

Figure Legends:

Fig. S1:

Preliminary gene structure of *Aplysia* H_v channel genes. CDS: coding region, exon borders are indicated.

AcH_v1 scaffold00253

CDS	195..1040	
Exon 1	1..194	517424..517617
Exon 2	193..356	524625..524790
Exon 3	356..464	528150..528258
Exon 4	463..700	528668..528904
Exon 5	696..1440	530671..531415

AcH_v2 scaffold00057

CDS	449..1468	
Exon 1	1..272	702415..702144
Exon 2	273..633	697725..697365
Exon 3	631..755	632843..632719
Exon 4	756..897	625404..625263
Exon 5	895..1029	623446..623312
Exon 6	1026..1084	622455..622397
Exon 7	1080..1221	617783..617642
Exon 8	1220..1336	613702..613586
Exon 9	1335..1423	612130..612042
Exon 10	1422..2591	610782..609613

AcH_v3 scaffold00078

CDS	3328..6270	
Exon 1	1..120	439851..439970
Exon 2	120..473	458948..459301
Exon 3	474..3394	460584..463504
Exon 4	3390..3517	522792..522919
Exon 5	3518..4451	550319..551252
Exon 6	4450..4505	582759..582814
Exon 7	4502..4644	589380..589522
Exon 8	4643..4759	592841..592957
Exon 9	4759..4833	595045..595120
Exon 10	4830..10253	598366..603788

Fig. S2:

Aplysia EST expression analysis

	juvenile CNS	Pedal-Pleural Ganglia	homologous channels in other molluscs
AcH_{v1}	SAMN00150086	SAMN00150073 SAMN00150074	hemocyte (<i>Crassostrea gigas</i>) larvae (<i>Lottia gigantea</i>) CNS (<i>Melibe leonina</i>)
AcH_{v2}	EB327294 EB299171	EB210826	hemocyte (<i>Crassostrea gigas</i>) larvae (<i>Lottia gigantea</i>)
AcH_{v3}	EB295811 EB313783 EB337456		CNS (<i>Tritonia diomedea</i>) larvae (<i>Lottia gigantea</i>)

Fig. S3

Sequence alignments of the three *Aplysia californica* channels

N-terminal domain
(no significant homology)

S1 domain

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AcHv1  VIHSQKFMVFIIIVLVVLCCLMVIAELLFDLEIVKL
AcHv2  FLKTNLVQYSTIIALVILCLIIVMELLIDMNIIV
AcHv3  LLHSKYVVILVILVTTCALVIAELLIDLSSVKK
..... : * * : * * : : * * : :

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S1-S2 linker
(no significant homology)

S2-S4 domains & linkers

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AcHv1  HHYIPKIFHYGSLGILSLFLIEIGLRIFVLRDLDFFRKHKLELFDVAVVIVSFILDIVFRD---NEDAATGVGLLIILLLMVTIVNGIVLSVQKQAEK
AcHv2  REKAHVLRHALSLTILSIFMVEVCVKIYVEGKHLKQKAEVFDIIVVIVSFTLDITFSFVSVSKAASEAAGLMVILLLMVTIINGVIMSVKLDANK
AcHv3  DMEIAHKLHYASVAVVSILLIEVTMKIICAGSHFLKRRKIEVFDIIVVASVIVDLIFIKGLNQFPVDDSI FVLAFLLELVIVVNSLVMVAVIDHEHV
      : * * : : * : : : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : : * : :

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C-terminal domain
(no significant homology)

Direct alignment of the four transmembrane domains (S1-S4) including the cytosolic linkers. The extracellular S1-S2 loops show no significant homology. All three channels have an aspartate (selectivity filter) in the middle of S1.

Fig S4:

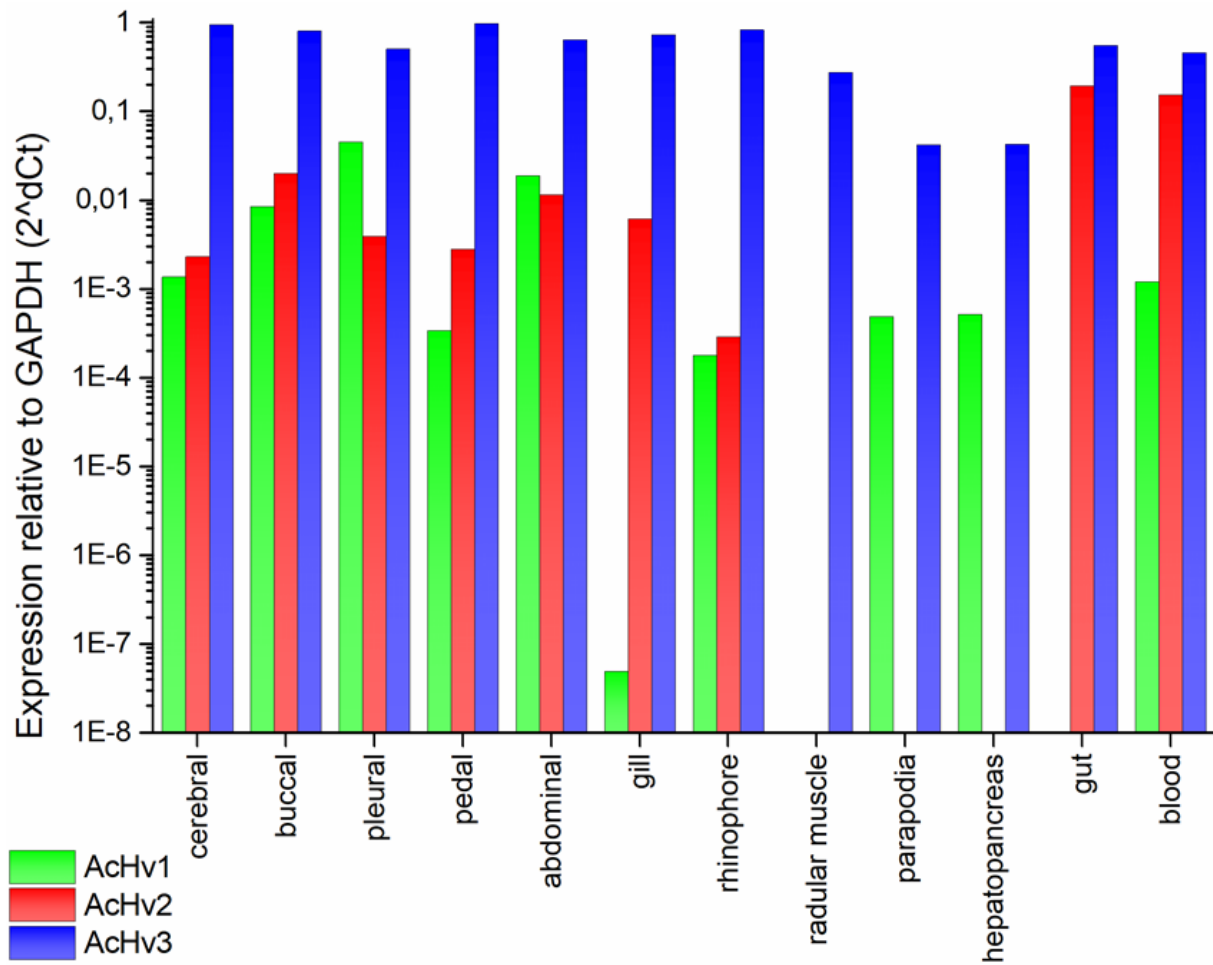
***Aplysia* H_v channels: Loop and domain length**

Type	total length	NTD	S1-S2	S2-S3	S3-S4	CTD
		int	ext	int	ext	int
ACh_v1	281	53	14	17	8	114
ACh_v2	339	73	79	17	11	82
ACh_v3	980	35	299	3	8	534
hH _v 1	273	100	12	17	8	55
NpH _v 1	239	54	10	17	8	69
KvH _v 1	248	39	41	17	8	65

Number of amino acids in different regions of the ACh_v1-2 channels and H_vs of three additional species. NTD: N-terminal domain, CTD: C-terminal domain, loops between the four transmembrane regions are indicated. hH_v1: human; NpH_v1: *Nicoletia phytophila*; KvH_v1: *Karlodinium veneficum*.

