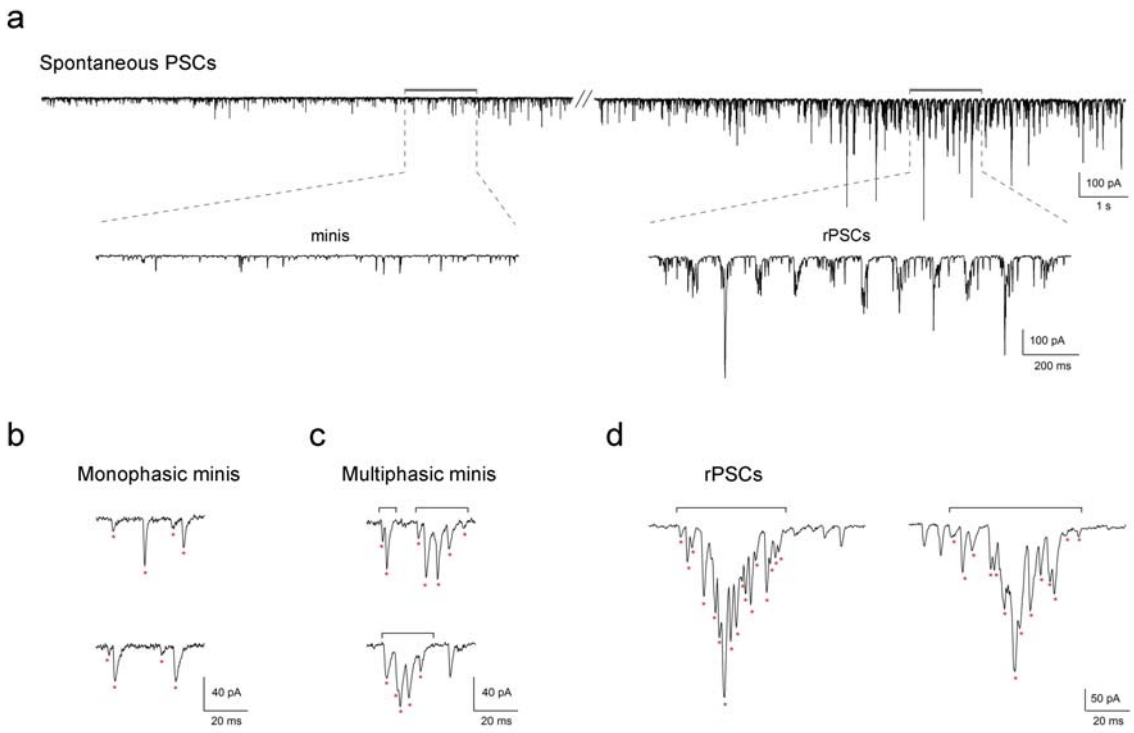
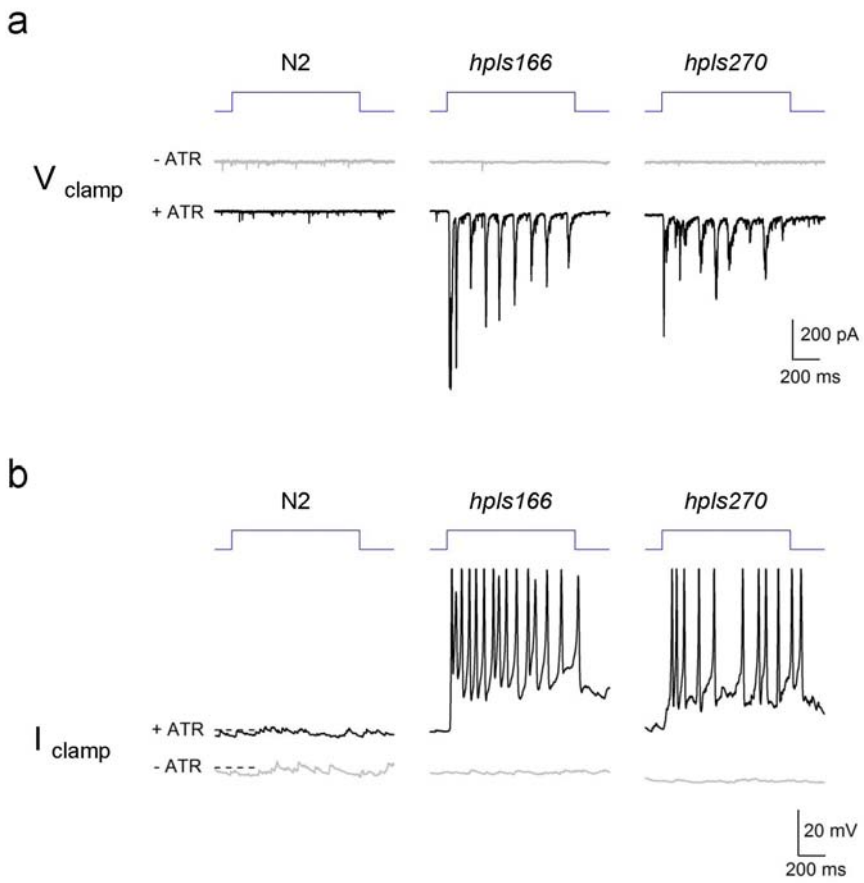
Supplementary Figure 1



Supplementary Figure 1. Difference between miniature PSCs versus rPSCs events.

