesicles that, by analogy with other systems$^{15}$, may represent a larger ‘refilling pool’ of recently generated vesicles available for tethering and release (Extended Data Fig. 8d). Thus, skate cells may be better equipped to continuously supply vesicles for tonic release, reflected by the non-saturating, linear change in $C_m$ in response to increasing the voltage of the stimulus.

To determine the number of vesicles released in response to a single $V_m$ spike or oscillation, we first integrated $I_{CaV}$ ($Q_{CaV}$) elicited by stimuli of varying duration, thereby establishing a relationship between $Q_{CaV}$ and $C_m$ (Fig. 4e). Shark and skate cells responded equally to identical voltage stimuli, further suggesting that $I_{CaV}$ is similar in both cell types and that $K^+$-channel identity dictates the oscillation phenotype and resulting amplitude of $I_{CaV}$ (Extended Data Fig. 9b–d). We next fit $Q_{CaV}$, induced by simulated $V_m$ spikes or oscillations to $Q_{CaV}-C_m$ relationships, and used the specific capacitance for a pure lipid membrane with diameter equal to that of an electrosensory cell vesicle to estimate fusion events from measured changes in $C_m$. Notably, our calculations suggest that shark cells released at least ten times more vesicles in response to one $V_m$ spike compared to skate cells subjected to a single oscillation event (Fig. 4f). Furthermore, with as other ribbon synapses, the large storage pool could facilitate a sustained release of vesicles in response to repetitive $V_m$ spiking, further amplifying the signals$^{14}$. Taken together, these properties should render sharks acutely sensitive to incoming signals by greatly amplifying vesicular release to even very brief stimuli. By contrast, skate cells may better encode stimulus variation with tunable voltage oscillations and more graded vesicle release.

We next asked how differences in cellular dynamics might contribute to sensation at the organismal level. Elasmobranchs preferentially

Fig. 3 | Voltage dynamics in electrosensory cells. a, $V_m$-dependent spiking in shark electrosensory cells and smaller voltage oscillations from skate cells at the indicated membrane potentials (dotted lines) achieved by current injection. b, Average voltage oscillation amplitude, normalized amplitude, and frequency at various membrane voltages for shark (red) and skate (blue) electrosensory cells. Values for oscillations from skate cells were significantly different for voltages at which activity was observed, whereas shark-cell activity changed only slightly at more depolarized voltages. c, Control, Nifedipine and 4-AP. d, Voltage dynamics in shark electrosensory cells and smaller voltage oscillations from skate cells at the indicated membrane potentials (dotted lines) achieved by current injection. e, Average voltage oscillation amplitude, normalized amplitude, and frequency at various membrane voltages for shark (red) and skate (blue) electrosensory cells. Values for oscillations from skate cells were significantly different for voltages at which activity was observed, whereas shark-cell activity changed only slightly at more depolarized voltages. n = 5, P < 0.01 for control at –60 mV versus all other voltages, two-way ANOVA with post hoc Tukey test. The mean ± s.e.m. for resting membrane voltages ($V_{rest}$) for shark (red, –66.2 ± 2.7 mV) and skate (blue, –54 ± 1.8 mV) are indicated as bars on each graph. C, Control.
respond to low frequency electrical signals produced by their prey, but direct comparison of frequency selectivity between species is confounded by behavioural and physiological differences. We therefore measured changes in ventilatory rate as a basic, time-locked physiologic metric that is well-validated in electroreceptive elasmobranch species and readily observed in response to sensory cues, such as weak electrical stimuli or odors, (Supplementary Videos 1 and 2). When presented with electrical stimuli of identical strength, the ventilation rates of sharks increased similarly at all stimulus frequencies (Fig. 4g). By contrast, the ventilation rate of skates maximally increased at low frequencies, resembling voltage oscillations in their electrosensory cells and signals emitted by their electric organ (Fig. 4g). In both species, maximal electrically induced ventilatory responses were similar to those evoked by food odors as a comparative control (Fig. 4g). As such, shark electrosensation may act as a threshold detector for broad frequencies, potentially reflecting its role in predation. By contrast, skate sensation appears more specifically tuned to enable the detection of signals from prey as well as frequencies in the range of conspecific electric-organ discharges.

Electrosensation has independently evolved in many taxa to facilitate particular behaviours ranging from predation to communication. By analysing related species that use electrosensation for distinct purposes, we found that subtle molecular variations considerably alter cellular properties that could ultimately mediate differences in behaviour. Our results suggest that molecular tuning of \( V_m \) oscillations in electrosensory cells is important for the initial detection and discrimination of salient electrical signals, although anatomical characteristics and processing by the central nervous system probably contribute to additional signal filtering (Extended Data Fig. 10). This observation is reminiscent of other sensory modalities in which sensory cells or their receptors are modified to mediate the detection of relevant stimuli. For example, expression and regulation of ion channels enable hair cells—which are developmentally related to electrosensory cells—to produce \( V_m \) oscillations of various amplitudes and frequencies to mediate detection of particular auditory signals.

### Online content

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0160-9.