Fig S5:

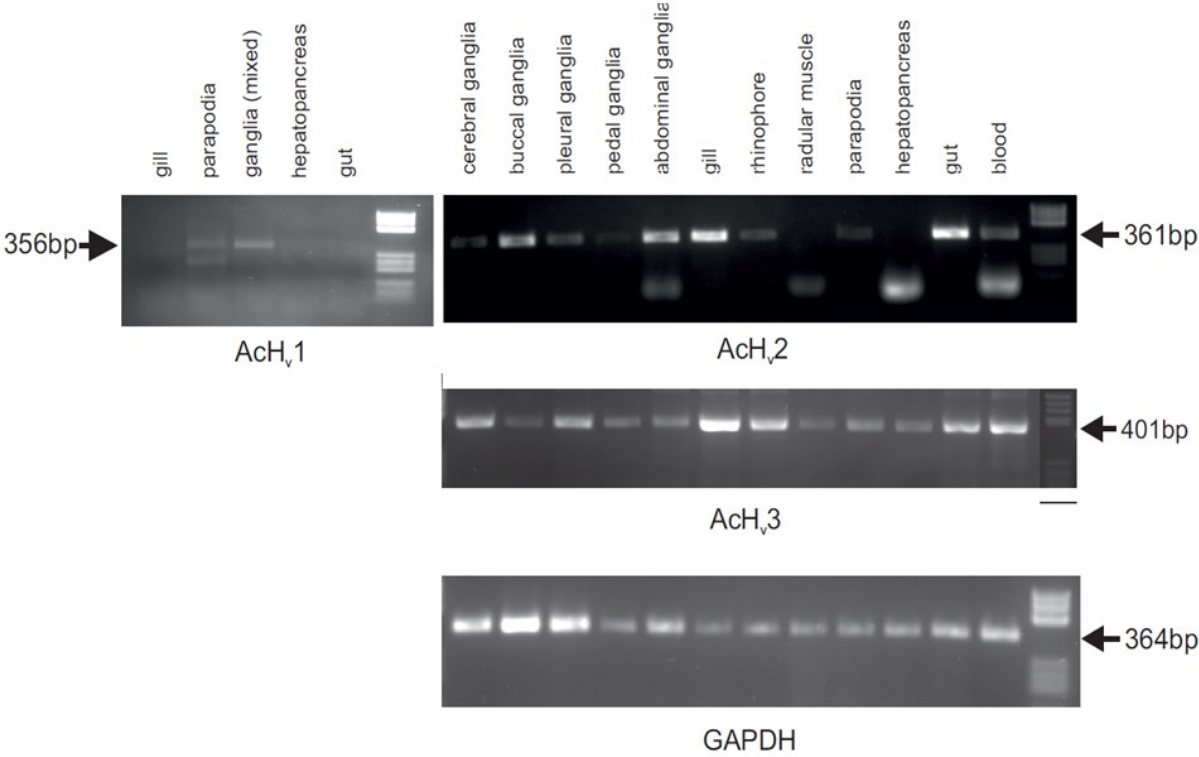
RT-PCR quantification of AcHv1, AcHv2, and AcHv3, in *Aplyisa californica* tissue samples



AcHv1, 2, 3 RT-PCR quantification relative to GAPDH in 12 different tissues of 4 animals with up to 5 individual measurements. Numbers are given in $2^{-\Delta Ct}$, where 1 would be equal to the expression of GAPDH.

Fig S6:

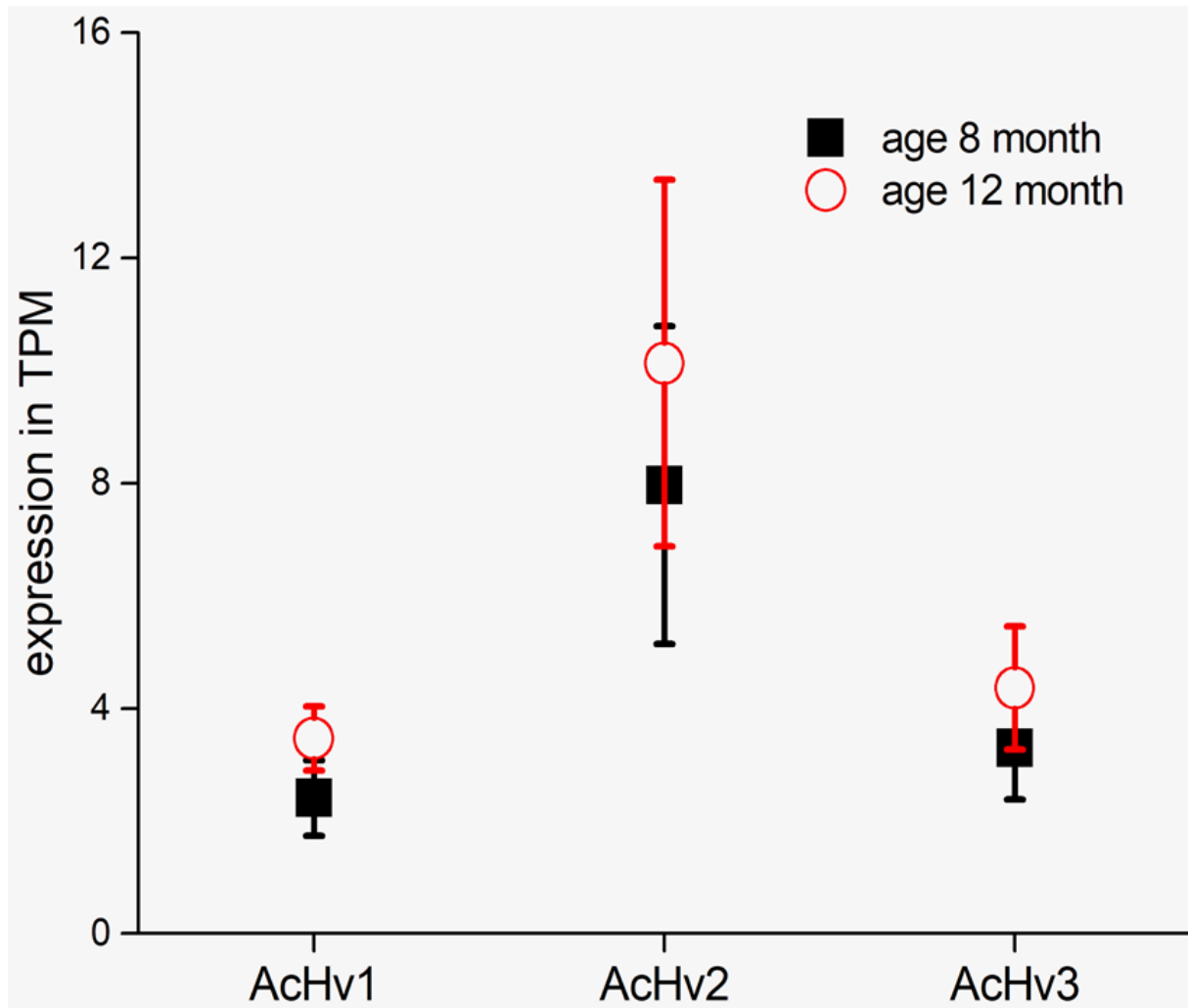
Expression of ACh_v1, ACh_v2, and ACh_v3 in comparison to GAPDH



Independent mRNA analysis of the expression of ACh_v1, ACh_v2, ACh_v3, and GAPDH from 3 animals. Data is in good agreement with the RT-PCR data from Fig. S5.

Fig. S7:

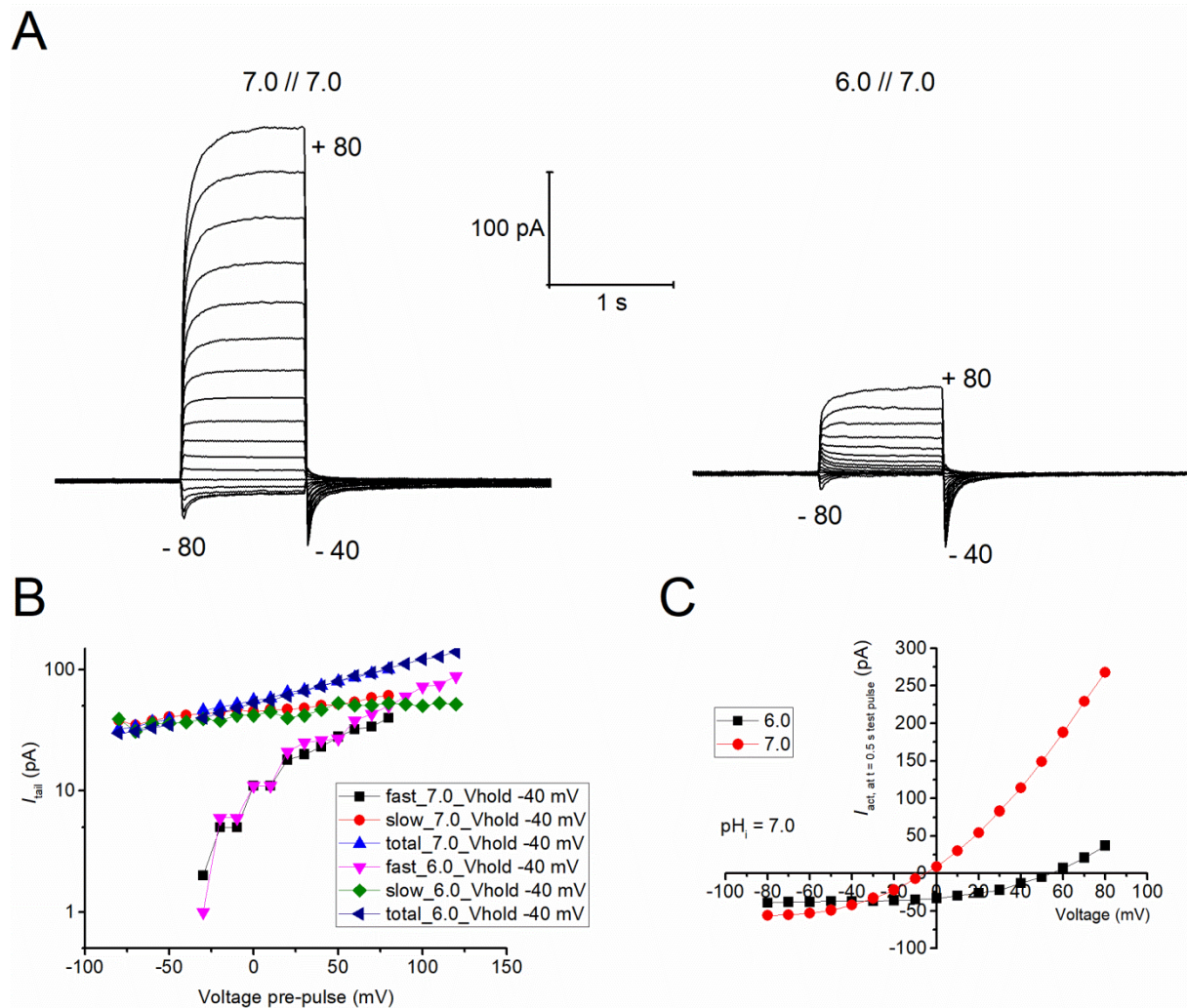
Expression of AcHv1, AcHv2 and AcHv3 dependent on animal age:



Expression in Transcripts Per Million (TPM) in pleural ganglion sensory neurons obtained from 6 sexually mature animals at age 8 months (black squares) and at age 12 months (red circles), data depicted in mean and S.E.M. AcHv2 appears to be upregulated by age ($p = 0.0618$); AcHv1 and AcHv3 are not obviously affected by aging (AcHv1 $p = 0.2555$, AcHv3 $p = 0.4710$ students t-test)

Fig. S8:

Tail current amplitude analysis of ACh_v3

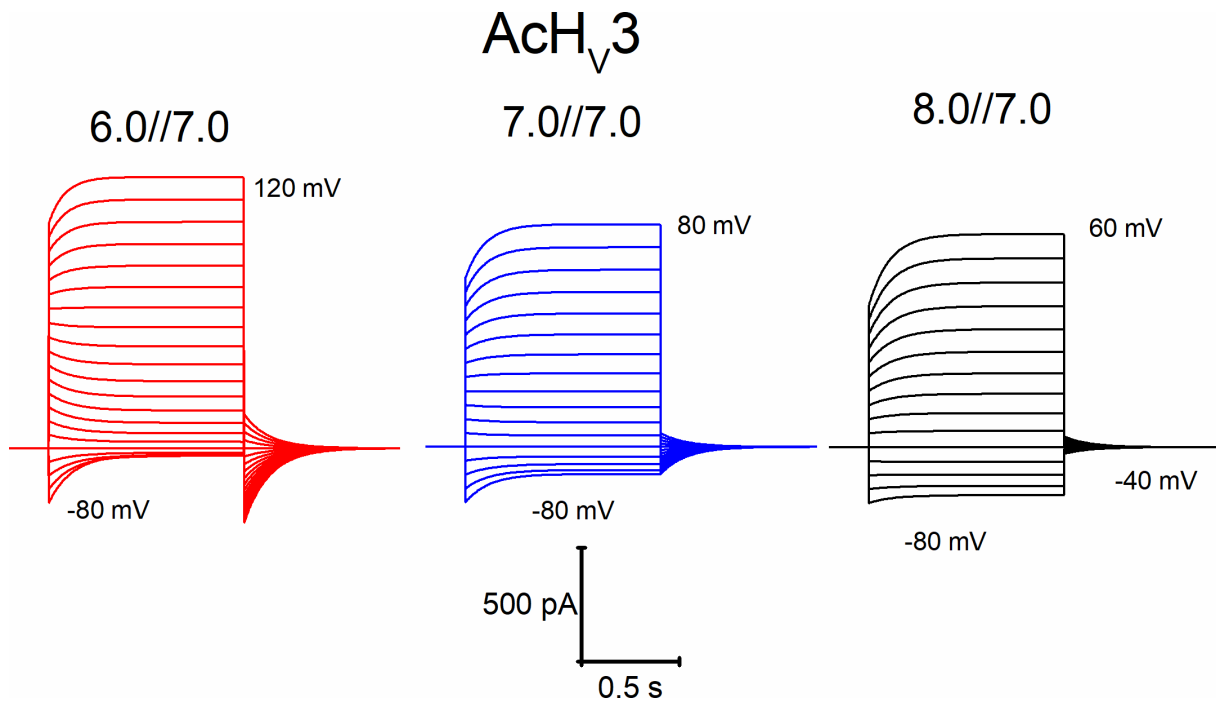


A) Two current families with pulses from -80 mV to + 80 mV in 10 mV increments and a holding potential of -40 mV at pH_o 7.0 or 6.0 and pH_i 7.0.

B) Tail current amplitude in pA at both pH_o 7.0 and 6.0. Tail currents showed a fast and a slow component. The slow component amplitude was calculated from the intersection of the extrapolated current to the starting time point of repolarisation to holding potential. The fast component was directly measured at the identical time point. Total current is the sum of slow and fast components. The currents are affected by pH_o. However, the reversal potential was -8 mV at pH_o 7.0 and +55 mV at pH_o 6.0. **C)** Current voltage plot, current 0.5 s after the pulse was plotted at each voltage. Reversal potential can be estimated from the individual curves.

Fig S9:

HH model of AChV_3 currents



Currents from the simple two states model. Note that there is no pH-dependence of gating or pH-dependence of conductance implemented. Overall the shape of the currents is presented in accordance with figure 4. Holding potential is -40 mV at all pH_o .

Fig. S10:

EST-coverage of predicted coding region:

AcHv1: 76.9 % (100 % exon-intron boundaries within coding sequence)

FC764689 3-219

AcHv2: 100% (100% exon-intron boundaries within coding sequence)

EB327294 1-186

EB299171 122-270

FC764689 168-304

EB210826 290-339

AcHv3: 62.0 % (100% exon-intron boundaries within coding sequence)

EB337456 1-64

EB295811 96-323

HS121401 338-520

EB313783 797-929

EST-coverage of the predicted coding region of the three *Aplysia californica* paralogs. Of all three channels the whole exon-intron boundaries are covered by the EST sequences. Therefore, all exons are included. Not EST covered parts of the genomic DNA was verified by mollusk genome sequences other than *Aplysia californica*. A detailed analysis mollusk H_v channels will be presented in a upcoming publication.

Fig. S11:

Aplysia californica voltage-gated hydrogen channel 1-like
(LOC101848758) **AcH_v1**

XM_005100609

TSA: GBBG01064906 GBDA01021710 GBCZ01104187

MKLDGLRKMQDDLVKVIERDDTSTVTSDSDETIARGPKTLRETLDVVIHS
QKFMVFIIVLVVLDCLMVIAELLFDLEIVKLGEHHYIPKIFHYGSLGII
SLFLIEIGLRIFVLRDLDFKHKLELFDVAVVIVSFILDIVFRDNE DAATG
VGLLIILRLRVTRIVNGIVLSVQKQAEKKIEREKHLREECEQELAKFRE
YCMAQAEAEIEVLQGLLHKHNIEFTTNKITRPE SRVQVDVVAEVNSMTAVA
ETDIPLSPSQPGEQEISLSSGDNVTDVAVVVV-