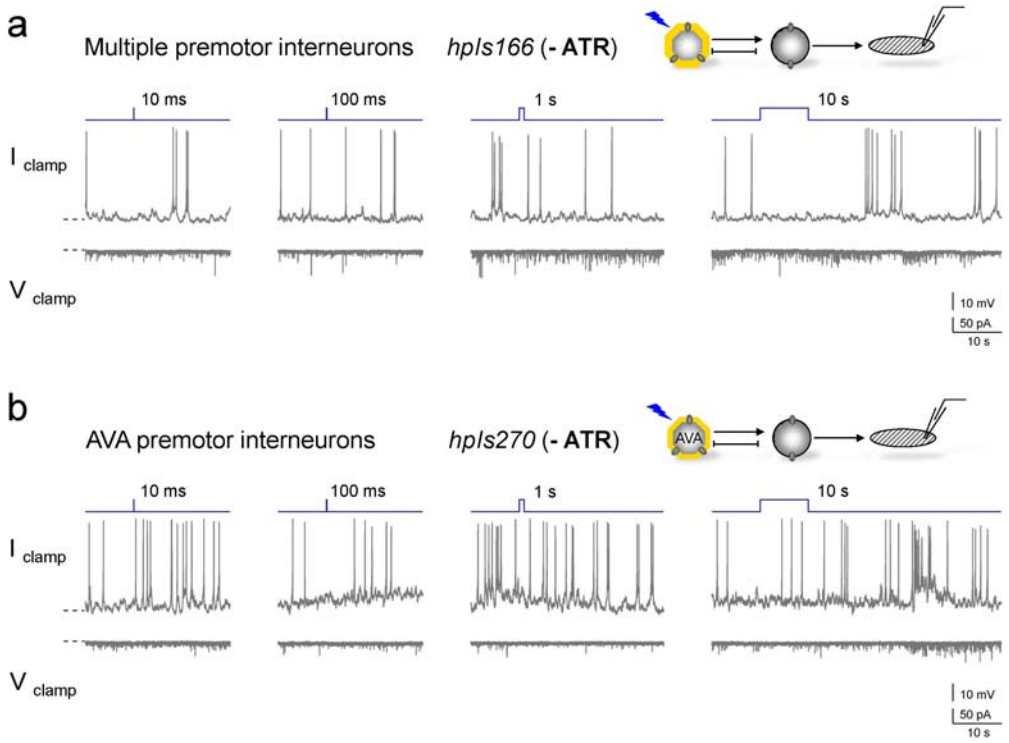
(**a**) Example traces of spontaneous miniature PSCs (minis, left), and rhythmic bursting PSCs (rPSCs, right) in wild-type animals. Body wall muscles were held at -60 mV. The enlarged view of each trace is shown below. (**b,c**) Sample traces of monophasic and multiphasic miniature PSCs events. (**d**) Sample traces of single rPSCs events.

Supplementary Figure 2



Supplementary Figure 2. rPSCs correlate with action potentials at body wall muscles. (**a**) Representative rPSCs traces, from animals of various genetic backgrounds (N2, *hpIs166* and *hpIs270*), cultured with or without *all-trans* retinal (+ATR, -ATR, respectively), upon 1 second blue light stimulation. Body wall muscles were held at -60 mV. (**b**) Representative action potential traces recorded from these animals. Body wall muscles were held at 0 pA. Dash lines denote -25 mV.