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METHODS

Animals and cells. Male and female catfish charkhs (Scyliorhinus retifer) and little skates (Leucosagia erinacea) were provided by the Marine Biological Laboratory (Woods Hole, MA, USA) and their use was approved by the UCSF Animal Care and Use Committee. Animals were euthanized with tricaine methanesulfonate (MS222, 1 g l−1). Ampullary organs of adult animals were removed from the hyoid cluster (skates) or buccal and supraorbital clusters (sharks) on ice and further dissected by removing most of the canals and aerifer nerve fibres. Ampullae were treated with papain for less than 5 min and then electroosmotic cells were mechanically dissociated over the recording chamber. Isolated electroosmotic cells were identified by the presence of their single kinocilium. HEK293T cells (American Type Culture Collection, ATCC) were grown in DMEM, 10% fetal calf serum and 1% penicillin/streptomycin at 37 °C in 5% CO2. Cells were transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. Cell lines were verified from the ATCC but were not further tested for identity or mycoplasma contamination during our studies. 100 ng of Kc1.3 or BK constructs were co-expressed with 0.3 μg GFP. Mock transfection experiments (0.3 μg GFP) were performed as controls, in which minimal voltage-activated outward current was observed.

Whole mount preparations. Juvenile fish were euthanized with an overdose of MS-222 in artificial seawater and fixed in 4% paraformaldehyde for at least 24 h. The cartilage matrix and electrosensory tubules were stained using Alicant Blue (20 mg Alicant Blue SGX in 30 ml glacial acetic acid and 70 ml 100% ethanol) and bone was stained using Alizarin Red following previously published methods26. Molecular biology. kcnma1 and kcnma1a from shark ampullary organs were synthesized according to GenScript. Human KCNMA3 was a gift from L. Salkoff (Addgene 16195). Chimaera synthesis and mutagenesis were carried out and verified by GenScript or by the 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). Capacitance and associated ion current measurements were amplified and digitized with an EPC10 amplifier in lock-in mode (HEKA) and Patchmaster software (HEKA). Unless stated otherwise, whole-cell data were filtered at 1 kHz and sampled at 10 kHz, and single-channel data were filtered at 5 kHz and sampled at 50 kHz. Data were leak subtracted online using a P/4 protocol, except for data obtained using voltage ramp protocols, and membrane potentials were corrected for liquid junction potentials. Electroosmotic cell recordings were performed using borosilicate glass pipettes polished to 8–10 MΩ. For heterologous expression experiments in HEK293, recordings were performed using pipettes polished to 2–3 MΩ.

The extracellular solution was a modified elasmobranch Ringer’s solution containing (in mM): 136 K-gluconate, 4 KCl, 1 K-EGTA, 1 HEDTA, 10 HEPES, 10 glucose, pH 7.3. Calculated concentrations of buffered glucose, pH 7.3. The extracellular solution contained (in mM): 136 K-gluconate, 4 KCl, 1 K-EGTA, 1 HEDTA, 10 HEPES, 10 glucose, pH 7.3. The extracellular solution contained (in mM): 250 NaCl, 6 KCl, 4 CaCl2, 1 MgCl2, 10 glucose, 360 urea, pH 7.6; for recording capacitance changes (in mM): 250 NaCl, 6 KCl, 4 CaCl2, 1 MgCl2, 10 glucose, 360 urea, pH 7.6; for measuring KV1.3 gating currents, 500 μM Cd2+, 100 μM Zn2+, 100 μM Bay K, 10 μM nifedipine, 5 μM mibebradil, 1 μM 4-AP, 100 nM ibiotoxin, 10 mM TEA−, 10 μM NS11021, 100 μM quinidine, 25 mM α-oxodentrotoxin, 10 mM nargamotox, 1 μM UK78282, 25 mM Guangxitoxin-1E, 20 μM IXE991. Pharmacological effects were quantified by differences in normalized peak current from the same cell after bath application of the drug (Ipeakinactivation/Icontrol).

METHODS

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as a control. Ionic pore-blocker stocks were prepared in standard extracellular solution and diluted before use. Unless stated otherwise, the following concentrations were used: 100 or 500 μM Cd2+, 1 μM Bay K, 10 μM nifedipine, 5 μM mibebradil, 1 μM 4-AP, 100 nM ibiotoxin, 10 mM TEA−, 10 μM NS11021, 100 μM quinidine, 25 mM α-oxodentrotoxin, 10 mM nargamotox, 1 μM UK78282, 25 mM Guangxitoxin-1E, 20 μM IXE991. Pharmacological effects were quantified by differences in normalized peak current from the same cell after bath application of the drug (Ipeakinactivation/Icontrol).

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Isothermal capacitance was measured in response to 200-ms voltage pulses in 10-mV increments from a −100 mV holding potential. G–V relationships were derived from I–V curves by calculating G according to the following: G = Ipeak/(V − Vrev), and fit with a Boltzmann equation. Voltage-dependent inactivation was measured during −20 mV voltage pulses after a series of 2-s pre pulses ranging from −100 to 60 mV in 10-mV increments. Voltage-dependent inactivation was quantified as INmax, with IN occurring at the activating voltage pulse after a −100 mV prepulse. IN was measured in response to 200-ms voltage pulses in 10-mV increments from a −100 mV holding potential. Voltage-dependent inactivation was measured during 40-mV voltage pulses after a series of 5-s pre pulses ranging from −100 to 60 mV in 10-mV increments. Cumulative inactivation was measured in response to 50-ms, 40-mV pulses every 5 ms. Activation kinetics were determined by fitting the initial rising phase of currents activated by various voltages with single exponentials. Deactivation kinetics were fit with a single exponential upon repolarization to various voltages in 10-mV increments from 40 mV to −100 mV after 40-mV pre pulses of the indicated durations. The reversi-ble potentiation of KV channels was measured using a similar protocol that stepped in 10-mV increments from 40 mV to −120 mV. Single-channel currents were measured from the middle of the noise band between closed and open states or calculated from the difference between Gaussian-fitted closed and open peaks on all-points amplitude histograms for each excised patch record. Conductance was calculated from the linear slope of I–V relationships. In current-clamp mode, current injection was used to bring membrane potential to various values and then was fixed. In this case, membrane potential was defined as the base of the spiking or oscillating activity. Alternatively, brief current injection was delivered to determine the effects of pharmacological inhibitors on spiking activity.

