

Arrhythmogenic effects of mutated L-type Ca²⁺-channels on an optogenetically paced muscular pump in *Caenorhabditis elegans*

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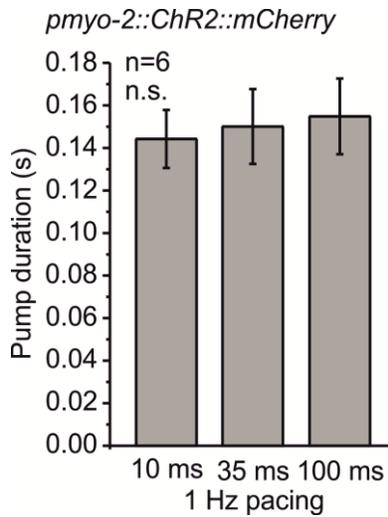
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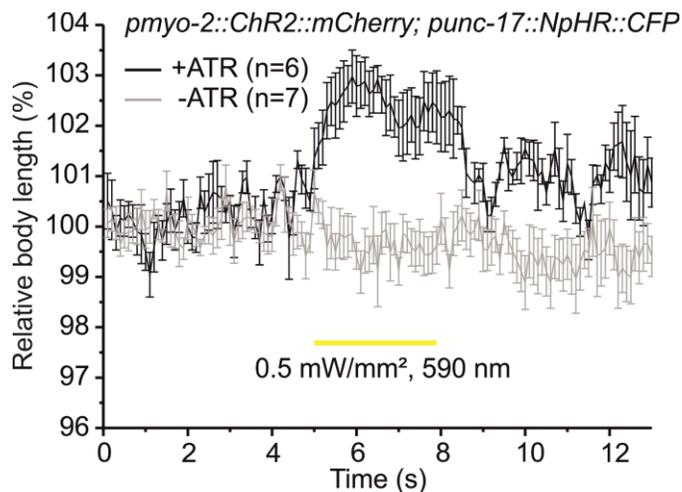
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Supplementary Information



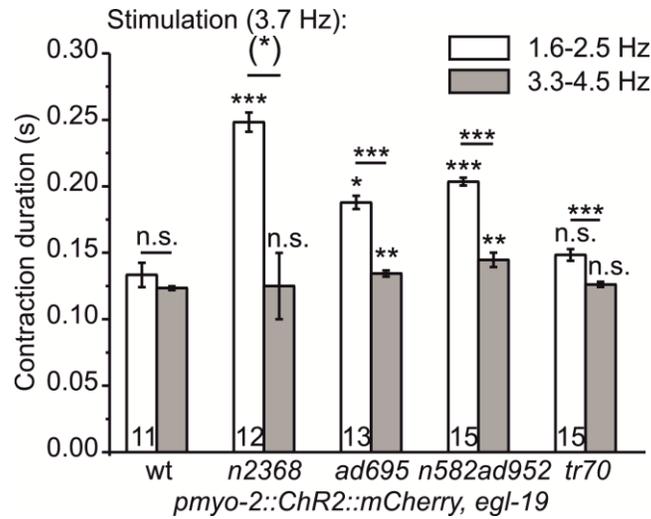
Supplementary Fig. S1: Pump duration is not altered by length of stimulation light pulse (10 - 100 ms)

Pacing with 10, 35 and 100 ms (470 nm) has no effect on pump duration as deduced from EPGs.