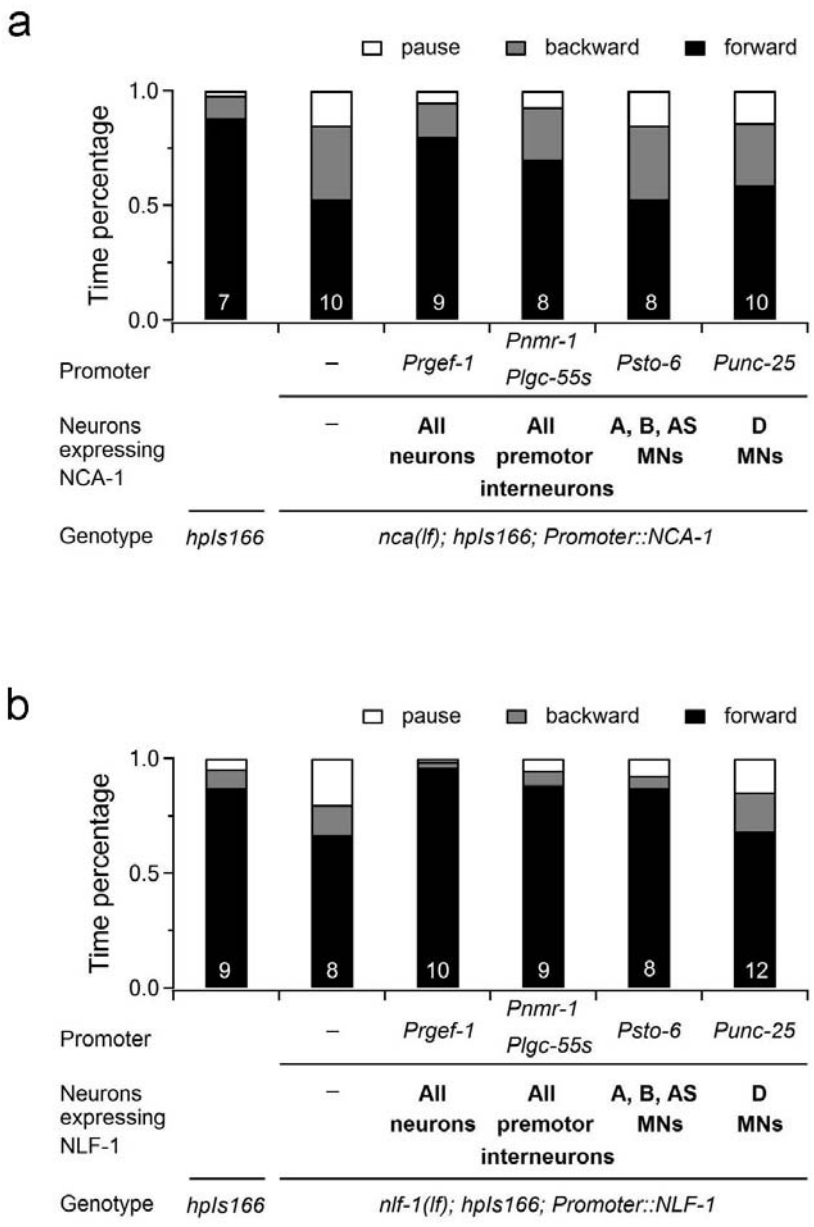
Supplementary Figure 3



Supplementary Figure 3. No persistent activity was observed in the absence of ATR.

(**a,b**) Recording of the same transgenic strains (**a** *hpIs166*, **b** *hpIs270*) and by the same stimulation protocols, as in Figure 1**c**, cultured in the absence of *all-trans* retinal (-ATR). V_{clamp} : 10 ms (n = 7), 100 ms (n = 7), 1 s (n = 10), 10 s (n = 7); I_{clamp} : 10 ms (n = 64), 100 ms (n = 6), 1 s (n = 6), 10 s (n = 5) (**a**, quantified in Figure 1**d**); V_{clamp} : 10 ms (n = 6), 100 ms (n = 6), 1 s (n = 8), 10 s (n = 9); I_{clamp} : 10 ms (n = 7), 100 ms (n = 7), 1 s (n = 7), 10 s (n = 7) (**b**, quantified in Figure 1**f**).