Qion and Qoff represents the integral of non-linear gating current measured during and after voltage pulses from holding potentials of −100 or 0 mV. Qon was quantified only from cells with no ionic current. ON gating-current kinetics were quantified by single exponential fits of the slope of decreasing outward current elicited by voltage pulses in 10-mV increments from a −100 mV holding potential. OFF gating-current kinetics were calculated by single-exponential fits of the slope of increasing negative current elicited by voltage pulses in 10-mV increments from a −100 mV holding potential. Gating current dependence on deactivation of KV was also assessed by single-exponential fits of currents upon repolarization to various voltages in 10-mV increments from 20 mV to −100 mV after 40-mV pre pulses of the indicated durations. Deactivation of gating currents was also measured at −100 mV with exponential fits after a series of 40-mV voltage pulses of varying duration from 0.5 ms to 30 ms.

Capacitance measurements were performed using a 15-mV, 1.5-kHz sinusoidal stimulation protocol applied from −90 mV before and after depolarization pulses of various lengths to acquire pre- and post-stimulus capacitance values. Cells were discarded when the series resistance (Rs) changed and exceeded the membrane resistance (Rm) or if membrane conductance (Gm) varied greatly after depolarizing voltage pulses. Whole-cell ion currents were filtered at 1 kHz and sampled at 10 kHz. Gating currents and capacitance measurements were filtered at 1 kHz and sampled at 20 kHz. Capacitance records were filtered at 100 Hz during offline analyses. Changes in capacitance were measured by averaging capacitance over 200 ms after the depolarizing voltage step and subtracting the averaged capacitance before depolarization. Intracellular ATP was included in all experiments and 100 nM ibiotoxin, 1 mM 4-AP and intracellular Cs+ were used to block K+ currents. The integral of ICa/Vpeak (QCa) was used to account for variability in the kinetics of ICa. To calculate vesicle release, we fit QCa/Vpeak induced by simulated spike- or oscillation-voltage protocols to QCa/Vpeak − Cm relationships. When identical voltage protocols were used to elicit ICa/Vpeak = the same from skate and shark electroosmotic cells, consistent with the similar ICa in these cells.

Transcriptional sequencing and analysis. Poly A+ RNA was extracted from ampullary electroosmotic cells, non-electroreceptor covered skin, muscle and forebrain of an adult catfish charkh then was reverse-transcribed using SuperScript III kit (Invitrogen). Sequencing libraries were prepared using the Illumina TruSeq Stranded RNA Sample 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 between 30 million and 40 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 blastx tool from NCBI blast (version 2.2.31) using a maximum E value of 1 × 10⁻5. Reciprocal blastx alignments (using zebrafish protein sequences that aligned to catshark 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 FPKM.

In situ hybridization histochemistry. Adult chain catsharks were euthanized with an overdose of MS-222 in artificial seawater and transcardially perfused with PBS followed by 4% paraformaldehyde. Ampullary organs were dissected from the buccal and supraorbital clusters and cryo-protected in 30% sucrose in PBS overnight. Cryostat sections (15-μm thick) were probed with digoxigenin-labelled cRNA for shark CaV1.3 and fluorescein-labelled cRNA for shark BK and Kv1.3 receptors. Probes were generated by T7/T3 in vitro transcription reactions using a 500-nucleotide fragment of CaV1.3 cDNA (nucleotides 3700 to 4200), a 325-nucleotide fragment of BK cDNA (nucleotides 636 to 961) and a 470-nucleotide fragment of Kv1.3 cDNA (nucleotides 670 to 1140). 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 methods. After hybridization and detection, sections were covered with a coverslip and co-stained with DAPI as a nuclear marker (Prolong Gold Antifade Mountant with DAPI; Invitrogen).

Transmission electron microscopy. Tissue samples from the catshark and skate hyoid capsule with electrosensory cells were fixed in 2% glutaraldehyde, 1% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4, postfixed in 2% osmium tetroxide in the same buffer, stained en bloc with 2% aqueous uranyl acetate, dehydrated in acetone, infiltrated and embedded in LX-112 resin (Ladd Research Industries). Semithin sections stained with toluidine blue were prepared to orient and locate the area of interest. Samples were ultrathin sectioned (typically 100 nm) on a Reichert Ultracut S ultramicrotome and counter-stained with 0.8% lead citrate. Grids were examined on a JEOL JEM-1230 transmission electron microscope (JEOL USA, Inc.) and imaged with the Gatan Ultrascan 1000 digital camera (Gatan Inc.). All measurements were performed in Image J (NIH) on electron micrographs adjusted for brightness and contrast (Photoshop CS6, Adobe Systems). Measurements of vesicles followed published methods, with populations attached to the ribbon structure (attached), in proximity to the synapse (readily releasable) and freely filling cytosolic space (refilling).

