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Oxidation of a potassium channel causes progressive sensory function loss during ageing.

SUPPLEMENTARY INFORMATION

Table S-1 Electrophysiological properties of ΔNIRD KVS-1 channels in heterologous expression systems (CHO, HEK 293)

|          | σ_{120} (pA/pF) | τ_{120} (ms) | V_{1/2} (mV) | V_S (mV) | n  |
|----------|----------------|--------------|--------------|----------|----|
| ΔNIRD    |                |              |              |          |    |
| Cnt.     | 210 ± 23       | 11 ± 1.0     | 47.1 ± 2.1   | 19.7 ± 1.1 | 9  |
| CHT      | 248 ± 29       | 88 ± 9.1**   | 44.6 ± 2.7   | 19.4 ± 1.2 | 12 |
| DTT      | 227 ± 27       | 13 ± 1.3     | 46.9 ± 2.2   | 20.6 ± 1.4 | 14 |
| Cnt.     | 273 ± 36       | 9.7 ± 1.0    | 45.7 ± 2.8   | 20.9 ± 1.4 | 6  |
| H_2O_2   | 317 ± 51       | 94 ± 9.6*    | 44.3 ± 2.5   | 20.2 ± 1.6 | 5  |
| DTT      | 291 ± 47       | 16 ± 1.7     | 47.4 ± 2.8   | 19.0 ± 1.6 | 5  |
| ΔNIRD-C95S |            |              |              |          |    |
| Cnt.     | 202 ± 19       | 10 ± 9.4     | 46.1 ± 2.2   | 20.0 ± 1.3 | 8  |
| CHT      | 200 ± 26       | 19 ± 1.8*    | 44.6 ± 1.4   | 19.1 ± 1.5 | 10 |
| DTT      | 207 ± 25       | 11 ± 1.1     | 47.7 ± 2.1   | 21.2 ± 1.5 | 10 |
| Cnt.     | 289 ± 41       | 9.6 ± 1.0    | 45.8 ± 2.4   | 21.2 ± 1.8 | 6  |
| H_2O_2   | 292 ± 48       | 16 ± 1.9*    | 47.3 ± 2.9   | 20.6 ± 1.5 | 6  |
| DTT      | 299 ± 50       | 13 ± 1.7     | 45.5 ± 2.3   | 19.9 ± 1.7 | 6  |
| ΔNIRD+MPS-1 |          |              |              |          |    |
| Cnt.     | 107 ± 15       | 7.4 ± 0.9    | 35.6 ± 2.8   | 20.5 ± 1.6 | 5  |
| CHT      | 111 ± 16       | 94 ± 14*     | 37.4 ± 3.0   | 20.0 ± 1.7 | 5  |
| ΔNIRD-C95S+MPS-1 |      |              |              |          |    |
| Cnt.     | 124 ± 16       | 8.6 ± 1.2    | 36.1 ± 2.5   | 19.4 ± 1.6 | 5  |
| CHT      | 120 ± 18       | 14 ± 2.0     | 35.9 ± 3.2   | 19.5 ± 1.5 | 5  |

Currents were elicited by voltage jumps from –80 mV to +120 mV in 20 mV increments. Current densities, σ_{120}, were calculated by normalizing the peak current at +120 mV to the cell capacitance. Time constants at +120 mV, τ_{120}, were calculated by fitting macroscopic currents to a single exponential function:
\[ I(t) = I_0 + I_1 e^{-t/\tau} \]  \hspace{1cm} (S-1)

The half-maximal voltage, \( V_{1/2} \) and the slope factor \( V_s \) were calculated by fitting macroscopic conductance-V relationships \((G)\) to the Boltzmann equation:

\[ G(V) = \frac{I}{V - V_{\text{rev}}} = \frac{G_{\text{Max}}}{1 + e^{(V_{1/2}-V)/V_s}} \]  \hspace{1cm} (S-2)

where \( I \) is the macroscopic peak current, \( V \) is the applied voltage, and \( V_{\text{rev}} \) is the computed Nernst potential for \( K^+ \) at the experimental concentrations (–87 mV). CHT and DTT were applied on the same cell (as shown in Fig. 1). CHT was supplied for 5 minutes and DTT for 5–10 minutes. \( \text{H}_2\text{O}_2 \) and DTT were applied on the same cell. \( \text{H}_2\text{O}_2 \) was applied for 2–3 minutes and DTT for 5–10 minutes. Wild type KVS-1 channels were expressed in CHO cells. \( \Delta \text{NIRD} \) and \( \Delta \text{NIRD-C95S} \) channels were expressed in CHO cells and in HEK cells. Channels expressed in CHO cells were tested for susceptibility to CHT, channels expressed in HEK cells were tested for susceptibility to \( \text{H}_2\text{O}_2 \).