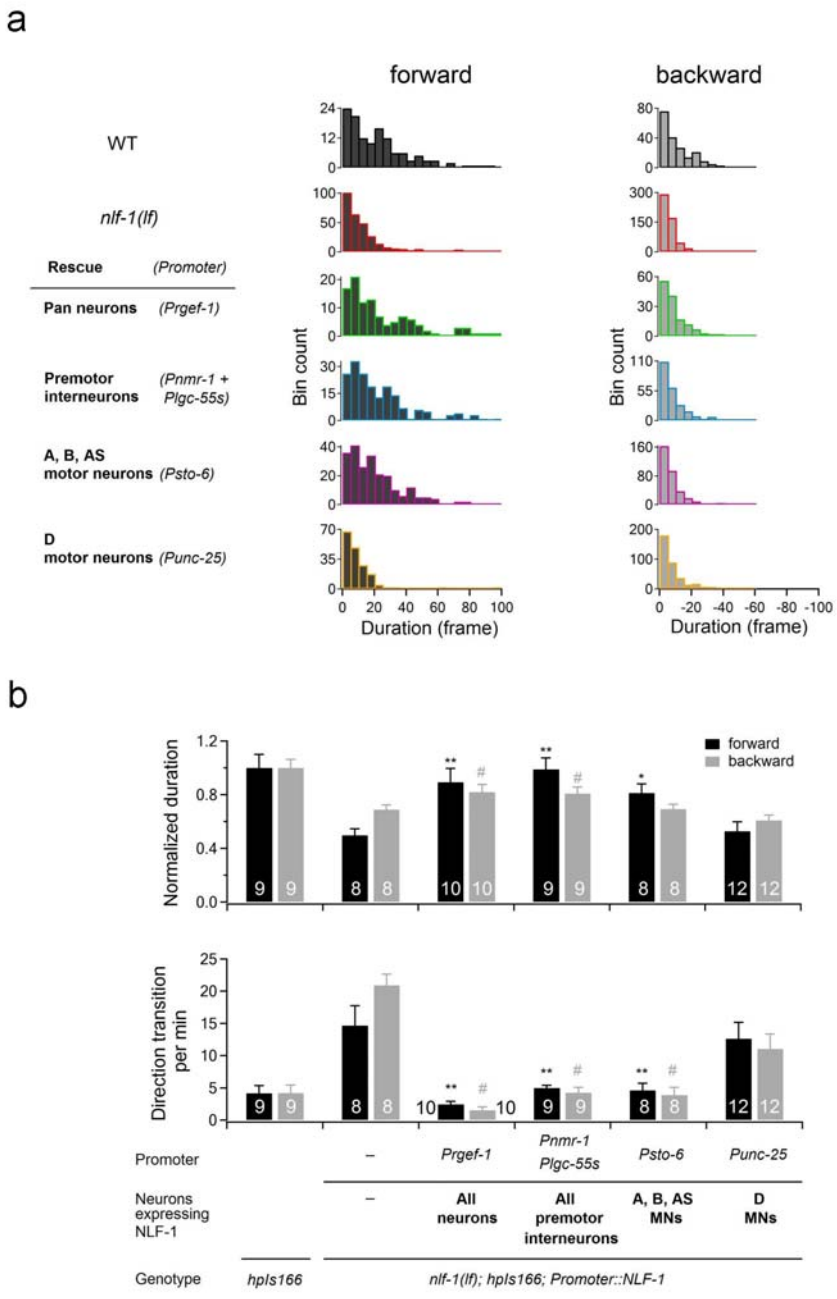
Supplementary Figure 4



Supplementary Figure 4. A key requirement of NCA in premotor interneurons.

(**a**) Under the same assay conditions (Methods), the spontaneous motor activity was reduced in *nca(lf)* mutants, represented by the increased percentage of time animals spent in the pausing (white bars, centroid moved between -1 and 1 pixel/second) mode. The reduced motor activity was rescued by restoring the NCA channel expression in premotor interneurons, but not in motor neurons. (**b**) Similar defects were observed in *nlf-1* mutant animals. Restored expression of NLF-1 in either premotor interneurons or excitatory motor neurons could rescue the overall activity level. All strains were analyzed in the *hpIs166* transgenic background.