To measure ribbon-shape variation, the difference between the traced distance of the ribbon and the distance of a line drawn from the start to the end of the ribbon was divided by the distance of that line. All values represent a positive difference of the ribbon and the distance of a line drawn from the start to the end of the ribbon. In one of four circles (diameter 5.5 cm), all equally spaced from the centre of the tank. After an initial recording of baseline ventilation frequency, individual fish were stimulated at 2, 5, 10, 25, 100, 150 and 200 Hz for 5 min, in randomized order. Each frequency was tested 10 times. All were exposed to a plume of Mysis shrimp odorant, and sharks were presented with squid odorant, to measure responses to natural food stimuli. To prevent habituation to the stimuli, an interval of 20 min without electrical stimuli was used between each trial. A digital video camera (Panasonic HC-V770) was positioned above the tank and used and measure ventilatory responses, as characterized by cyclical movement of spiracles or gills. Measurements were randomized and made blind to stimulation conditions.

Statistical analysis. Data were analysed with Clampfit (Axon Instruments), Patchmaster (HEKA) or Prism (Graphpad). Data are represented as mean ± s.e.m. and n represents independent experiments for the number of cells in electrophysiology, quantified structures from histological analysis, or behavioural trials. 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 on the basis of the number of independent experiments required for statistical significance and technical feasibility.

Behavioural analysis. In an isolated location and under normal lighting conditions, individual juvenile skates (n = 6) and sharks (n = 5) of both sexes were allowed to freely move and habituate for 20 min in an ambient temperature, seawater-filled cylindrical acrylic tank (diameter 28 cm). A sinusoidal electrical stimulus (100 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 powered by a function generator (Tone Generator Pro, Performance Audio) was randomly positioned and obscured by sand substrate in one of four circles (diameter 5.5 cm), all equally spaced from the centre of the tank. After an initial recording of baseline ventilation frequency, individual fish were stimulated at 2, 5, 10, 25, 100, 150 and 200 Hz for 5 min, in randomized order. Each frequency was tested 10 times. All were exposed to a plume of Mysis shrimp odorant, and sharks were presented with squid odorant, to measure responses to natural food stimuli. To prevent habituation to the stimuli, an interval of 20 min without electrical stimuli was used between each trial. A digital video camera (Panasonic HC-V770) was positioned above the tank and used and measure ventilatory responses, as characterized by cyclical movement of spiracles or gills. Measurements were randomized and made blind to stimulation conditions.

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Statistical analysis. Data were analysed with Clampfit (Axon Instruments), Patchmaster (HEKA) or Prism (Graphpad). Data are represented as mean ± s.e.m. and n represents independent experiments for the number of cells in electrophysiology, quantified structures from histological analysis, or behavioural trials. 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 on the basis of the number of independent experiments required for statistical significance and technical feasibility.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability. Deep sequencing data are archived in the Gene Expression Omnibus under accession number GSE103977. The GenBank accession numbers for the α subunit are: CaV1.3 MF959522, Kv1.3 MF959523 and BK MF959524. Other data are available from the corresponding author upon reasonable request.