Statistically significant differences from control are indicated with * (0.01 < \( P < 0.05 \)) and ** (\( P < 0.01 \)).
**Table S-2** Electrophysiological properties of native ASER currents

|                  | $I_{80}$(pA) | $V_{1/2}$ (mV) | $V_s$ (mV) | n  |
|------------------|--------------|----------------|------------|----|
| **Day four**     |              |                |            |    |
| $N2$.            | 67 ± 6.1     | 29.7 ± 1.9     | 20.7 ± 1.0 | 38 |
| tm2034           | 64 ± 9.1     | 36.3 ± 1.8**   | 16.0 ± 1.0**| 23 |
| Wild type-KVS-1  | 66 ± 7.5     | 30.9 ± 2.4     | 19.9 ± 1.3 | 13 |
| C113S-KVS-1      | 62 ± 5.6     | 30.2 ± 2.2     | 20.3 ± 1.1 | 25 |
| age-1(hx546)     | 65 ± 6.3     | 30.2 ± 1.9     | 21.8 ± 1.4 | 15 |
| **Day twelve**   |              |                |            |    |
| $N2$.            | 110± 12*     | 29.8 ± 1.6     | 20.0 ± 1.5 | 29 |
| tm2034           | 61 ± 7.8     | 37.0 ± 1.9**   | 15.4 ± 1.1**| 14 |
| Wild type-KVS-1  | 102± 12*     | 30.1 ± 2.3     | 20.1 ± 1.3 | 24 |
| C113S-KVS-1      | 66± 6.0      | 30.3 ± 2.8     | 19.8 ± 1.6 | 23 |
| age-1(hx546)     | 70 ± 7.3     | 31.4 ± 2.3     | 21.1± 2.0  | 15 |

Currents were elicited by voltage jumps from –80 mV to +80 mV in 20 mV increments. $I_{80}$ indicates the steady-state current at +80 mV.

The half maximal voltage, $V_{1/2}$ and the slope factor $V_s$ were calculated by fitting macroscopic conductance-V relationships (G) to eqn. (S-2) with $V_{rev.} = −89$ mV. Statistically significant differences from control are indicated with * $(0.01 < P < 0.05)$ and ** $(P < 0.01)$. 
Fig. S-1 (a) Representative whole-cell currents evoked in a four days old N2 tm2034 ASER neuron by single voltage jumps from -80 mV to +80 mV in the presence of 0.25 mM H2O2 in the pipette. Right, fractional current, in the absence (n = 22) or presence of 0.25 mM H2O2 (n = 7) in the pipette. (b) Representative whole-cell currents in a twelve days old N2 tm2034 ASER neuron. Right, fractional current, in 4 days old (same as in (A)) and 12 days old neurons (n = 14). (c) Representative potentials evoked in 4 days old N2 (red) and tm2034 (blue) ASER cultured neurons in response to a 12 pA current injection. (d) Rise times in 4 days old N2 (squares) and tm2034 (triangles) neurons. n = 8 cells and n = 5 cells for respectively, N2 and tm2034.
Fig. S-2 Fluorescence microscopy images taken from Pkvs-1::gfp transgenic nematodes showing GFP signals in neurons of the head including the amphid neurons (in two different focal plans), in the ventral cord neurons, in the anal depressor muscle (arrow) and vulva. Images were taken with an Olympus BX61 microscope equipped with a digital camera.
FULL METHODS

Molecular Biology
Cysteine mutants (C113S, ΔNIRD-C95S, C151S, C209S, C283S and C172S-C173S), were constructed by polymerase chain reaction (PCR) using Pfu polymerase (Stratagene) and wild-type or ΔNIRD (obtained by deleting the first 18 amino acids, [1]) KVS-1 cDNA in the pCI-neo vector (Promega).

Strains
Strain used were Bristol (N2), tm2034 (kvs-1 KO; outcrossed four times) age-1(hx546) and eat-4(ky5). We constructed: tm2034(PKVS-1::KVS-1)(myo-2::gfp), termed wild-type-KVS-1, tm2034(PKVS-1::C113S-KVS-1)(myo-2::gfp), termed C113S-KVS-1, tm2034(PKVS-1::KVS-1)(myo-2::gfp)(gcy-5::gfp)(rol-6), tm2034(PKVS-1::C113S-KVS-1)(myo-2::gfp)(gcy-5::gfp)(rol-6) and age-1(hx546)(gcy-5::gfp)(rol-6), tm2034(Pflp-6::KVS-1)(myo-2::gfp)(gcy-5::gfp) and tm2034(Pflp-6::C113S)(myo-2::gfp)(gcy-5::gfp).