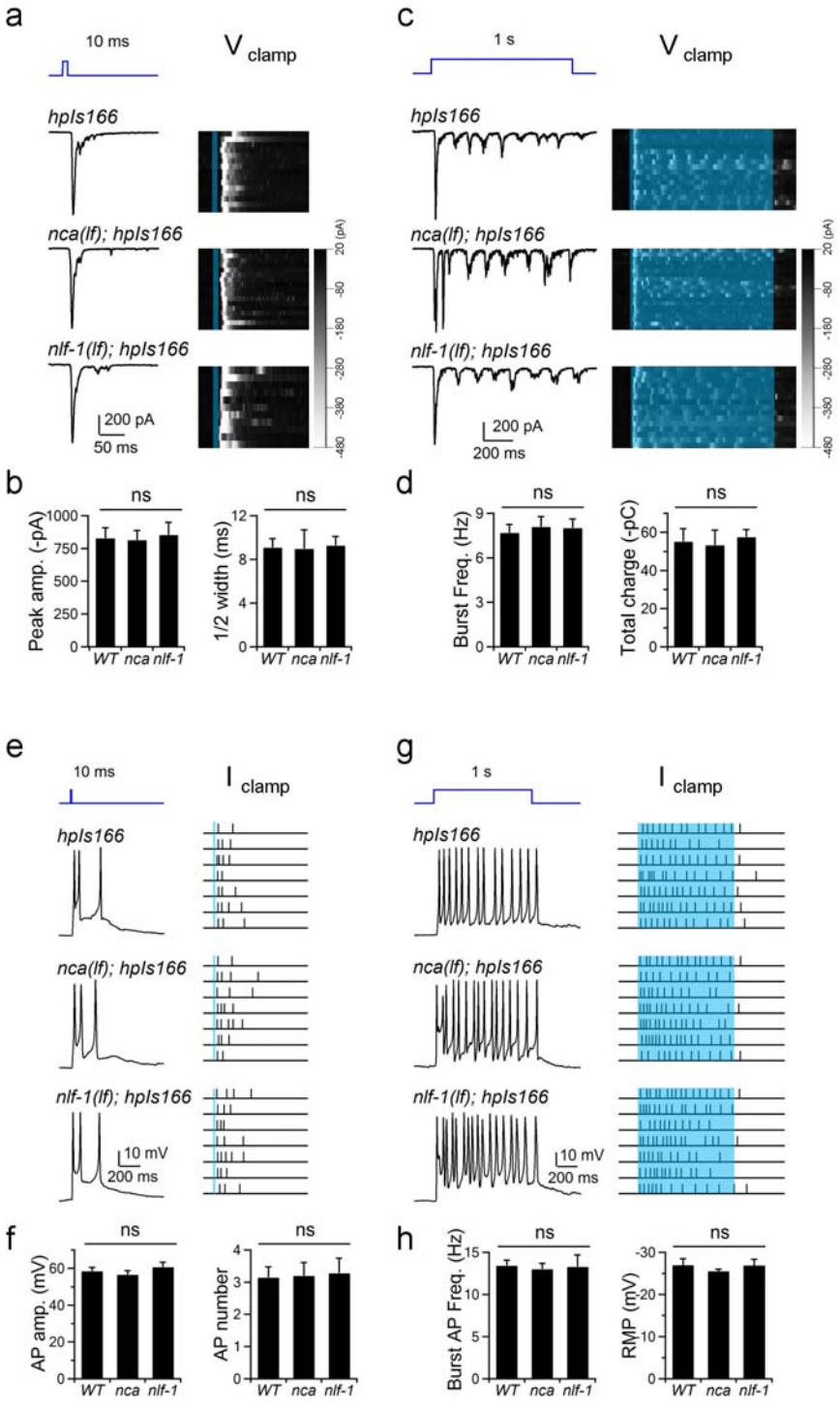
Supplementary Figure 5



Supplementary Figure 5. NLF-1 is required for sustained locomotion.

(**a**) Histogram of the duration of sustained directional movement (forward and backward) in animals of different genetic backgrounds. The duration of sustained forward and backward movements was significantly reduced in *nlf-1(lf)* mutant animals. This defect was rescued by restoring expression of NLF-1 in premotor interneurons (*Pnmr-1 + Plgc-55s*). (**b**) Quantification of the average duration and frequency of directional transition. NLF-1 expression in premotor interneurons was necessary for increasing the duration (both forward and backward) for *nlf-1(lf)* mutants. Restoring expression of NLF-1 in cholinergic motor neurons (*Psto-6*) can partially rescues the forward duration, but not backward duration, whereas expression of NLF-1 in GABAergic motor neurons (*Punc-25*) did not rescue the duration of forward or backward movement. All strains were analyzed in the *hpIs166* transgenic background. * $P < 0.05$, ** $P < 0.01$, # $P < 0.05$, against *nlf-1(lf)* mutants by the Mann-Whitney U test. Error bars, SEM.

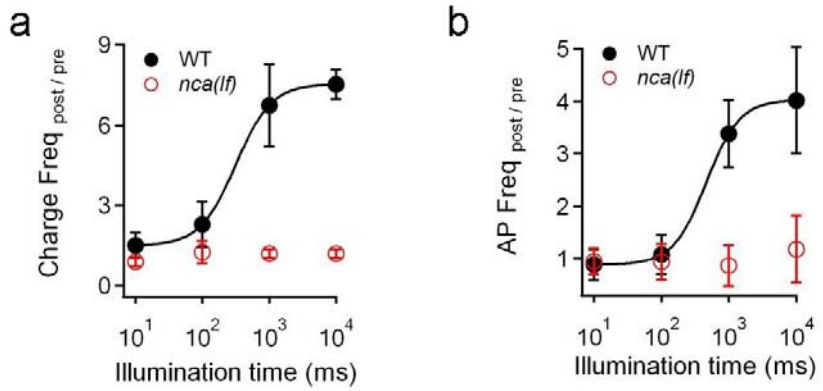
Supplementary Figure 6



Supplementary Figure 6. Similar evoked activity in wild-type, *nca(lf)* and *nlf-1(lf)* animals.

(**a**) Left panel, representative traces of rPSCs evoked during 10ms-blue light stimulation in wild-type (WT), *nca(lf)* and *nlf-1(lf)* animals, respectively. Right panel, raster plots of rPSCs evoked by 10ms-blue light stimulation from each sample. (**b**) Quantifications of the peak amplitude and half-width of rPSCs. There are no significant difference between WT animals and *nca(lf)* and *nlf-1(lf)* mutants. WT (n = 15), *nca(lf)* (n = 17), *nlf-1(lf)* (n = 11). (**c**) Left panel, representative traces of rPSCs evoked during 1s-blue light stimulation in wild-type, *nca(lf)* and *nlf-1(lf)* animals, respectively. 1s-blue light stimulation induced rPSCs in all three genotypes. Right panel, raster plot of individual recordings. (**d**) Quantifications of the frequency and total discharge of rPSCs. There are no significant difference between wild-type animals, *nca(lf)* and *nlf-1(lf)* mutants. WT (n = 16), *nca(lf)* (n = 19), *nlf-1(lf)* (n = 15). (**e-h**) Example traces and quantification of APs evoked by 10ms- and 1s- blue light stimulation, in I_{clamp} configuration. There was no significant difference among these genotypes. WT (n = 12), *nca(lf)* (n = 7), *nlf-1(lf)* (n = 9) (**f**). WT (n = 18), *nca(lf)* (n = 9), *nlf-1(lf)* (n = 7) (**h**). All recordings were performed in the *hpIs166* background (wild-type animals in this set of experiments). Muscles were held at -60 mV in V_{clamp} and 0 pA in I_{clamp}, respectively. ns, no significant difference against wild-type animals by the Mann-Whitney U test. Error bars, SEM.

