Crosby Lab Code

by

Christelinda Laureijs

Scripts for analyzing evoked currents and action potentials in R

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Statistical analysis

Table 1: A multivariate repeated measures ANOVA using Pillai's Trace shows that current amplitude varies significantly with time, and there are no significant interactions between factors like sex and treatment.

Parameters	df	Pillai's Trace	F	*p*-value
(Intercept)	1, 57	0.91	578.34	< 0.001
Treatment	3, 57	0.02	0.36	0.78
Sex	1, 57	0.01	0.48	0.49
Treatment:Sex	3, 57	0.03	0.54	0.66
Times	4, 54	0.38	8.26	< 0.001
Treatment:Times	12, 168	0.15	0.73	0.72
Sex:Times	4, 54	0.03	0.43	0.79
Treatment:Sex:Times	12, 168	0.26	1.31	0.22

Levene's test

Univariate Shapiro

Box's M

Multivariate normality

T-tests

Plot eEPSCs

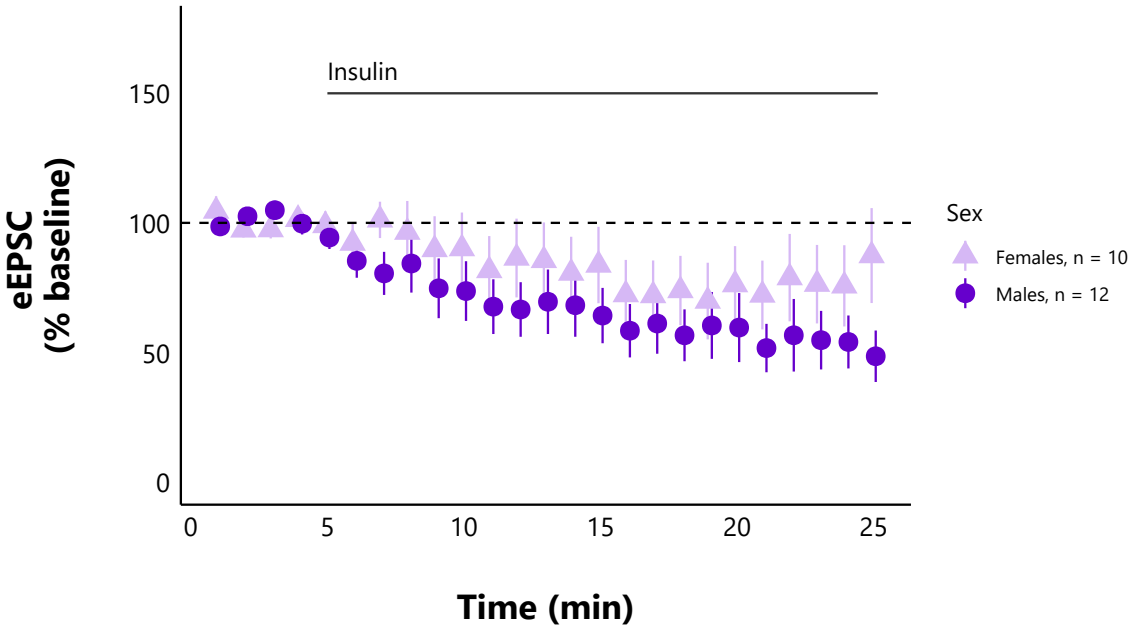

Figure 1: Insulin significantly decreases excitatory evoked post-synaptic current (eEPSC) amplitude over time in both sexes, with no significant differences between the sexes. I exposed DMH neurons to 500 nM of insulin from 5 minutes and onward. Each point represents the mean eEPSC amplitude (± the standard error) across all cells and *n* represents the number of unique cells. The asterisks indicate a statistically significant decrease in current amplitude relative to the baseline (t-test; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). The representative traces consist of eEPSCs from one cell averaged over the baseline period (0 to 5 min) and last interval (20 to 25 min) of the recording, and the scale bar represents 50 pA/20 ms.