Construction of transgenic animals
The promoter of kvs-1. A 3162 bp fragment of intronic sequence upstream exon 2 (termed the promoter of kvs-1 or Pkvs-1) was amplified by PCR from the C53C9 cosmid and subcloned into the pPD95.75 Fire vector (P_kvs-1::gfp construct), using BamH I and Xma I restriction sites. Primers: 5’-
CGCGGATCCATGATGCTTCTTCATGAT-3' and 5'-
TCCCCCCGGGGGCAATTTGGTTGCTGAA-3'. \( P_{kvs-1::gfp} \) was injected into the syncitial gonads of adult \( tm2034 \) hermaphrodites. \( P_{kvs-1::gfp} \) yielded GFP signals in several amphid neurons including the ASEs, vulva, ventral cord motor-neurons and anal depressor muscle [2] (Fig. S-2).

The \( flp-6 \) promoter. 2481 bp of genomic DNA (the promoter of \( flp-6 \), termed \( P_{flp-6} \)) were amplified by PCR using the following primers: 5'-
AAGCTTACTGCATAAAAAAACAACAAAAAAATAA-3' and 5'-
CCCGGGATTCTGGAATAATCATATTGTTTTCAAAAT-3' and subcloned in a construct containing wild-type KVS-1 or C113S KVS-1 cDNA in pPD95.75 using Hind III and SAM I restriction sites.

Wild-type-KVS-1 and C113S-KVS-1 transgenic worms For transgenic expression of KVS-1, Xma I and KPN I restriction sites were added by PCR in wild-type and C113S KVS-1 cDNA (5'-TCCCCCCGGGATGAGCACGGAAAGGCTG-3' and 5'-CGGGGTACCAACGATTCTGCCACATCAAT-3') and subcloned in \( P_{kvs-1} \) in pPD95.75. The constructs were injected into the syncitial gonads of adult \( tm2034 \) hermaphrodites. Transformant lines for \( wild-type-KVS-1 \) and \( C113S-KVS-1 \) were each stabilized by a mutagenesis-induced integration into a chromosome by irradiating 40 animals with \( \gamma \)-ray with 4000 rads for 40 minutes. The progeny were checked for 100% transmission of the marker (\( myo-2::gfp \)) and also for the presence of the transgene by PCR amplification. Two lines for \( wild-type-KVS-1, tm2034(P_{kvs-1::KVS-1})(myo-2::gfp) \) (#3 and #4) and two lines for \( C113S-KVS-1, tm2034(P_{kvs-1::C113S-KVS-1})(myo-2::gfp) \) (#7 and #11) were outcrossed four
times. These lines gave similar results in chemotaxis assays. The data presented in this study were obtained with lines #3 and #11. For electrophysiology lines #3 and #11 were injected with the P{gcy-5::gfp} construct. Because these lines express GFP in the pharynx, the transformation marker was rol-6. Thus, for electrophysiology, strains used were tm2034(P{KVS-1::KVS-1}(myo-2::gfp)(gcy-5::gfp)(rol-6), tm2034(P{KVS-1::C113S-KVS-1}(myo-2::gfp)(gcy-5::gfp)(rol-6) and age-1(hx546)(gcy-5::gfp)(rol-6).

P{flip-6::wild-type-KVS-1} and P{flip-6::C113S-KVS-1} transgenic worms. The constructs were linearized and injected (3 ng/µl) into the syncitial gonads of adult tm2034 hermaphrodites together with the two linearized transformation markers myo-2::gfp and gcy-5::gfp (3 ng/µl each) and 50 ng/µl of genomic DNA digested with Sca I [3].

Behavioral assays

Age-synchronization. Nematodes were grown in standard 10 cm NGM plates + OP50 E. coli until a large population of gravid adults was reached (3-5 days). The animals were collected in 50 ml Falcon tubes, washed in M9 buffer (22 mM KH₂PO₄, 22 mM NaH₂PO₄, 85 mM NaCl, 1 mM MgSO₄), and treated with 10 volumes of basic hypochlorite solution (0.25 M NaOH, 1% hypochlorite freshly mixed; no significant differences were observed in bleach-free preparations, obtained by isolating laid eggs with SDS/NaOH). Worms were incubated at room temperature for 10 minutes, then the eggs (and carcasses) collected by
centrifugation at 400g for 5 minutes at 4 °C, incubated overnight in M9 buffer and seeded on standard NMG plates.

Behavioral tests were performed without knowledge of the worms’ genotype.