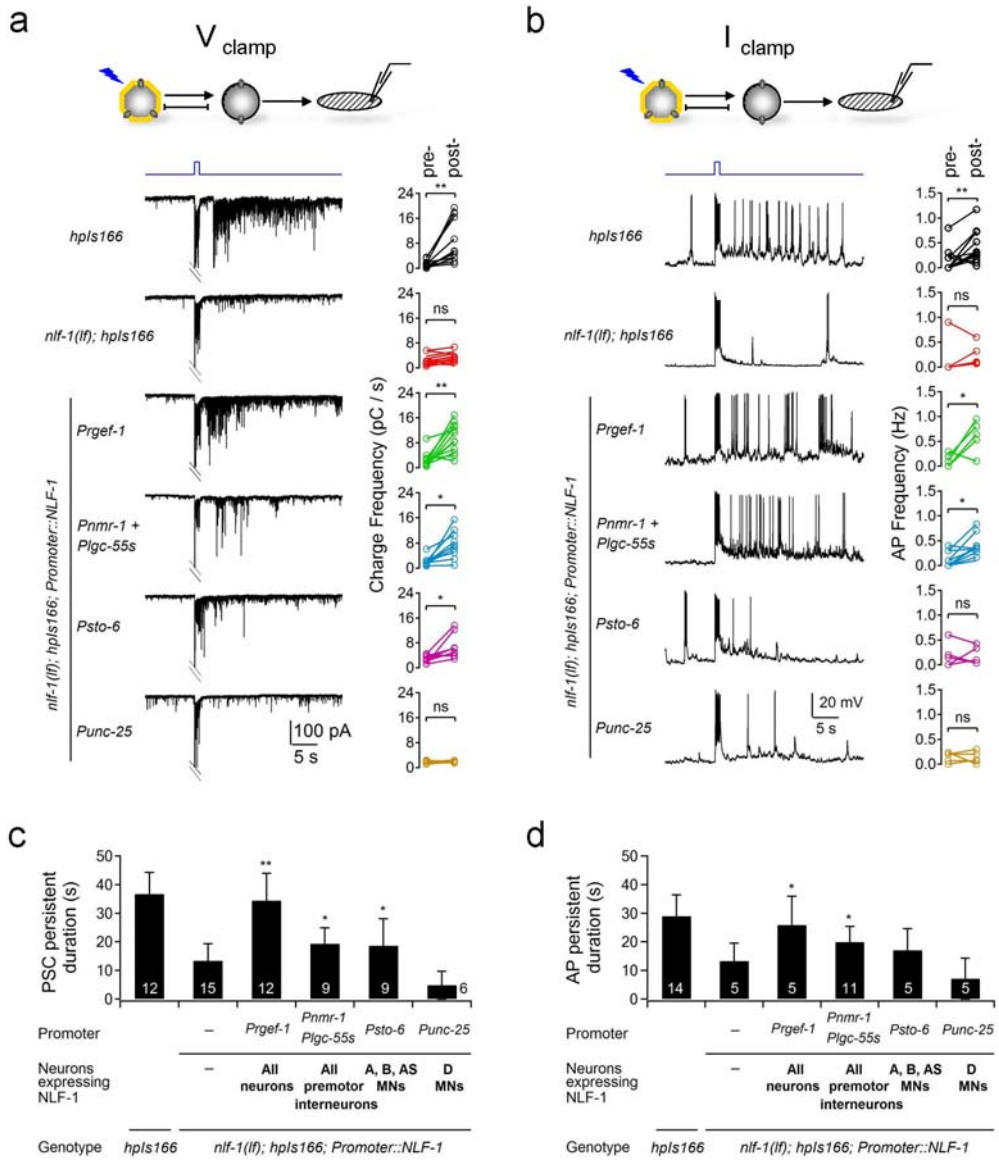
Supplementary Figure 7



Supplementary Figure 7. *nca(lf)* mutants exhibit no persistent neural activity.

nca(lf) mutants did not exhibit increased charge frequency (rPSCs; **a**) or firing frequency (action potentials; **b**), with illumination times from 10ms to 10s. V_{clamp} : 10 ms (WT, n = 10; *nca(lf)*, n = 13), 100 ms (WT, n = 7; *nca(lf)*, n = 11), 1 s (WT, n = 12; *nca(lf)*, n = 16), 10 s (WT, n = 10; *nca(lf)*, n = 21) (**a**); I_{clamp} : 10 ms (WT, n = 14; *nca(lf)*, n = 7), 100 ms (WT, n = 9; *nca(lf)*, n = 7), 1 s (WT, n = 14; *nca(lf)*, n = 7), 10 s (WT, n = 7; *nca(lf)*, n = 4) (**b**). Error bars, SEM. Data were collected from more than four biological and technical replicates (independent culture preparations). Error bars, SEM.