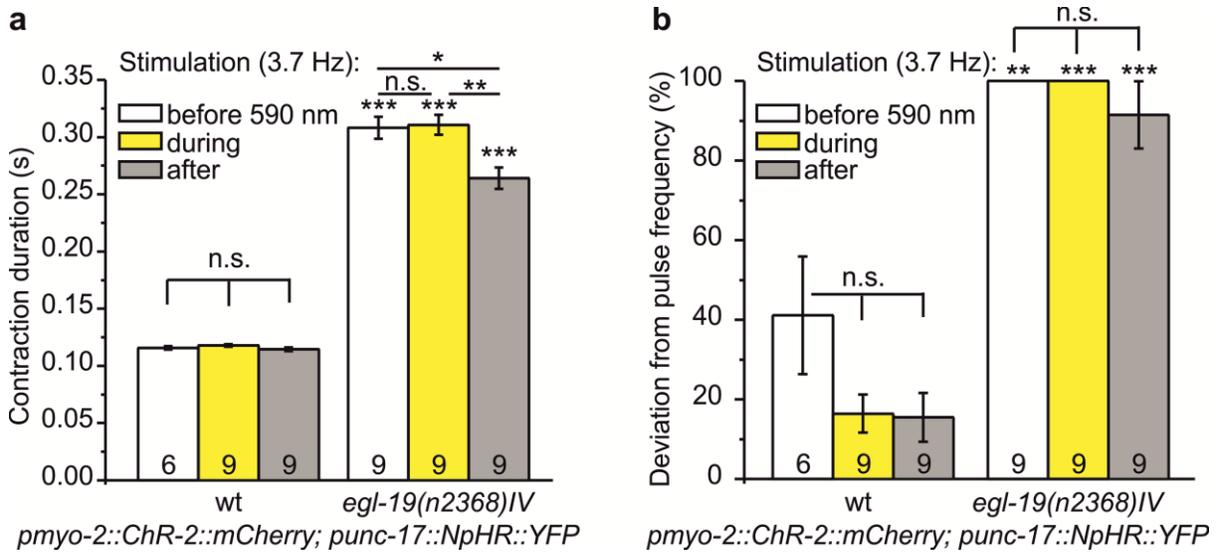
Supplementary Fig. S2: Cholinergic neuron hyperpolarization induces body relaxation

Animals expressing ChR2 in PMCs and NpHR (halorhodopsin) in cholinergic neurons were illuminated with 570-610 nm light (0.5 mW/mm²) for 3 s. Analysis of averaged (\pm S.E.M.), normalized body length deduced from videos (10 fps) verifies that cholinergic neuron hyperpolarization is effective and induces muscle relaxation (black trace, n=6 animals treated with ATR). No effects are observed in animals grown the absence of ATR (grey trace, n=7 animals). Illumination period is indicated by a yellow bar.



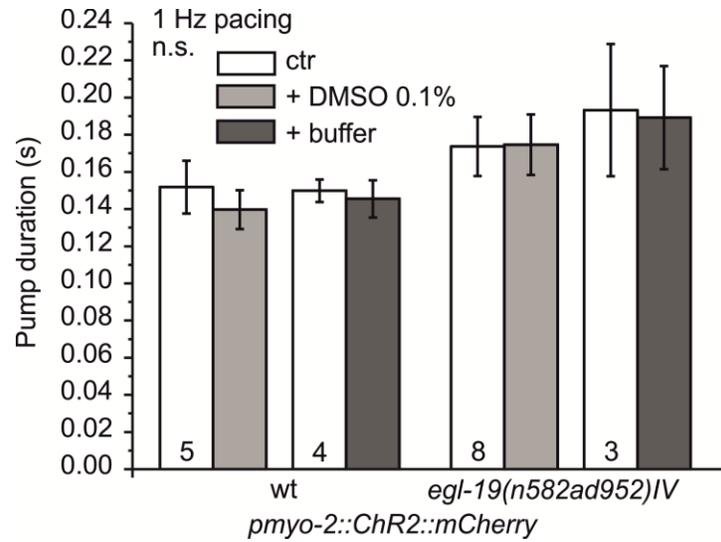
Supplementary Fig. S3: Detailed analysis of contraction duration obtained by kymographic analysis

Detailed analysis shows strongly increased contraction durations only for the 2 Hz pumping periods of *egl-19* mutants compared to wild type (wt). Statistically significant differences: t-test with Bonferroni correction (***P<0.001; **P< 0.01).



