

Supplemental Digital Content 1. Syntaxin1A-mediated Resistance and Hypersensitivity to Isoflurane in *Drosophila melanogaster*

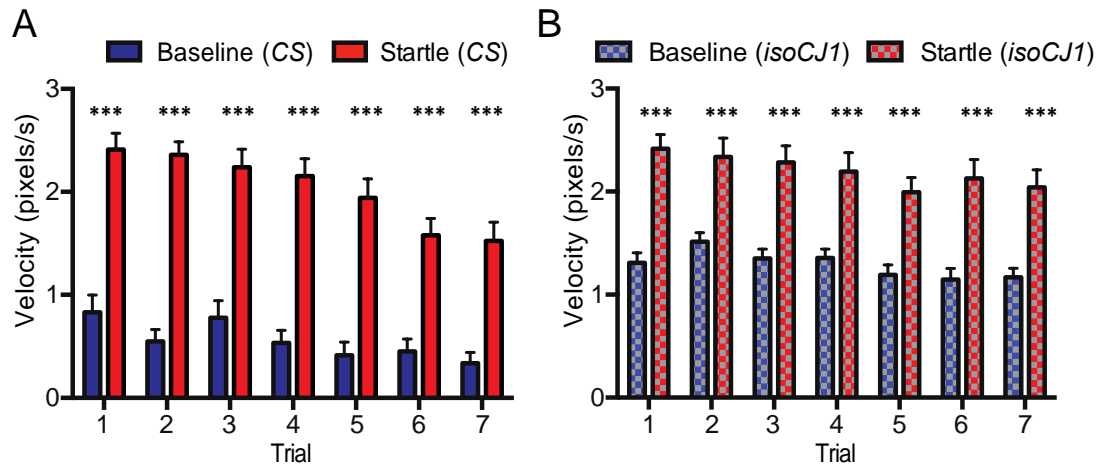


Fig. 1. Startle-induced locomotion assay baseline and startle responses without isoflurane gas.

Startle-induced locomotion in flies across seven consecutive startle stimuli, separated by 10 min; stimulus = 500ms vibration, delivered every 1s. Data displayed is mean \pm SEM. (A) Velocity (pixels/s) of wild-type (*Canton-S*, [CS]) flies for baseline (blue) and startle (red) response. (B) Velocity (pixels/s) of *isoCJ1* for baseline (blue, checkered) and startle (red, checkered) response. Both *CS* and *isoCJ1* show a significant increase in velocity following the startle stimulus across all seven trials.

*** $P < 0.001$, t -test comparing means.

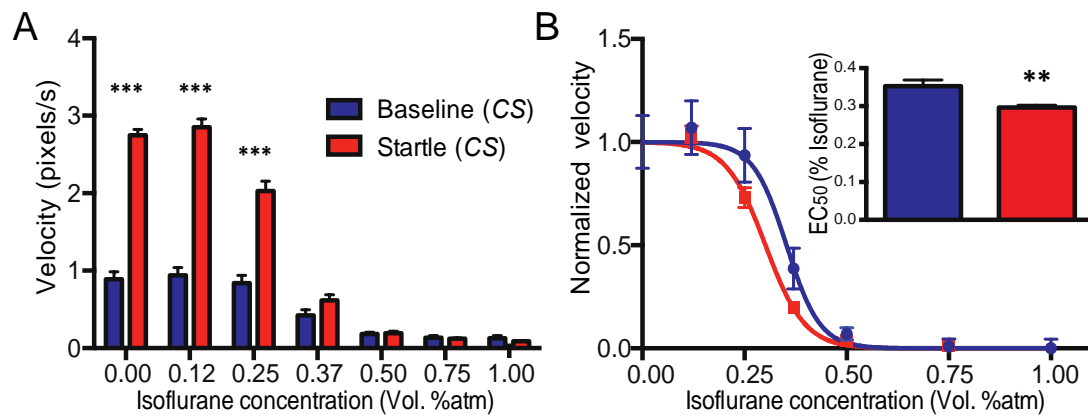


Fig. 2. Startle-induced locomotion assay baseline and startle responses with isoflurane gas.

(A) Raw velocity measures for baseline (blue) and startle (red) for wild-type (*Canton-S*, [CS]) flies under increasing concentrations of isoflurane. Data displayed is mean \pm SEM. The significant increase in velocity for the startle is lost after 0.25vol% isoflurane. *** $P < 0.001$, t -test comparing means. (B) Nonlinear regression of normalized baseline velocity (blue) or normalized startle velocity (red) under increasing isoflurane concentrations (vol. % atm). Error bars represent SEM. Inset: estimated EC₅₀ \pm standard error of the estimate (SEE) for baseline (blue) and startle-induced velocity (red) in wild-type female flies. ** $P < 0.01$, calculated by extra sum-of-squares F test between estimated EC₅₀ ($n = 60$).