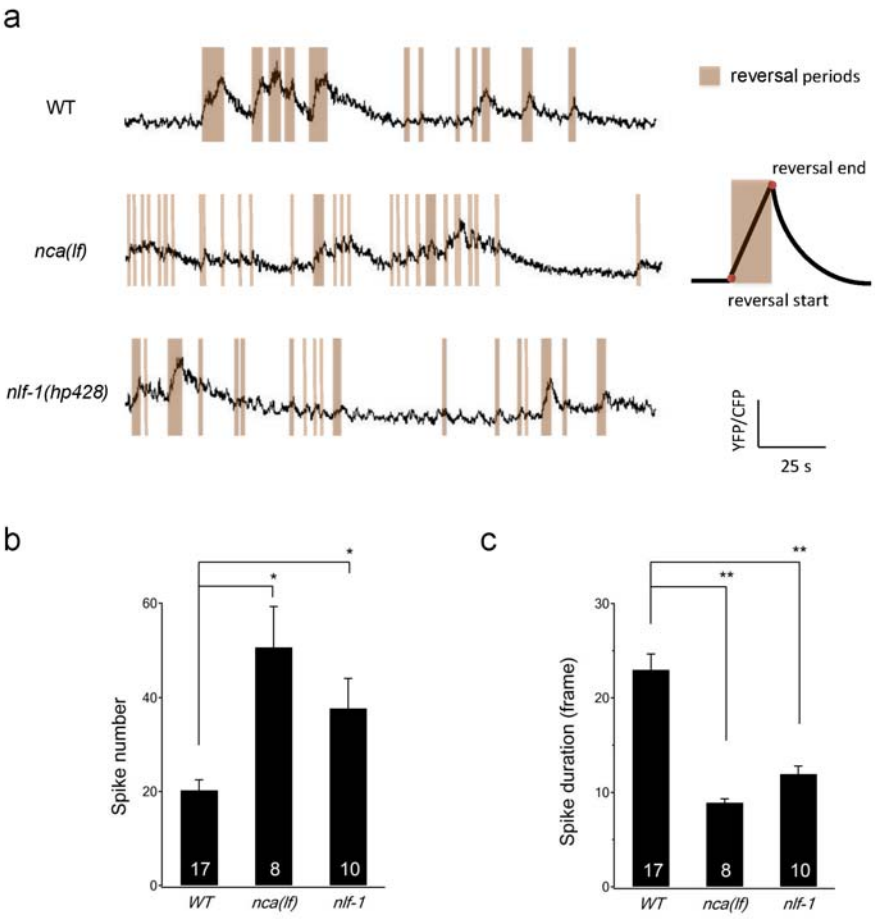
Supplementary Figure 8



Supplementary Figure 8. NLF-1 is required for persistent activity.

(**a**) Left panels, representative traces of persistent rPSCs after 1s-blue light stimulation. Right panels, rPSCs charge frequency before and after the stimulation. Connected lines represent the recording from same preparation. (**b**) Quantification of the duration of persistent rPSCs. Restoring expression of NLF-1 in premotor interneurons provided the best restoration of persistent neural activity in *nlf-1* mutants. Restoring expression of NLF-1 in all cholinergic motor neurons (*Psto-6*) partially rescued the deficit, whereas restored NLF-1 expression in GABAergic motor neurons (*Punc-25*) did not rescue the defects. (**c,d**) Representative traces and quantification of AP frequency before and after the stimulation. The schematics of the recording configuration (V_{clamp} (**a**) and I_{clamp} (**c**)) were shown. All recordings were performed in *hpIs166* background (wild-type animals in this set of experiments). Muscles were held at -60 mV in V_{clamp} and at 0 pA in I_{clamp}, respectively. * $P < 0.05$, ** $P < 0.01$, ns, no significant difference against *nlf-1(lf)* mutants by the Mann-Whitney U test. Error bars, SEM.