A) Chemotaxis assays. Experiments were performed as described previously [2]. Briefly, a chunk of agar 0.5-cm in diameter was removed from 10-cm plates and soaked in the attractant for 2 hours. Lysine and biotin were used at concentrations of 0.5 M and 0.2 M, respectively. Chunks were put back in the plate overnight to allow equilibration and formation of a gradient. Roughly 20 age-synchronized worms were placed between the test spot and a control spot on the opposite side of the plate. 10 µl of 20 mM NaN3 was placed on both spots. After one hour, animals on the test/control spot were counted, and a chemotaxis index, C.I., calculated as the number of animals at the test spot ($N_{\text{test}}$) minus the number of animals at the control spot ($N_{\text{cnt.}}$), divided by the total number of animals ($N$). A positive C.I. indicates attraction:

$$C.I. = \frac{N_{\text{test}} - N_{\text{cnt.}}}{N}\quad (S-3)$$

An experiment was carried out with roughly 100 worms/genotype distributed in 5 test plates.

To study chemotaxis during ageing, experiments were started with ~600-1000 age-synchronized worms per genotype (mean life span ~ 20 days). Worms were examined every day until death and were scored as dead when they were no
longer able to move even in response to prodding with a platinum pick. Each
day, worms were transferred to a fresh plate containing bacteria. The
experiments with Pflp-6::wild-type-KVS-1 and Pflp-6::C113S-KVS-1, were
started with ~200-300 age-synchronized worms.

B) Thrashing assay. Experiments were performed as described previously [2].
Briefly, age-synchronized worms were picked in a drop of M9 buffer on an agar
plate. After 2 min of recovery, thrashes were counted for 2 min. A thrash was
defined as a change in the body bend at the mid-body point.

C) Solid substrate assay. Worms were filmed using a digital Photometrics
Cascade 512B camera connected to a Leica MZ16 microscope at a rate of 3
frames per second. Speed was calculated as the distance covered by a point
located in the mid-body of the animal in one second. The average speed of a
worm was calculated by averaging 10 or more individual measurements.

SOD assay

Worms were grown as described above, lysed in 50 mM TRIS-HCL (pH =8.0)
and SOD activity was assessed using the Superoxide Dismutase Assay II kit
(Calbiochem).

Electrophysiology

a. Heterologous expression systems
Data were recorded with an Axopatch 200B amplifier (Axon) a PC (Dell) and Clampex software (Axon). Data were filtered at $f_c=1$ kHz and sampled at 2.5 kHz.

**Heterologous expression system.** Chinese Hamster ovary (CHO) and Human Embryonic Kidney 293 (HEK 293) cells were plated/transfected as described before [2] and used 24-36 hours post-transfection. Bath solution was (in mM): 4 KCl, 100 NaCl, 10 Hepes (pH=7.5 with NaOH), 1.8 CaCl$_2$ and 1.0 MgCl$_2$. Pipette solution: 100 KCl, 10 Hepes (pH=7.5 with KOH), 1.0 MgCl$_2$, 1.0 CaCl$_2$, 10 EGTA (pH=7.5 with KOH). CHT and DTT were added fresh prior the experiment from 1 M stocks in H$_2$O. Offset potentials due to series resistances ($\leq 5$ mV) were not compensated for when generating current-voltage relationships.

**b. Primary cultures**

Cultured ASER cells were prepared as described before [4]. Briefly, gravid adult worms were lysed using 0.5 M NaOH and 1% NaOCl (no significant differences were observed in bleach-free preparations, obtained by isolating laid eggs with SDS/NaOH). Released eggs were washed three times with sterile egg buffer containing 118 mM NaCl, 48 mM KCl, 2 mM CaCl$_2$, 2 mM MgCl$_2$, and 25 mM Hepes (pH 7.3, 340 mosM), and adult carcasses were separated from washed eggs by centrifugation in sterile 30% sucrose. Eggshells were removed by resuspending pelleted eggs in a sterile egg buffer containing 1 unit/ml chitinase at room temperature for 1.5 hours. Embryos were resuspended in L-15 cell culture medium containing 10% fetal bovine serum, 50 units/ml penicillin, and 50 µg/ml streptomycin (Sigma) and dissociated by gentle pipetting. Intact embryos,
clumps of cells, and larvae were removed from the cell suspension by filtration.

Dissociated cells were plated on glass cover slips previously coated with peanut lectin (0.1 mg/ml) dissolved in water. For electrophysiological recordings bath solution was (in mM): 145 NaCl, 5 KCl, 1 CaCl$_2$, 5 MgCl$_2$, 10 Hepes/NaOH (pH = 7.50, and 20 D-glucose. Pipette solution: 125 potassium gluconate, 18 KCl, 0.7 CaCl$_2$, 2 MgCl$_2$, 2 MgATP , 10 EGTA/KOH, and 10 Hepes/KOH (pH = 7.5).

Currents were repeatedly elicited 3-5 times (with the same voltage-stimulus) and digitally averaged on line. Leak currents were recorded in the cell-attached configuration before establishing the whole-cell configuration and were digitally subtracted during analysis. Offset potentials due to series resistance (≤ 2 mV) were not compensated for when generating current-voltage relationships.
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