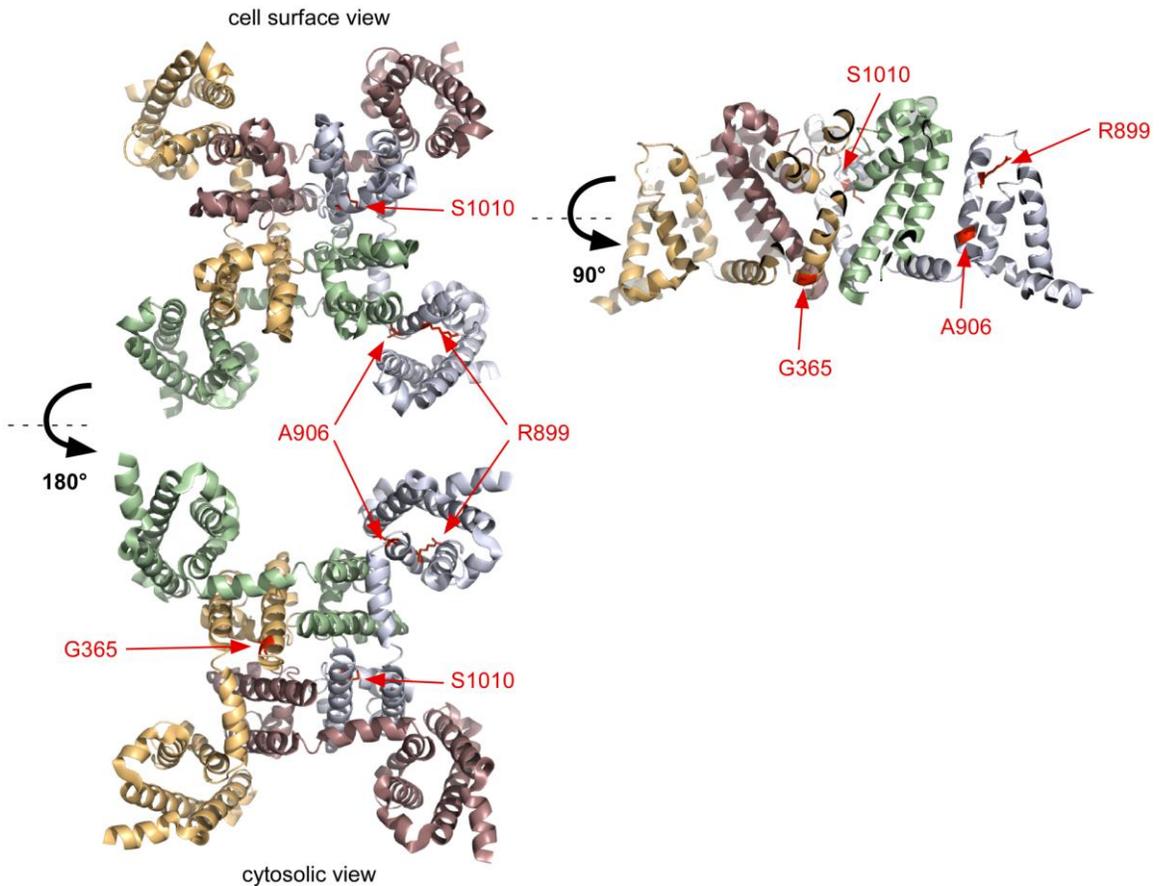
Supplementary Fig. S4: No neuronal effect on contraction duration and deviation from 3.7 Hz pumping of *egl-19* mutants

A) Contraction duration as measured by kymographs upon ongoing ChR2 PMC photostimulation at 3.7 Hz, combined with photoinhibition of cholinergic neurons by NpHR (yellow), in *egl-19(n2368)* mutants, compared to wild type (wt). **B)** Fraction of time the animals in A) showed deviation from the pace frequency before or after cholinergic neuron photoinhibition. Statistically significant differences: t-test with Bonferroni correction (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$).



Supplementary Fig. S5: No effect of 0.1% DMSO on pump duration

Pump duration was analyzed by EPGs of 1 Hz optically paced pharynxes, of allele *n582ad952* and compared to wild type (wt), in control conditions, or 2 min after incubation with 0.1% DMSO, or after application of buffer (EmD50).



