Supplementary Information

A synaptic DEG/ENaC ion channel mediates learning in *C. elegans* by facilitating dopamine signalling

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Supplementary Materials and Methods

Molecular Biology

To generate the pasic-1GFP and pasic-1ASIC-1N::GFP reporter constructs, DNA fragments derived from the *asic-1* locus were PCR-amplified using appropriate oligonucleotide primers and fused to GFP. For the pasic-1GFP reporter, oligonucleotide primers

5’CCCAAGCTTCAATCAGTCACCCAGGTGTAC3’ and

5’CGCGGTACCTGCTCCGCAAAGTCAACGAC3’ were used to amplify a 2 Kb DNA fragment encompassing the promoter of *asic-1*, which was digested with HindIII and KpnI and inserted into the pPD95.77 plasmid vector (Fire et al, 1990). For pasic-1ASIC-1N::GFP, a 2.2 Kb DNA fragment including the *asic-1* promoter plus the intracellular amino-terminus of ASIC-1 was PCR-amplified using primers

5’CCCAAGCTTCAATCAGTCACCAGGTTGTAC3’ and

5’CGCGGTACCTGCTCCGCAAAGTCAACGAC3’, digested with HindIII and BamHI and inserted into the pPD95.77. To generate the full-length pasic-1ASIC-1::GFP reporter, the complete coding region of the *asic-1* gene were PCR-amplified using the
oligonucleotide primers 5’TCCCCCGGGGAAAGAACAGCTAAAACG3’ and
5’GAACCGGTTTATCAAGATTAAACCGTC3’, which include Smal and AgeI
restriction sites respectively. The resulting 4.2 Kb DNA fragment was subcloned into the
pCRII-Topo plasmid vector (Invitrogen, Carlsbad, USA). The fragment was then fused to
GFP by transferring to the pPD95.77 plasmid vector (Fire et al, 1990). The asic-1 promoter
was amplified separately using primers
5’ACGCGTCGACTTAGGGTCTGTTATTTGAGT3’ and
5’TCCCCCGGGCATTGCTGACTGCTGAAATTT3’, which include SalI and Smal
restriction sites respectively. The resulting 2 Kb fragment was first, inserted into the pCRII-
Topo vector and subsequently transferred to the pPD95.77-ASIC-1::GFP plasmid to
generate pPD95.77-asic-1ASIC-1::GFP. To label synaptic vesicles in asic-1-expressing
neurons, a pasic-1DsRED::SNB-1, reporter fusion was constructed in multiple steps, as
follows. First, plasmid pDsRED2-C1 (Clontech Laboratories, Inc, Mountain View, USA)
was digested with AgeI and EcoRI to excise a 0.7 Kb DNA fragment, containing the
DsRED coding sequence, which was subsequently inserted between the AgeI and EcoRI
sites on pPD95.77 to generate pPD95.77dsRED. Second, the SNB-1 coding region was
PCR-amplified directly from genomic DNA with primers 5’CGG
GGTACCGAATTTCGGACTCGAGATGCCGGC3’ and
5’CGGGGTACCCATGACCTTTTCTCAGCCCATCAAACG3’, digested with EcoRI
and inserted at the EcoRI site of pPD95.77DsRED to generate pPD95.77DsRED::SNB-1.
Third, the asic-1 promoter region was PCR-amplified directly from genomic DNA with
primers 5’CGCGGATCCCTTGTCTCGACTTAATTTACATAATTAGGT3’and
5’CGCGGTACCATTGTTGGCCTGAAATTTGATC3’, digested with BamHI and KpnI
and inserted between the BamHI and KpnI sites on pPD95.77DsRED::SNB-1 to generate pasic-1DsRED::SNB-1. To monitor neurotransmitter exocytosis, super-ecliptic pHluorin was fused at the carboxy-terminus of *C. elegans* synaptobrevin and driven by the asic-1 promoter (pasic-1SNB-1::SEpHluorin). The pasic-1SNB-1::SEpHluorin reporter fusion was constructed as follows: First, the super-ecliptic pHluorin coding region was PCR-amplified from plasmid pGEX-2T-SEpHluorin (Miesenbock et al, 1998) with primers 5’CGGGGTACCGGATCCACCGGTGGAAGT3’ and 5’CCGGAATTCACCGGTTTTGTATAGTTC3’, digested with KpnI and EcoRI and inserted between the KpnI and EcoRI sites on pPD95.77 to generate pPD95.77SEpHluorin. Second, the asic-1 promoter region was PCR-amplified directly from genomic DNA with primers 5’CGCGGATCCTTCGACACTAATTTACAATTAGGT3’ and 5’CGCGGTACCATTTGGTGGCCTGAAATTTGATC3’, digested with BamHI and KpnI and inserted between the BamHI and KpnI sites on pPD95.77SEpHluorin to generate pasic-1SEpHluorin. Finally, the SNB-1 coding region was PCR-amplified directly from genomic DNA with primers 5’CGG GGTACCGAATTCGGACGCTCAAGGAGATGCCGGC3’ and 5’CGGGGTACCGAATTCTTTTCCTCCAGCCCATAAAACG3’, digested with KpnI and inserted at the KpnI site of pasic-1SEpHluorin to generate pasic-1SNB-1::SEpHluorin. Reporter constructs were injected into the gonads of wild type animals together with pRF4, a plasmid that carries the rol-6 (su1006), dominant transformation marker. At least three independent, transgenic lines were obtained for each plasmid construct and roller hermaphrodites were examined for reporter fusion expression. For transgene complementation of asic-1(ok415), the wild-type asic-1 locus was PCR-amplified with primers 5’AGTCTGCTACTACTTCTGACT3’ and
5’TTCGACATCACCGATTCTACCACA3’ from cosmid ZK770 (Coulson et al, 1995). The resulting 5.8 Kb DNA fragment was injected into the gonads of asic-1(ok415) together with the pmyo-2GFP transformation marker. Five independent transgenic lines were isolated and examined in behavioural experiments. For genetic ablation of asic-1-expressing cells, wild type animals were injected with pasic-mec-4(u231), a plasmid carrying the toxic, gain-of-function mec-4(u231) allele (Driscoll & Chalfie, 1991) under the control of the asic-1 promoter, together with the pmyo-2GFP transformation marker. To generate the pasic-mec-4(u231) genetic ablation construct, the promoter and part of the first exon of the asic-1 gene were PCR-amplified from genomic DNA with primers