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Extended Data Fig. 1 | Properties of shark $I_{\text{CaV}}$. a, Top, isolated shark ampullary organs with attached canals and nerve fibres (scale bar, 100 μm); bottom, a representative electrosensory cell patch-clamp experiment (scale bar, 10 μm). b, Left, $I_{\text{CaV}}$ currents elicited by increasing voltage pulses from $-100$ mV; right, average current–voltage ($I$–$V$) relationship ($n = 7$). c, $I_{\text{CaV}}$ exhibited an L-type CaV pharmacological profile: Peak currents were regulated by Bay K (agonist), Cd$^{2+}$ (blocker) and nifedipine (antagonist), but not by mibefradil (T-type antagonist). $n = 4$. $P < 0.0001$ for control versus all treatments except mibefradil, one-way ANOVA with post hoc Bonferroni test. d, $I_{\text{CaV}}$ conductance–voltage ($G$–$V$) relationship (black) with half-maximal activation voltage ($V_{a1/2}$) of $-54.6 \pm 1.2$ mV. Inactivation–voltage relationship (grey) with half-inactivation potential ($V_{h1/2}$) of $-82.9 \pm 1.4$ mV. A large window current was observed between $-70$ mV and $-40$ mV. $G$–$V$ relationships were established from current measurements during voltage pulses delivered in 10-mV increments from $-100$ mV. Inactivation was measured at a $-20$ mV test pulse after a series of voltage prepulses. $n = 7$. e, CaV α-subunit mRNA expression in shark ampullary organs. Bars represent FPKM. f, $I_{\text{CaV}}$ elicited by a 2-s depolarizing voltage step to $-30$ mV exhibits little inactivation. Representative of $n = 5$. g, CaV1.3 alignment revealed that the IVS2–S3 motif that confers low voltage threshold in skate CaV1.3 is conserved in the shark orthologue. h, Expression of CaV auxiliary subunits in shark ampullary organs. Bars represent FPKM. i, Average $I_{\text{CaV}}$ current density and voltage-activation threshold was similar in shark and skate electrosensory cells. $n = 6$. All data are represented as mean ± s.e.m, $n$ denotes cells.
Extended Data Fig. 2 | Properties of shark \( I_{\text{KV}} \). a, Currents elicited by 500-ms voltage ramps in shark (red) or skate (blue) electrosensory cells in the presence of intracellular Cs\(^+\) (left) or K\(^+\) (right). The insets on the right show the inward \( I_{\text{CaV}} \) in the presence of intracellular K\(^+\). b, Average \( I_{\text{CaV}} \) current density elicited by voltage ramps was similar for both shark and skate cells in the presence of intracellular Cs\(^+\), but larger in shark cells in the presence of intracellular K\(^+\). Outward K\(^+\) current density was significantly larger in shark cells. \( n = 5, P < 0.0001 \) for shark versus skate \( I_{\text{CaV}} \) or \( I_k \) with intracellular K\(^+\), two-tailed Student’s t-test. c, The percentage of \( I_{\text{CaV}} \) remaining in the presence of K\(^+\) compared with Cs\(^+\) is markedly greater in shark cells compared with those from skate. d, Inward currents elicited by increasing voltage pulses from –100 mV were not affected by IbTx or 4-AP, but were blocked by Cd\(^{2+}\). \( n = 5, P < 0.0001 \) for inward control currents versus Cd\(^{2+}\), two-way ANOVA with post hoc Tukey test. Peak inward currents were not affected by IbTx or 4-AP. e, Reversal potential for \( I_{\text{KV}} \) in shark electrosensory cells is near the reversal potential for selective K\(^+\) permeation (\( E_K \), blue arrow on the I–V plot). \( n = 5 \). Arrows indicate when currents were measured at the indicated voltages after an activating prepulse of 40 mV (also shown in expanded view). Extracellular Cd\(^{2+}\) was included to block \( I_{\text{CaV}} \) for biophysical studies of \( I_{\text{KV}} \). f, \( I_{\text{KV}} \) currents elicited by a voltage protocol to obtain the \( G-V \) relationship. Arrows indicate when tail currents were measured at –30 mV after voltage steps that increased in 10-mV increments from –100 mV (expanded view within inset). Representative of \( n = 11 \). g, Voltage-dependent inactivation properties of \( I_{\text{KV}} \). The arrow indicates when inactivation was measured during 40-mV pulses after a series of prepulses that increased in 10-mV increments from –100 mV. \( V_{1/2} \) was –5.5 ± 1.7 mV and inactivation was incomplete. \( n = 6 \). h, mRNA expression of the \( K_v \) channel auxiliary subunits in shark ampullary organs. Bars represent FPKM. i, mRNA expression of \( kcnab3 \) isoforms. The major isoform studied is indicated in red. Other low-expression ampullary isoforms were similar. The only isoform that was appreciably expressed outside of ampullae was truncated and found in the brain (grey). All data are represented as mean ± s.e.m. \( n \) denotes cells.
Extended Data Fig. 3 | Properties of shark BK. a, mRNA expression of major K⁺-channel α-subunits (Kcna3, Kv1.3) and (Kcna1, BK) in shark and skate electrosensory cells. b, Average K⁺ current density, 4-AP-sensitive current (I_{KV}), and IbTx-sensitive current (I_{BK}) in shark and skate electrosensory cells. n = 5, P < 0.0001 for shark versus skate cells for all comparisons, two-tailed Student’s t-test. c, Co-localization of CaV1.3 (red) and BK (green) transcripts within shark ampullary organs. Nuclei were stained with DAPI (blue). Scale bar, 100 μm. Representative of n = 4. d, In the presence of 4-AP, a relatively small outward current remained that was insensitive to IbTx and was slightly increased by NS11021 at very positive voltages. n = 5, P < 0.05 for control versus NS11021 at 70 or 80 mV, two-way ANOVA with post hoc Tukey test. e, BK alignment revealed that residues found to reduce conductance in skate BK are conserved in the shark orthologue. f, Heterologously expressed shark and skate BK had relatively small single-channel conductance compared with mouse BK. Shark BK = 109 ± 4 pS; skate BK = 105 ± 5 pS; mouse BK = 259 ± 12 pS. n = 5, P < 0.0001 for mouse versus shark or skate BK, two-way ANOVA with post hoc Tukey test. g, 1 μM Ca²⁺ increased open probability in shark BK expressed in excised inside-out patches, and 10 μM NS11021 increased open probability of channels in excised outside-out patches. NS11021 modulation was blocked by 100 nM IbTx. Holding voltage was 80 mV. NP₀: basal, 0.0061 ± 0.0014; Ca²⁺, 0.11 ± 0.011, NS11021, 0.24 ± 0.026. n = 5, P < 0.0001 for basal versus Ca²⁺ or NS11021, two-tailed Student’s t-test. All data are represented as mean ± s.e.m, n denotes cells or tissue sections.
Extended Data Fig. 4 | Properties of shark \(K_V\). a, Voltage-activated currents recorded in HEK293 cells expressing shark (red) or human (black) \(K_V^{1.3}\). Arrows indicate when currents were measured at \(-30\) mV after voltage pulses that increased in 10-mV increments from \(-100\) mV. The inset shows expansions of measured currents following arrows. b, Left, normalized currents elicited by a 40-mV voltage pulse demonstrated that expressed shark \(K_V\) channels open more slowly compared with human orthologues. Right, average activation kinetics were slower for shark \(K_V\) compared with human orthologues in response to voltages from 20 mV to 50 mV. \(n = 6\), \(P < 0.0001\) for contribution of orthologue identity to series variance, two-way ANOVA with post hoc Bonferroni test. c, Deactivation kinetics of normalized currents from shark and human \(K_V\) channels at various repolarizing voltages after an activating prepulse of 40 mV. The arrow indicates when current properties were measured during the voltage protocol. d, Inactivation properties (left) and average inactivation–voltage relationships (right) of shark (red, \(V_{h/2} = 0.1 \pm 2.8\) mV) and human (black, \(V_{h/2} = -30.6 \pm 0.9\) mV) \(K_V^{1.3}\) channels. The arrow indicates when inactivation was measured during 40-mV pulses after a series of prepulses that increased in 10-mV increments from \(-100\) mV. \(n = 9\), \(P < 0.0001\) for \(V_{h/2}\), two-tailed Student’s \(t\)-test. e, \(I_{KV}\) was reduced by 4-AP or quinidine in native shark electrosensory cells or heterologously expressed shark \(K_V^{1.3}\). Currents were elicited by increasing voltage pulses from \(-90\) mV (native) or \(-100\) mV (heterologous). f, Pharmacological profile of shark electrosensory cell \(I_{KV}\) and heterologously expressed shark \(K_V^{1.3}\). Currents measured at peak amplitude were reduced by 4-AP or quinidine, but not by other treatments. Pharmacological modulation of native \(I_{KV}\) and shark \(K_V^{1.3}\) was similar. g, The short human isoform of \(K_V^{1.3}\) (short N-terminal truncation) was used to study gating currents because of enhanced expression, but channel properties are identical15. Similarly, we found that activation threshold and voltage-dependent inactivation, and deactivation were similar between long and short isoforms. \(n = 6\). All data are represented as mean ± s.e.m, \(n\) denotes cells.
Extended Data Fig. 5 | Shark Kv gating currents. a, Gating currents recorded in HEK293 cells expressing non-conductive shark (red) or human (black) Kv1.3 elicited by increasing voltage pulses in 10-mV increments from a −100-mV holding potential. b, Average charge (Q)–V relationships. Shark Kv1.3 V1/2 was −33.4 ± 0.5 mV, Ks = 6.1 ± 0.5 mV; and human Kv1.3 V1/2 was −54.25 ± 0.7 mV, Ks = 5.2 ± 0.6 mV. n = 9, P < 0.0001 for V1/2 two-tailed Student’s t-test. Dotted lines indicate associated G–V relationships. c, QON kinetics after voltage sensor activation from a holding potential of −100 mV were significantly slower in shark (red) compared with human (black). n = 7, P < 0.0001 for contribution of orthologue identity to series variance, two-way ANOVA with post hoc Bonferroni test. d, Representative OFF gating current (QOFF) kinetics during repolarization in 10-mV increments after a 40-mV prepulse. The arrow indicates when deactivation rates were measured and purple traces show deactivation at −50 mV. e, QOFF kinetics were significantly faster in shark (red) compared with human (black). n = 11, P < 0.0001 for contribution of orthologue identity to series variance, two-way ANOVA with post hoc Bonferroni test. f, Gating currents from shark (red) or human (black) Kv1.3 after decreasing voltage pulses in increments of 10 mV from a holding potential of 0 mV. Scale bars, 500 pA, 25 ms. g, Average charge (Q)–V relationships of downward voltage sensor movement (QOFF) in response to decreasing voltage pulses from a holding potential of 0 mV. Shark Kv1.3 V1/2 was −61.6 ± 1.9 mV and human Kv1.3 V1/2 was −110.9 ± 1.01 mV, n = 7, P < 0.0001 for contribution of orthologue identity to series variance, two-way ANOVA with post hoc Bonferroni test. h, QOFF kinetics of voltage sensor deactivation from a holding potential of 0 mV were significantly faster in shark (red) compared with human (black). n = 7, P < 0.0001 for contribution of orthologue identity to series variance, two-way ANOVA with post hoc Bonferroni test. All data are represented as mean ± s.e.m. i, Ion tail currents (indicating channel closure) deactivated faster in shark (red) compared with human (black) Kv1.3. Tail currents were measured at −100 mV after a series of activating voltage pulses that increased in 10-mV increments. Inset, arrows indicate when tail currents were measured after activating voltage pulses. Representative of n = 10. τ for deactivation from 60-mV pulse: shark = 1.5 ± 0.1 ms; human = 5.5 ± 0.4 ms. P < 0.0001, two-tailed Student’s t-test. All data are represented as mean ± s.e.m, n denotes cells.
Extended Data Fig. 6 | Shark Kv voltage sensor domain relaxation.