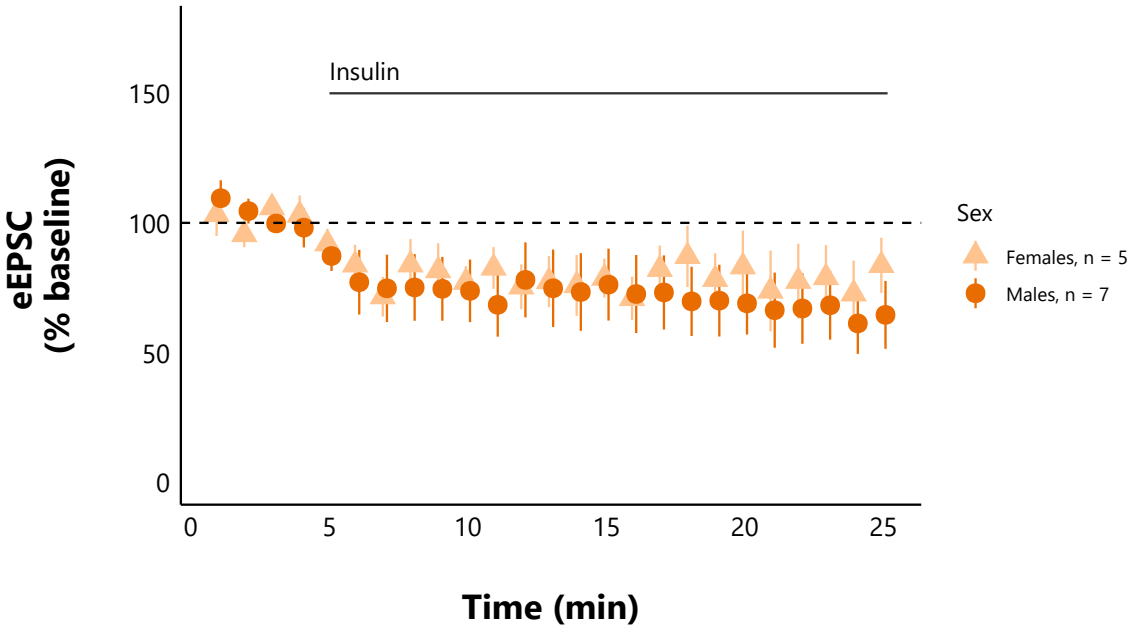

Figure 2: Insulin significantly decreases current amplitude over time in both sexes, even when the insulin receptor blocker HNMPA is applied. This suggests that insulin may not require insulin receptors to bind to DMH neurons and influence excitatory synaptic transmission. Each point represents the mean ± SE eEPSC amplitude and *n* represents the number of unique cells. The asterisks indicate a statistically significant decrease in current amplitude relative to the baseline (t-test; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). The representative traces consist of eEPSCs from one cell averaged over the baseline period (0 to 5 min) and last interval (20 to 25 min) of the recording, and the scale bar represents 50 pA/20 ms.