5’ACGCGTCGACTTCGACACTAATTTACAATTAGGTC3’ and
5’CGGGGTACCGACTCGTGTCGCCGCAAAGTCAACGAC3’. The resulting 2.4 Kb DNA fragment was digested with SalI and KpnI, and inserted into pmec-mec-4(u231) (Harbinder et al, 1997). For RNAi experiments, we constructed a plasmid that directs the synthesis of dsRNA corresponding to asic-1, in E. coli bacteria, which were then fed to animals, according to a previously described methodology (Kamath et al, 2001). A 1.5 Kb fragment of genomic DNA, encompassing 0.6 Kb of coding sequence, was amplified from an exon-rich region of the asic-1 gene, using primers

5’TCAAGTATCCACGTGTAGTAA3’ and 5’ATCTGGAAATGGTTGCTCGTC3’. The fragment was initially inserted into the pCRII-Topo vector (Invitrogen, Carlsbad, USA), excised with EcoRI, and subcloned to the pL4440 plasmid vector (Timmons et al, 2001). The resulting construct was transformed into HT115 (DE3) E. coli bacteria, deficient for RNase-E (Kamath et al, 2001). To augment RNAi, animals were reared for two generations on dsRNA-producing E. coli bacteria before examination. The following primers, which
amplify specifically asic-1 mRNA, were used for RT-PCR:

5'GTTTCGGATGAGGTTACGAAAC3', 5'AATTCTATCTTTACACCCGTA3' and
5'AGGTATTTCCACGAAATCATGGT3'.