**a,** $Q_{OFF}$ kinetics after either 10-ms or 1-ms activating prepulses of 40 mV. Purple traces indicate deactivation at $-50$ mV. Arrow indicates when current properties were measured during the voltage protocol.

**b,** Average $Q_{OFF}$ kinetics were faster in shark compared with human during deactivation after 40-mV prepulses of 25, 10, or 5 ms duration, but rates were similar after 1-ms activating prepulses. Kinetics were measured at voltages that decreased in 10-mV increments from 40 mV. $n = 9$, $P < 0.0001$ for contribution of orthologue identity to series variance at 25, 10, or 5 ms, but no significant difference was observed at 1 ms, two-way ANOVA with post hoc Bonferroni test.

**c,** Shark Kv1.3 $Q_{OFF}$ kinetics were relatively unaffected by the duration of the activating voltage pulse, whereas human Kv1.3 entered a proposed ‘relaxed’ state that resulted in the slowing of $Q_{OFF}$ with increasing pulse length. Deactivation was measured at $-100$ mV after a series of 40-mV voltage pulses of varying duration from 0.5 ms to 30 ms.

**d,** Average $Q_{OFF}$ kinetics in response to indicated voltage pulse lengths. $n = 6$, $P < 0.0001$ for comparison of orthologue $Q_{OFF}$ kinetics after a 30-ms voltage pulse.