Aplysia californica uncharacterized LOC101850633_X1-X4

XM_005093050 **AcH_v2**

TSA: GBBE01033180

MRMSRSIEYPSEKNGEPSCIEAEQRSGETKMLKSEQSQDEAETSDWSENE
DSHSGKLDANSCKGKLA AFLKTNLVQYSIIALVILDCLIIVMELLI DMNI
IVFPEDDPHPPGEGSSHPVAFASRSSNLTGDNHTVYPAHHIHTHHNS
SNLTMYGND SAHAAPVHHHTNKEKAEHVLHALSLTILSIFMVEVCVKIYV
EGKHM LKQKAEVFD AIVVIVSFTLDITFSFVSVSKAASEAAGLMVILRLV
RVTRIIINGVIMSVKLDANKKMEVHKKARRKLERENKRLQAKIERLEREVA
TLKQKMATSSSTPQMSFEMQSGLSVERSPSGEMRENSAQV-

Aplysia californica uncharacterized LOC101855857

XM_005094218 **AcH_v3**

TSA: GBAV01052985

MGDAEPRAHPRPRGQASPFMPRLRKRGEKLLHSKYVWILVIILTVD CAL
VIAELI L DLSSVKKTQGATEAMLSFVEKIIKKYPDEVAPLHSLTDVFEE
LNHADIVWNNTRNSRGHDLDPDLERNLHHHHKNRDALHTSVTTPTSVPV
VGGESVAEGWPLSSDTP LGVGNFSRALRTLWIQKRNYSSNSRFRRSNRND
SLRDSSAVLSRLLERTRLEIEKVL SKLSRRRKRSSSEETGGLTAEYEATDS
GEAGDTSRNSQELEDEVLNSDFFENNYGKNGKTYLSSLTGLIVKILTMQ
SNETGPILGGPARYAEAPLTVESAGKGEKGAHVSDQDILHKYRLEFHH
SEDMEIAHKLHYASVAVVSILLIEVTMKIICAGSHFLKRKIEVFD AIVV
ASVIVDLIFIKGLNQFPVDDSI FVLAFLLPKRVIVVNSLVM AVIDHEHV
KLRLLYSRKKKLDKTVETLRNEVDELKGMQDIRQFCIKEGIEASRIDSL
LGKFAPRRR KDSKFYTLV KLV M STASINNNNDNDNSVSSSSMENDLRDYA
NRDSVLNEATSNENTVTLKQYLSVPFFSGGNRSNTLDIESRGSGRSGGS
PSIYITSPASDDEAPVFSFDIAEDDDVMSNDQDDAGSQDDETSIQAGSD
AATI AVTSAE VNTVSPNVAFYVGSQSSLC SVHSQEDIRTWDTNDNYERN
FPMCGVPCASGGEDLAAAALDDVISNSPTVNSNSWGSPSRHYSRFLTVPCC
TLLSAVTNTTSTATSY P SSSNNTNNNSNSNNNNNACAETHPLLGDPPP
GQNMTRSVSDNCDVTATQYGARTCGGRYGSFVPMRRKPC LSEKRRSYARA
RSESIENQEFIP LMSQ GANKGRVRHHS DLEGRPSTRKDDLKRSRSHSPSP
MVLLGVP GQTKKYS D P P P S Y Q A A S R S M N D V S S A G K G S G N P H A N N L G K D G
RILRRSCLSLTSEGRKRRGKSPQRVSFKVS-

Amino acid sequence Maximum Likelihood method (implemented in Bio-Edit), version 3.6a2.1

Jones-Taylor-Thornton model of amino acid change

Fig. S12:

List of *Aplysia californica* H_v channels. GenBank and TSA accession numbers are shown. Transmembrane regions (yellow), selectivity filter (magenta), and voltage-sensor motif (R blue, W red) are indicated.

Identity/Homology within the core region (S1-S4) (in %)

			AcHv1	AcHv2	AcHv3
<i>Extatosoma tiaratum</i>	Insecta	GAWG01024136	39/59	35/60	28/51
<i>Nicoletia phytophila</i>	Insecta	KT780722	46/70	41/61	38/57
<i>Homo sapiens</i>	Mammalia	NM_001040107	45/66	40/60	33/56
<i>Mus musculus</i>	Mammalia	NM_001042489	43/59	42/59	33/52
<i>Karlodinium veneficum</i>	Alveolata	JN255155	27/41	27/42	26/46
<i>Lingulodinium polyedra</i>	Alveolata	KU752798	35/56	27/45	24/44
<i>Emiliana huxley</i>	Haptista	XP_005762299	28/42	28/47	24/38

Preliminary cladogram of ten different H_v channels