**Supplementary References**


Supplementary Figure Legends

Supplementary Figure 1. Phylogenetic Relations Among DEG/ENaC Proteins
The genetically characterized *C. elegans* proteins are shown in red (ASIC-1, DEG-1, DEL-1, FLR-1, MEC-4, MEC-10, UNC-8, and UNC-105). Other nematode proteins are shown with blue lines. Representative DEG/ENaC proteins from a variety of organisms, ranging from snails to humans, are also included (mammalian: red lines; fly: green lines; snail: orange line, zebrafish: purple line). Branch lengths represent relative evolutionary distance (calculated number of amino acid substitutions per site; 0.1 for the scale bar).

Supplementary Figure 2. Phylogenetic Relationships Among *C. elegans*, Zebrafish, Mouse and Human ASIC Family Members
ASIC-1, ASIC-2, C24G7.4, F23B2.3 and T28B8.5 are *C. elegans* proteins. Branch lengths represent relative evolutionary distance (calculated number of amino acid substitutions per site, 0.1 for the scale bar; z: zebrafish; m: mouse; h: human).

Supplementary Figure 3. M-Coffee-Generated Multiple Sequence Alignment of Nematode ASIC-1, Mouse and Human ASIC Isoforms
The ASIC-1 amino acid residues deleted in the *asic-1(ok415)* mutant are indicated by the red box. (z: zebrafish; m: mouse; h: human)

Supplementary Figure 4. The *asic-1* Locus
(A) *asic-1* intron-exon structure. mRNA-specific primers used for reverse transcription PCR (RT-PCR) are shown (a, b, c). The region deleted in the *asic-1(ok415)* mutant is indicated. By removing exons 6-8 and parts of the fifth and ninth *asic-1* exon, the *ok415* deletion eliminates the first cysteine-rich domain (CRDI) of the extracellular ASIC-1 region.
(B) Transmembrane domain and topology predictions for ASIC-1. The probability of cytoplasmic and non-cytoplasmic localization is plotted against amino acid sequence. The amino terminus (amino acids 1-37) and the carboxy terminus (amino acids 811-844) are predicted to be intracellular, while the central domain (amino acids 60-782) is predicted to be extracellular. Amino acids 38-59 and 783-810 form the hydrophobic transmembrane segments. The plot was generated using the Phobius algorithm (http://phobius.cgb.ki.se/).

(C) RT-PCR amplification of asic-1 mRNA from wild type and asic-1(ok415) mutant animals using two different primer sets. The ama-1 gene (RNA polymerase II, large subunit) is used as control.

**Supplementary Figure 5. Sinusoidal Locomotion Characteristics.**

Wild type and asic-1(ok415) mutant animals inscribe sinusoidal tracks with similar wavelength and amplitude on bacterial laws (see also Table 1).

**Supplementary Figure 6. Genetic Ablation of asic-1-Expressing Neurons.**

The cytotoxic mec-4(d) allele is expressed in dopaminergic neurons under the control of the asic-1 promoter. Images show vacuolated dying neurons in L1 larvae.

**Supplementary Figure 7. Differential Requirement for Dopamine in Basal Slowing and Conditioning Behaviours**

(A) Basal slowing behaviour of cat-2 mutants, defective in dopamine biosynthesis, after treatment with increasing concentrations of exogenously supplied dopamine (DA). Error bars denote S.E.M. values (n=15 in 3 experiments).

(B) Exogenous addition of dopamine restores the capacity for associative learning in cat-2 mutants at concentrations lower than those required for normal basal slowing behaviour. Bars depict chemotaxis indices towards isoamyl alcohol, calculated for either naïve (white bars) or conditioned animals (black bars), after treatment with increasing concentrations of exogenously supplied dopamine (DA). Error bars denote S.E.M. values (n=200 in 4 experiments).
Supplementary Figure 8. ASIC-1 is not Required for Adaptation.