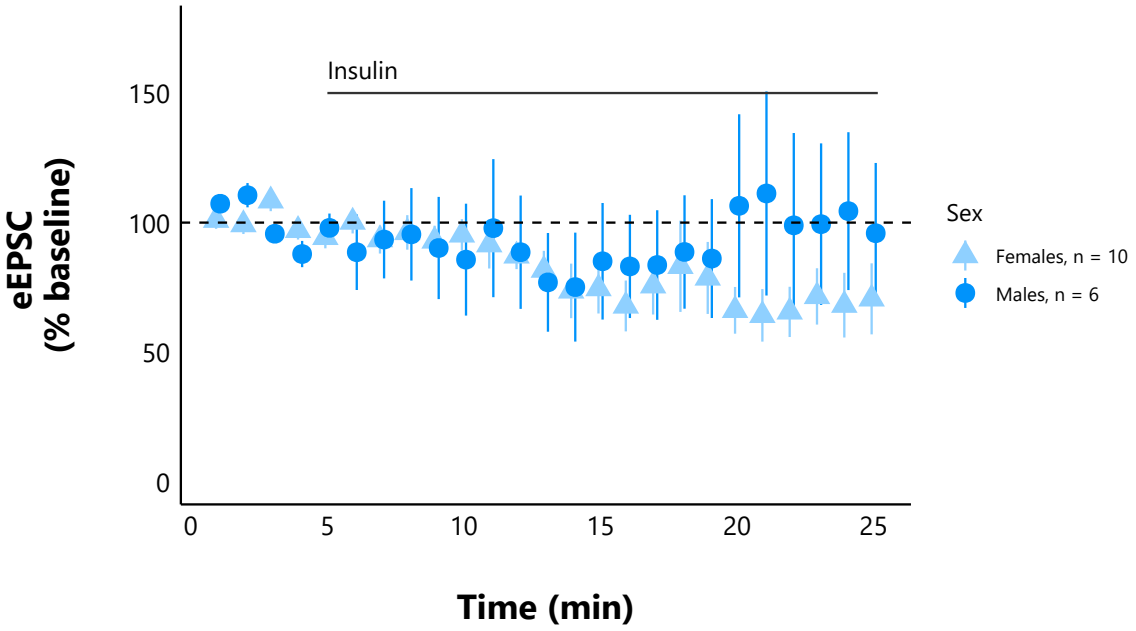

Figure 3: Insulin does not significantly decrease current amplitude over time with PPP present. PPP is an insulin-like growth factor 1 receptor antagonist. Since blocking these receptors reduced the changes in excitatory synaptic transmission seen in control recordings, insulin may require insulin-like growth factor 1 receptors to act on DMH neurons. Each point represents the mean ± SE eEPSC amplitude and *n* represents the number of unique cells. There are no asterisks because the decrease in eEPSC amplitude was not statistically significant relative to the baseline. The representative traces consist of eEPSCs from one cell averaged over the baseline period (0 to 5 min) and last interval (20 to 25 min) of the recording, and the scale bar represents 50 pA/20 ms.