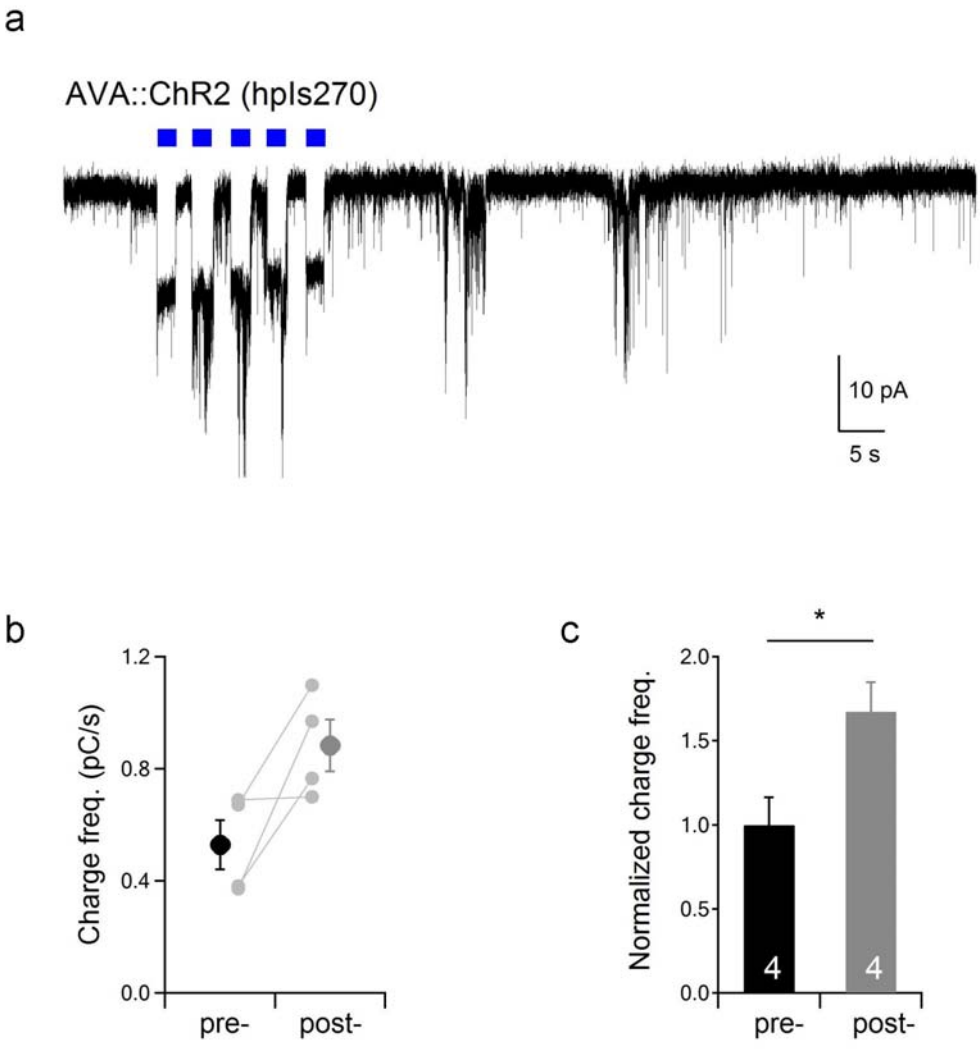
Supplementary Figure 9



Supplementary Figure 9. Fainter mutants exhibit reduced Ca^{2+} transient duration.

(**a**) Ca^{2+} transients in coimaged AVA/AVE indicated by the YFP/CFP ratio of a cameleon reporter in wild type and fainter animals. Brown bars indicate backing locomotion. (**b**) Quantification of the number of Ca^{2+} spikes. Fainter mutants exhibit high frequency initiation that correlates with increased number of Ca^{2+} spikes. (**c**) Quantification of the duration of Ca^{2+} increase. Fainter mutants exhibit reduced Ca^{2+} spike width. * $P < 0.05$, ** $P < 0.01$ by the Mann-Whitney U test. Error bars, SEM

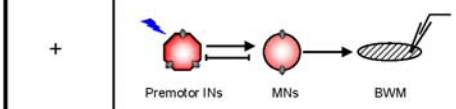
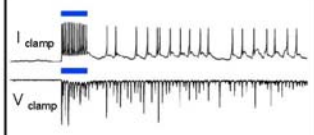
Supplementary Figure 10



Supplementary Figure 10. AVA exhibits persistent activity upon stimulation.

(**a**) AVA recording in a dissected *hpIs270* preparation (hold at -60 mV by whole cell patch clamping) upon a train of 2s 470nm light stimulation at 2s interval. Solutions used for AVA recording are the same as those used for the neuromuscular preparation. (**b**) Charge frequency of AVA was significantly increased after stimulation in 3 of 4 such preparations. Charge transfer during 10 second pre- to 40-60 second post-stimulation was quantified. Connected lines represent recordings from the same neuron before and after the stimulation. (**c**) Normalized charge frequency showed significantly difference before and after the stimulation. * $P < 0.05$ by Student's t test. Error bars, SEM.

Supplementary Table 1. Correlation between persistent activity and locomotion.

Gene	*Synapse & Circuit*	*Activity output*	*Behavior*
nca, nlf-1	Motor circuit	Persistent activity	Sustained locomotion
+			
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