 ASIC-1(ok415) mutant animals show normal chemosensory adaptation behaviour. Bars depict chemotaxis indices towards isoamyl alcohol calculated for animals that were either naïve or conditioned in the absence of food for the indicated time intervals. White bars denote wild type animals, black bars denote ASIC-1(ok415) mutants. Error bars denote S.E.M. values (n=150 in 3 experiments; ***: P<0.001, unpaired t-test).

Supplementary Figure 9. Subcellular Localization of ASIC-1 in Animals With UNC-104 Deficiency.

Images of unc-104(e1265) mutant transgenic animals carrying a full-length pasic-1::ASIC-1::GFP reporter fusion. An image of a wt animal carrying the same reporter is included as control (top). The punctate distribution of GFP in dopaminergic neuron processes is not affected in the mutants. White bars denote 10 microns.
**Supplementary Table 1.** Genetic ablation of *asic-1*-expressing neurons. Mosaic analysis of N2Ex[\(p_{asic-1}mec-4(u231)]\ Ex[p_{asic-1}GFP]\ double transgenic animals.

<table>
<thead>
<tr>
<th>Ablated neurons*</th>
<th>Chemotaxis index after conditioning†</th>
</tr>
</thead>
<tbody>
<tr>
<td>All 12 <em>asic-1</em>-expressing neurons</td>
<td>56.8±5.1</td>
</tr>
<tr>
<td>All 8 dopaminergic neurons</td>
<td>51.8±6.3</td>
</tr>
<tr>
<td>4 non-dopaminergic tail neurons</td>
<td>-4.3±4.1</td>
</tr>
<tr>
<td>2 ADE dopaminergic neurons</td>
<td>19.4±5.2</td>
</tr>
<tr>
<td>4 CEP dopaminergic neurons</td>
<td>39.8±5.6</td>
</tr>
<tr>
<td>2 PDE dopaminergic neurons</td>
<td>21.4±7.0</td>
</tr>
</tbody>
</table>

* Mosaic N2Ex[\(p_{asic-1}mec-4(u231)]\ Ex[p_{asic-1}GFP]\, double transgenic animals lacking the indicated neurons were grouped and assayed.
† Chemotaxis index (±S.E.M.) towards isoamyl alcohol after conditioning to isoamyl alcohol in the absence of food (n=50 for each mosaic subtype, 3 independent experiments).
Figure 4: Phobius posterior probabilities for ASIC-1.

A) A schematic representation of the genomic region around the ASIC-1 gene, highlighting the locations of the OK415 insertions (a, b, c).

B) A graph showing the posterior label probabilities for ASIC-1, with different colors indicating transmembrane, cytoplasmic, non-cytoplasmic, and signal peptide probabilities.

C) An agarose gel showing PCR products for wild-type (wt) and ASIC-1 (OK415) alleles, with markers indicating 902, 744, 495, 407, and 249 base pairs.
$p_{asic-1me4c-4(d)}$

Cephalic neurons

Tail neurons
Figure A: Bar chart showing the number of body bends per 20 seconds under different conditions: wt, cat-2(e1112) no DA, cat-2(e1112) +0.1 mM DA, cat-2(e1112) +2 mM DA, with Off Food and On Food treatments.

Figure B: Bar chart showing the chemotaxis index under different conditions: wt, cat-2(e1112) no DA, cat-2(e1112) +0.1 mM DA, cat-2(e1112) +2 mM DA, with Isoamyl alcohol and different treatment groups: Naive and Conditioned.
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Supplemental Figure 8

Supplemental Figure 8

Isoamyl alcohol

Chemotaxis index

Naive
Conditioned 1 hr
Conditioned 3 hrs

-25
0
25
50
75
100
Supplemental Figure 9

Comparison of fluorescence imaging between wild-type (wt) and unc-104(e1265) strains. Images show the expression of the pH-sensing transgene, pasic-1::ASIC-1::GFP.