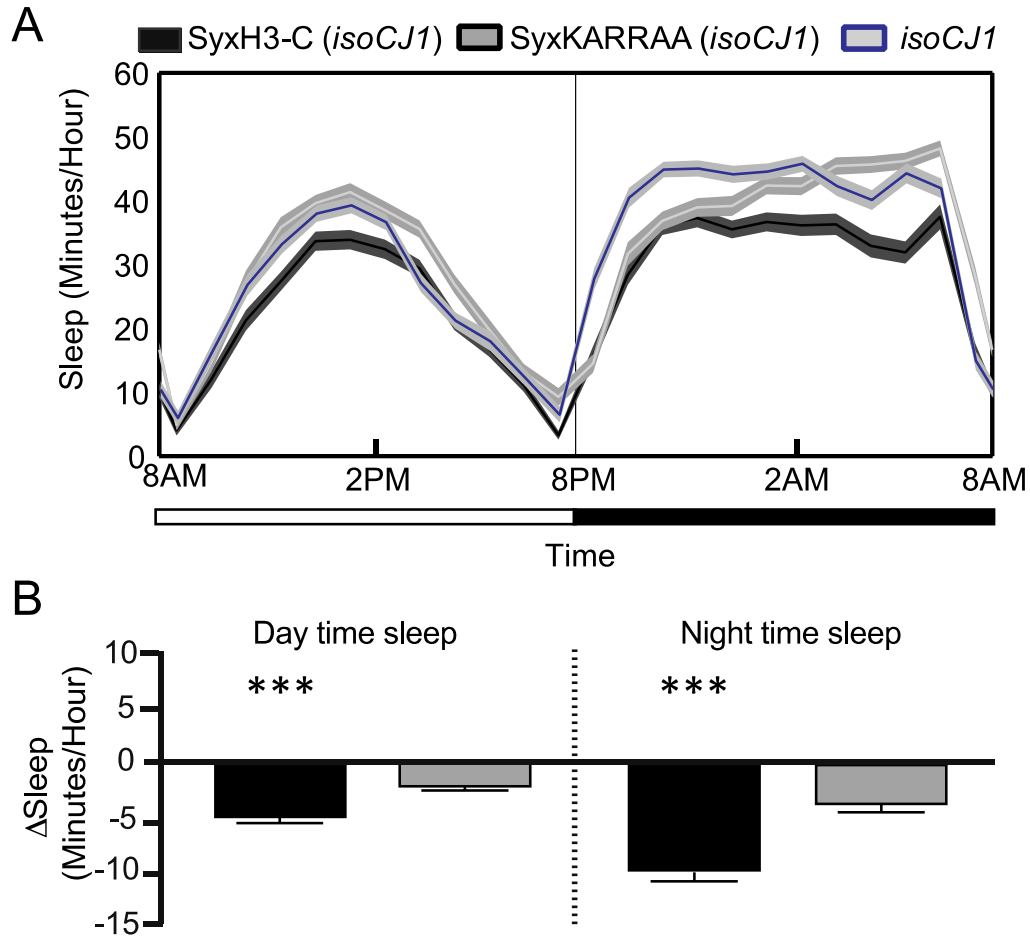


Fig. 3. Sleep duration is altered in *syxH3-C*.

(A) Average sleep (min/h) across 3 days in *isoCJ1* (blue), *syxH3-C* (black) and *syxKARRAA* (grey) with shaded area denoting error (\pm SEM) ($n = 34$ flies per genotype). Sleep is defined as inactivity for > 5 mins. (B) Difference in time spent asleep (Δ sleep min/h \pm SEM) compared to *isoCJ1* for day (left) and night (right) in *syxH3-C* (black) and *syxKARRAA* (grey) across 3 days. *** $P < 0.001$, t -test comparing means ($n = 34$ flies per genotype).