**e,** Hypothetical model of shark and human Kv1.3. Compared with its human orthologue, shark Kv1.3 exhibits reduced voltage sensor domain relaxation, which stabilizes pore opening in human Kv1.3. Reduced voltage sensor relaxation is indicated by dotted lines to suggest that this state (or states) may occur to a lesser extent in the shark orthologue. Thus, compared with human Kv1.3, the shark orthologue requires relatively less repolarizing voltage to more quickly return to a resting/closed state. All data are represented as mean ± s.e.m, $n$ denotes cells.
Extended Data Fig. 7 | Shark–human Kv chimaeric analyses.

**a**, Chimaeric shark–human V_{K1.3} channels reveal that shark Kv S1–S6 confers differences in activation voltage threshold, deactivation kinetics and inactivation. Top, the chimaera constructs analysed (shark in red, human in black). Middle, the arrow indicates when voltage-activated currents were measured at −30 mV after a series of voltage pulses that increased in 10-mV increments from −100 mV. Bottom, the arrow indicates when inactivation was measured during 40-mV pulses after a series of prepulses that increased in 10-mV increments from −100 mV.

**b**, Compared with wild-type human Kv, average G–V relationships for wild-type shark, shark (s)S1–S6, and sS1–S4 channels were similarly shifted to positive voltages with more gradual slopes and deactivation kinetics were faster. Va1/2 (mV) for wild-type human = −30.7 ± 0.5 mV, slope factor (K_a) = 4.7 ± 0.5 mV; wild-type shark V_{a1/2} = −5.4 ± 0.5 mV, K_a = 7.6 ± 0.4 mV; sS1–S6 V_{a1/2} = −9.7 ± 0.7 mV, K_a = 8.1 ± 0.6 mV; sS1–S4 V_{a1/2} = −6.5 ± 0.7 mV, K_a = 10.7 ± 1.2 mV. Average deactivation kinetics of wild-type shark, sS1–S6, and sS1–S4 Kv channels were faster than those of wild-type human channels. Substitution of human (h)S1–S6 or hS1–S4 into the shark Kv channel also partially shifted the activation threshold and deactivation kinetics. Channels containing hS5–S6 exhibited the strongest voltage-dependent inactivation—nearly as efficient as wild-type human Kv—whereas channels containing sS5–S6 displayed weaker inactivation. sS1–S4 had a smaller effect on inactivation. V_{1/2} for wild-type human = −34.6 ± 0.9 mV, wild-type shark = 0.1 ± 2.8 mV, hS1–S6 = −18.3 ± 0.4 mV, hS1–S4 = −12.3 ± 1.2 mV, sS1–S6 = −9.2 ± 0.8 mV, sS1–S4 = −16.0 ± 0.9 mV. n = 9 for each of wild-type shark, wild-type human, hS1–S6, hS1–S4 and sS1–S6, and 7 for sS1–S4.

**c**, Currents elicited from the indicated S5–S6 chimaeric channels in response to voltage protocols to access voltage-dependence for activation and inactivation.

**d**, sS5–S6 reduces voltage-dependent inactivation and hS5–S6 partially confers strong voltage-dependent inactivation in shark channels. n = 6. P < 0.0001 for hS5–S6 versus sS5–S6 or wild-type shark, sS5–S6 versus hS5–S6 or wild-type human, two-way ANOVA with post hoc Tukey test.

**e**, Top, S5–S6 substitution did not greatly affect voltage-dependent activation. n = 6 for hS5–S6, 7 for sS5–S6. Bottom, S5–S6 substitution did not affect deactivation kinetics. n = 7 for hS5–S6, 9 for sS5–S6. All data are represented as mean ± s.e.m, n denotes cells.
Extended Data Fig. 8 | Electrosensory cell ribbon synapse characteristics. a, Left, five highest expressed transcripts in shark ampullae. The Ca\(^{2+}\)-binding protein parvalbumin 8 is the most highly expressed and is enriched in ampullae compared with other examined tissues. Right, five highest expressed ATPase transcripts in shark ampullae. A plasma membrane Ca\(^{2+}\) ATPase is highly expressed and enriched in ampullae. Bars represent FPKM. b, Expression of transcripts associated with ribbon synapses in shark and skate ampullae. Expression of vGluT3 and EAAT1 suggests that the synapse could be glutamatergic. c, Transmission electron micrograph of skate ribbon synapse with arrows indicating electrosensory cell, synaptic ribbon and afferent nerve terminal. Distinct vesicular pools are coloured: blue, attached to ribbon; green, refilling; yellow, readily releasable. An orange dotted line indicates the 150-nm region in which the readily releasable pool was quantified. Scale bar, 500 nm. d, Quantification of attached vesicles, ribbon vesicle density, ribbon shape variation and vesicle diameter was similar between shark and skate electrosensory cells. The readily releasable pool, quantified by number of vesicles 150 nm from the synapse, was significantly larger in shark versus skate electrosensory cells. n = 18, P < 0.0001, two-tailed Student’s t-test. The refilling pool density, quantified as detached cytosolic vesicles, was significantly larger in skate electrosensory cells. n = 20 shark and 21 skate, P < 0.0001, two-tailed Mann–Whitney test. Shark ribbons were more parallel to the plasma membrane in comparison to skate ribbons that were often more perpendicular. For angle quantification n = 20 shark and 21 skate ribbons, P < 0.0001, two-tailed Mann–Whitney test. All data are represented as mean ± s.e.m, n denotes counted structures.
Extended Data Fig. 9 | Shark electrosensory cell vesicular release characteristics. 