Supplementary Fig. S6: Structure model of NavAb as EGL-19 surrogate with positions of the analyzed *egl-19* mutations indicated

Structural model of NavAb (PDB file 3RVZ), as a surrogate for the EGL-19 structure, with relevant, mutated amino acids superimposed (red) over positions of NavAb that align with the EGL-19 sequence (see **Supplementary Fig. S7**).

1st module

```
EGL-19          50 -aqrkplrqtnvversersllclslnnpirkklcisivewkpfeflilfmicanciaiayqppagdsdy
Cav1.2 human   100 -----pprallcltlnknpirracisivewkpfefiilltlfancivalaiyipfeddsna
navAB (3RVZ)    1 mdykdddkgslvprgshmylrit-----nivessfftkfiilylivngitmtgletsktfmgsgf

EGL-19          119 kntlletieyfvivvftiecvlkivamgfmfhpsaylrnawnildfiivvlgvstilskmsiq-----
Cav1.2 human   155 tnsnlerveyflfiiftveaflkviaygllfhpnaylrngwnlldfiivvvgflfsailegatkadganal
navAB (3RVZ)    61 vyttl--fnqivitiftieilriyv----hrisffkdpwslfdffvvaislvpt-----ss-----

EGL-19          183 ----gfdvkalrafrvrlrplrlvsgvpslqvlnailramipllhiallvfvlilyaiiglelfcgkl
Cav1.2 human   225 ggkgagfdvkalrafrvrlrplrlvsgvpslqvlnsilikamvpllhiallvfvlilyaiiglelfcgkm
navAB (3RVZ)    112 ----gfei--lrvlrvlrlfrlvtavpqrkivsalisvipgmlsvialmtlffiyifaimatqlf----

EGL-19          248 hstcidpa--tqqlaakdptpcgtdtegsafkcpqpsdsltngvrwecssnttppgnngitnfdnfgla
Cav1.2 human   295 hktcynqegiadvpaeddpascalet-ghgrqcq-ngtvckpg-----wdgpkhgitnfdnfafa
navAB (3RVZ)    171 -----gerfp-----ew-----fgtlges

EGL-19          316 mltvfqcvsllegwtdvmyvndavg-rewpwiyfvttlvilgsffvlnlvlgvlsgefskerekararglf
Cav1.2 human   353 mltvfqcitmegwtdvlyvndavg-rdwpwiyfvttliligffvlnlvlgvlsgefskerekakargdf
navAB (3RVZ)    185 fytlfqvmtlesws--mgivrplmevypawvfipfifvvtfvminlvvaicvdamaillnqkeeqhiid
                                                    G365 S372

EGL-19          385 q-----kfre----kqleedlkgv-----
Cav1.2 human   422 q-----klre----kqleedlkgvldwitqaedidpe
navAB (3RVZ)    253 evqshedninneiiklreeivelkeliktslkn-----
```