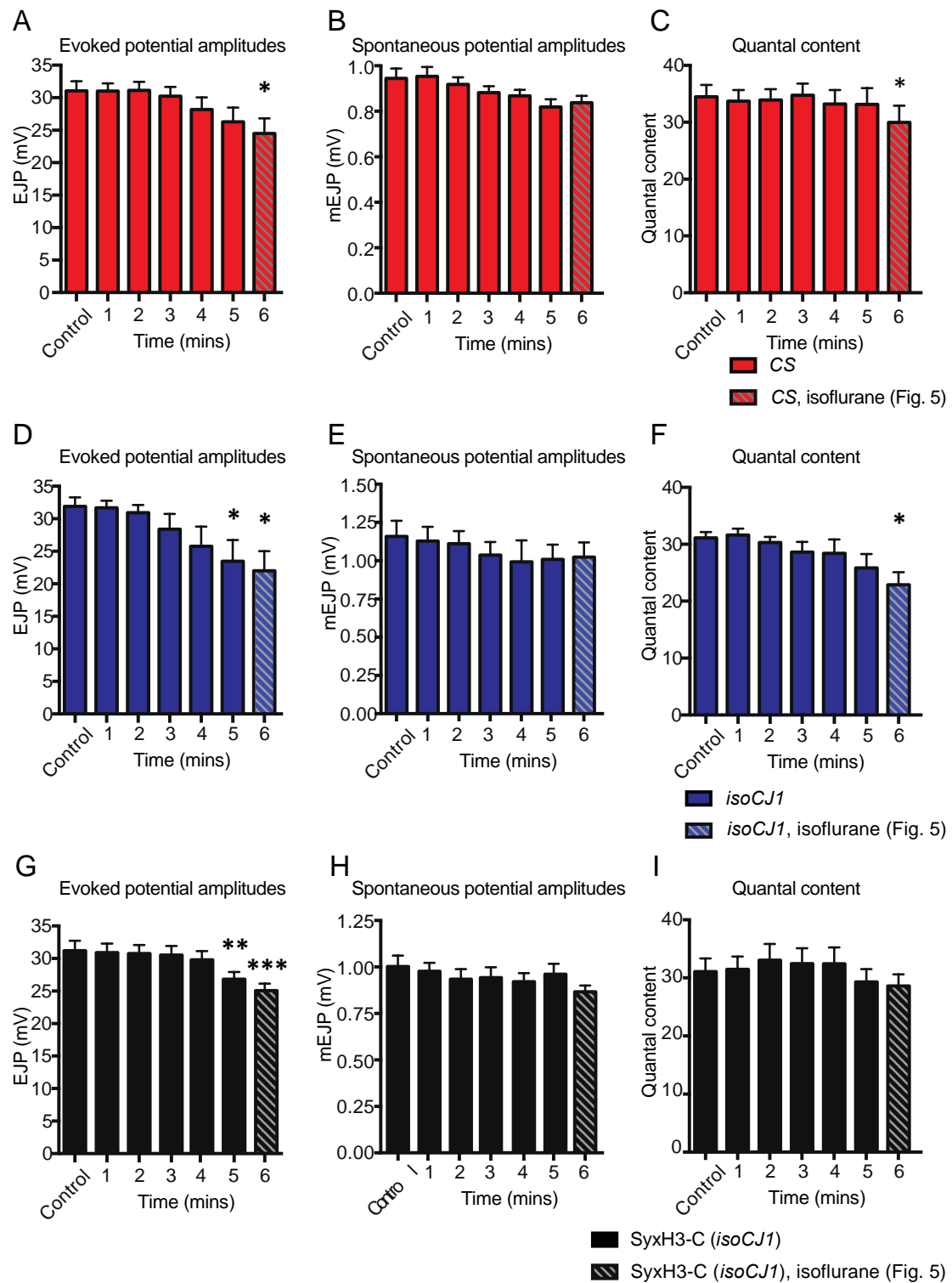


Fig. 4. Summary of evoked and spontaneous endplate potential amplitudes and quantal content before and during isoflurane perfusion.

(A) Excitatory junctional potential (EJP) amplitudes (mV) are shown before (control) and during isoflurane perfusion (time in min) in wild-type (*Canton-S*, [CS]). Red

shaded box denotes data used for figure 5E and G in article. (B) Miniature excitatory junctional potential (mEJP) amplitudes (mV) are shown before (control) and during isoflurane perfusion (time in min) in wild-type (*CS*). Red shaded box denotes data used for figure 5F and G in article. (C) Quantal content is shown before (control) and during isoflurane perfusion (time in min) in wild-type (*CS*). Red shaded box denotes data used for figure 5G in article. (D) EJP amplitudes (mV) are shown before (control) and during isoflurane perfusion (time in min) in wild-type (*isoCJI*). Blue shaded box denotes data used for figure 5G in article. (E) mEJP amplitudes (mV) are shown before (control) and during isoflurane perfusion (time in min) in wild-type (*isoCJI*). Blue shaded box denotes data used for figure 5G in article. (F) Quantal content is shown before (control) and during isoflurane perfusion (time in min) in wild-type (*isoCJI*). Blue shaded box denotes data used for figure 5G in article. (G) EJP amplitudes (mV) are shown before (control) and during isoflurane perfusion (time in min) in *syxH3-C*. Black shaded box denotes data used for figure 5G in article. (H) mEJP amplitudes (mV) are shown before (control) and during isoflurane perfusion (time in min) in *syxH3-C*. Black shaded box denotes data used for figure 5G in article. (I) Quantal content is shown before (control) and during isoflurane perfusion (time in min) in *syxH3-C*. Black shaded box denotes data used for 4G. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; all statistics shown are one-way ANOVA with Dunnett's multiple comparisons test.