**a.** Top, currents and capacitance changes in response to a 10 ms, \(-20\) mV voltage pulse in shark and skate electrosensory cells. Scale bars, 50 pA, 200 ms. Bottom, representative capacitance changes in response to the indicated durations of a voltage stimulus of \(-20\) mV. Scale bars, 25 fF, 200 ms. **b.** \(-20\) mV voltage pulses of various durations induced similar integrated \(I_{CaV}(Q_{Ca^{2+}})\) in shark or skate electrosensory cells. \(n = 6\). **c.** \(I_{CaV}\) elicited by simulated voltage spikes in shark electrosensory cells and smaller voltage oscillations in skate cells. \(K^+\) currents were blocked by intracellular Cs\(^+\), extracellular 4-AP and IbTx. **d.** Voltage-clamp protocols developed to simulate shark electrosensory cell spiking induced the same amount of \(Q_{Ca^{2+}}\) in shark or skate cells. Similarly, voltage protocols that simulated smaller skate electrosensory cell voltage oscillations induced the same amount of \(Q_{Ca^{2+}}\) in shark or skate cells. \(Q_{Ca^{2+}}\) elicited by simulated voltage spikes was larger than \(Q_{Ca^{2+}}\) elicited by simulated oscillations. \(n = 10\) for shark and 5 for skate. All data are represented as mean ± s.e.m, \(n\) denotes cells.
Extended Data Fig. 10 | Schematic representation of ampullae of Lorenzini distribution in two elasmobranch species. a, The dorsal surface of the chain catshark (*S. retifer*), with black dots corresponding to individual ampullary pores and blue lines representing canal structures. The buccal and supraorbital clusters from which electrosensory cells were obtained are indicated with arrowheads. This schematic was prepared from photographs of four individual fish. b, The ventral surface of the catshark. c, Photograph of ampullary pores, visible on the ventral rostrum of an adult catshark. d, The dorsal surface of the little skate (*L. erinacea*). The hyoid capsules from which electrosensory cells were obtained are indicated with arrowheads. This schematic was prepared from photographs of four individual fish. e, The ventral surface of the skate. f, Photograph of a cleared Alcian blue-stained skate, revealing the ampullary canals from the ventral surface of the skate.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
|-----|-----------|
| ☑   | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☑   | An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☑   | The statistical test(s) used AND whether they are one- or two-sided |
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| ☑   | A description of all covariates tested |
| ☑   | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☑   | A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☑   | For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable. |
| ☑   | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☑   | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☑   | Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated |
| ☑   | Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection
- Transcriptional profiling: Trinity suite for de novo assembly v2.1.0, NCBI Blast (v2.2.31) with a maximum E value of 1X10-5, RSEM v1.1.17
- Cell physiology: pClamp v10, Clampfit, PatchMaster, Prism v7
- Cellular structural measurements (ribbons): ImageJ v1.47
- Behavioral experiments: iMovie 10.1.2

Data analysis
- Prism v7 was used for statistical analyses.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data
Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Deep sequencing data are archived under GEO accession number GSE103977. The GenBank accession numbers for the α subunit are: CaV1.3 MF959522, KV1.3 MF959523, and BK MF959524. Other data are available from the authors upon request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample sizes were chosen based on pilot experiments and prior experiences of the investigators in similar previously published experimental preparations. Behavioral experiments (n=10 under each stimulus condition) exceed previously published sample sizes for elasmobranch sensory behavioral experiments.

Data exclusions
Data were excluded from behavioral experiments when basal ventilation frequency differed from the mean for each species by more than 2 standard deviations.

Replication
All attempts at replication were successful.

Randomization
Fish were chosen in random order for behavioral analyses and were placed back in their home aquaria for at least 24 hours between testing.

Blinding
Investigators were blinded during behavioral data analysis phases.

Materials & experimental systems

Policy information about availability of materials

n/a

Involved in the study

- Unique materials
- Antibodies
- Eukaryotic cell lines
- Research animals
- Human research participants

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
ATCC

Authentication
Authenticated from vendor (ATCC).

Mycoplasma contamination
Cell lines were negative for mycoplasma, as validated by ATCC.

Commonly misidentified lines
(See ICLAC register)
HEK293T is listed among the ICLAC misidentified cell lines; however, these cells were used only as representative mammalian expression systems, regardless of specific mammalian cell line identity. Cells were used solely in transient heterologous ion channel expression and patch clamp recordings.
Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials

Adult little skates (Leucoraja erinacea) and chain catsharks (Scyliorhinus retifer) of unknown age were used in cell physiology, transcriptional profiling, and histological analyses. Male and female fish were used, approximately 40 cm (skates) and 50 cm (catsharks) in total length. Adult skates weighed approximately 700 g, and catsharks weighed approximately 400 g.

For behavioral experiments, juvenile skates were at least 10 days post hatching, with a mean length of 10 cm and mass of 4.1 g of both sexes. Juvenile catsharks were at least 14 days post hatching, with a mean length of 12 cm and a mass of 3.8 g of both sexes.

Method-specific reporting

|   | Involved in the study |
|---|-----------------------|
| n/a |                       |
| ☒ | ChIP-seq              |
| ☒ | Flow cytometry        |
| ❌ | Magnetic resonance imaging |