2nd module

```
EGL-19          440 -vedvrpskwaarmk-rlekl-----nrrcrracrllvksqtfywlvillvllntlvl---tsehy
Cav1.2 human   480 aggdiegecgarlahriskskfsrywrwnrfrckrcraavksnvfywlviflvflntlti---asehy
navAB (3RVZ)    1 -mdykdddkgslvprgsh-----ylr----itnivessfftkfiilylivngitmtgletsktf

EGL-19          496 gqsewldhfgtmanlffvilfsmemllkmyslgfttyttsqfnrfdcfvissilefvlvfydlmkplgv
Cav1.2 human   547 ngpnwltvqdtankallalftaemllkmyslglqayfvslnrfdcfvvcggitetilvetkimsplgi
navAB (3RVZ)    56 mqs--fgvyttlfnqivitiftieilriy-vhrisffkdpwslfdffvvaislvp-----tssgf

EGL-19          566 svlrsarllrifkvtkywtslrnlvssllnsrslslllllffivifallgmqvfvggkfnfnpgqpkp
Cav1.2 human   617 svlrcvrlrlrifkitywnslslvasllnsrslslllllfflfiifvllgmqlfggkfnfdemqtr-
navAB (3RVZ)    114 eilrvlrvlrlfrlvtavpqrkivsalisvipgmlsvialmtlffiyifaimatqlfgerf---pew---

EGL-19          636 ranfdtfgvalltvtfqltgedwntvmyhgiesfgvgvtlgvi---vciiyivlfcignyillnvflaia
Cav1.2 human   547 rstfdnfpqslttvfiqiltgedwnsvmydgmaygppsfpglm---vciiyifilfcignyillnvflaia
navAB (3RVZ)    178 ---fgtlgesfytlfqvmtlesw-----smgivrplmevypawvfipfifvvtfvminlvvaic

EGL-19          703 vdnladadsltnaekeeeqgeiegedeefee-gedegeehgmdep---egdeemtsarprrm
Cav1.2 human   753 vdnladaesltsaqkeeee---ekerklartaspekkelvekpavgeskeekielksit-
navAB (3RVZ)    236 vdama---iln---qkeeqhidedvqshed-ninneiklreei---velkeliktslkn-
```

3rd module

```
EGL-19          750 --eemtsarprrmsevpaaastvkpikasslflshtnsfrvfcmvvnhsyftnavlfcilvssamlaa
Cav1.2 human   850 epempvgrprplselhlkekavmppeasaffifssnrfrlqchriivndtiftnlilfillssislaa
navAB (3RVZ)    1 -----mdykdddkgslvprgshmy-lritn-----ivessfftkfiilylivngitmtgl

EGL-19          818 edplqanstrnmilnyfd-----yfftsvftveitlkvivfglvfhkgsfcrnaf
Cav1.2 human   920 edpvqhtsfrnhilfyfdvfttftieialkilgnadyvftsiftleilkmtaygafllhkgsfcrnyf
navAB (3RVZ)    50 etsktfmqsfgyvttlfn-----qivitiftieilriyv----hrisffkdpw

EGL-19          868 nlldilvvavsltsfvlrtdamsvvkrlrvlrlrplraalnraakglkhvvcvivaiktignimlvtfml
Cav1.2 human   990 nlldllvsvslisfgiqsainvvkrlrvlrlrplraalnraakglkhvvcvivaiktignivivtll
navAB (3RVZ)    95 slfdffvvaisl-----vptssgfeilrvlrlrplraalnraakglkhvvcvivaiktignimlvtfml
                                                    R899 A906

EGL-19          938 qfmfaiigvqlfkgtfflcnldskmteaeergeyihyedgdpkpvskkrvsnndfnfdvngdamislf
Cav1.2 human   1060 qfmfacigvqlfkgtlytcsdsskqtaeackgnitytkdgedvndhpiqprswenskfddnvlaaammalf
navAB (3RVZ)    159 fyifaimatqlfgerf-----pew-----fgtlgesfytlf

EGL-19          1008 vvsstfegwqillyvaidneedkgpihnsrqavalffiafiiviaffmnnifvgfvivtfqnegereyen
Cav1.2 human   1130 tvstfegwqillyvaidneedkgpihnsrqavalffiafiiviaffmnnifvgfvivtfqnegereyen
navAB (3RVZ)    190 qvmleswsmgvl-----rplmevypawvfipfifvvtfvminlvvaicvdamaillnqkeeqh
S1010

EGL-19          1078 celdknqrkciefalkakphrryprnrlqyrvwvftsrafeyvifliivmntvslackhypssrgfed
Cav1.2 human   1200 celdknqrqcevayalkarprlrryprknqhgqykvvynstyfeylmfvlil-----
navAB (3RVZ)    250 i-idevq-----shedninneiiklre-----eeivelkelikt---slkn-----
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Supplementary Fig. S7: Sequence alignments of NavAb with each of the first 3 modules of EGL-19

Supplementary Video 1: Pharynx in electropharyngeogram setup; first spontaneous pumps, then optically paced at 1, 2 and 4 Hz