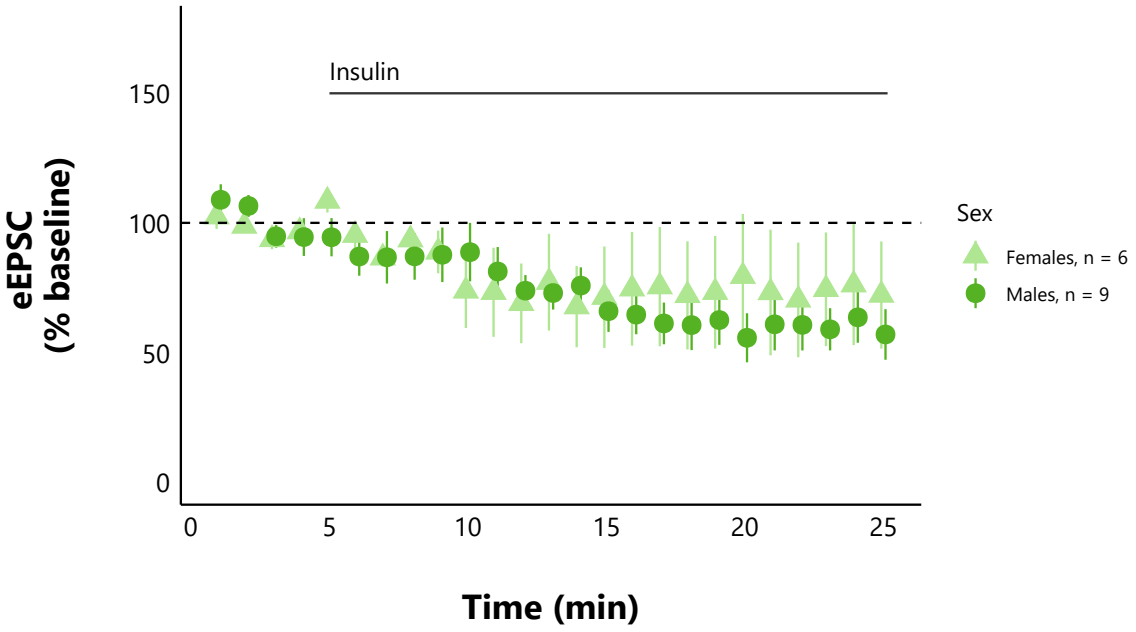

Figure 4: Insulin significantly decreases current amplitude over time in both sexes after a 24-hour fasting protocol. This suggests that insulin signalling in the DMH is associated with processes beyond appetite regulation. Each point represents the mean ± SE eEPSC amplitude and *n* represents the number of unique cells. The asterisks indicate a statistically significant decrease in current amplitude relative to the baseline (t-test; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). The representative traces consist of eEPSCs from one cell averaged over the baseline period (0 to 5 min) and last interval (20 to 25 min) of the recording, and the scale bar represents 50 pA/20 ms.

Cell Coordinates Plot

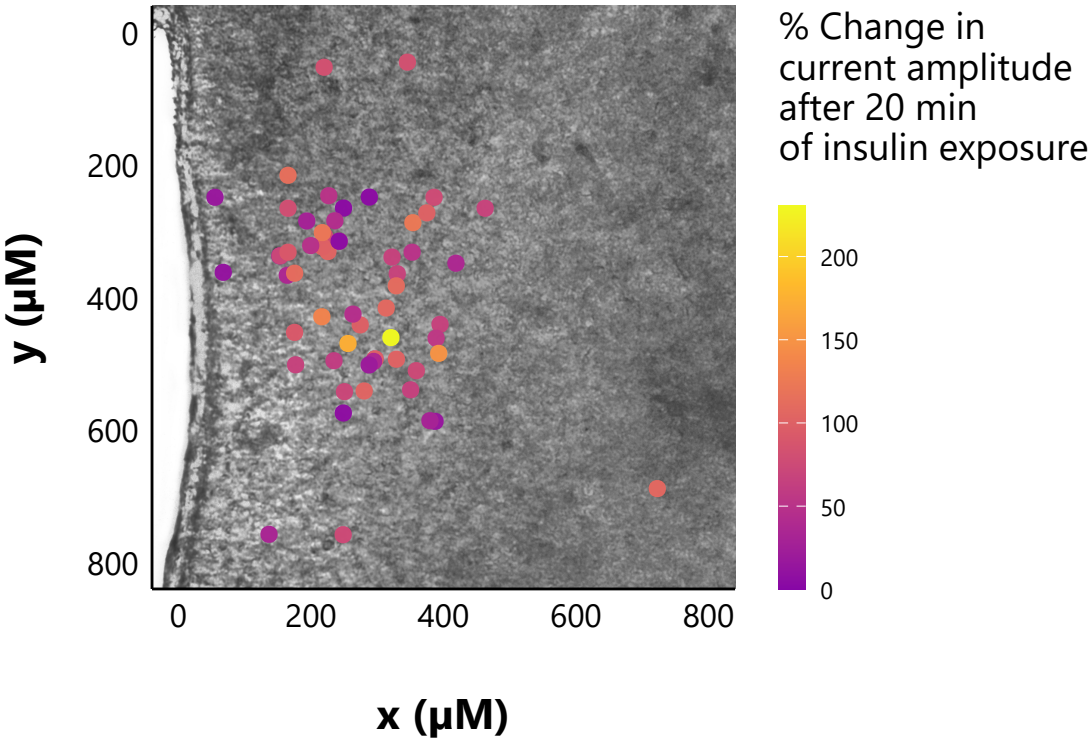

Figure 5: The neurons that I recorded were located in the DMH, and most neurons experienced a decrease in evoked current amplitudes (eEPSC) relative to baseline amplitudes. Each neuron is coloured according to the relative decrease in eEPSC amplitude after 20 minutes of insulin exposure. Neurons with similar responses to insulin did not cluster together, and there were no regions within the DMH that had a greater proportion of highly responsive neurons. In this figure (magnified 5X), the top of the third ventricle is located at (0,0).

Final Treatment Comparisons Plot

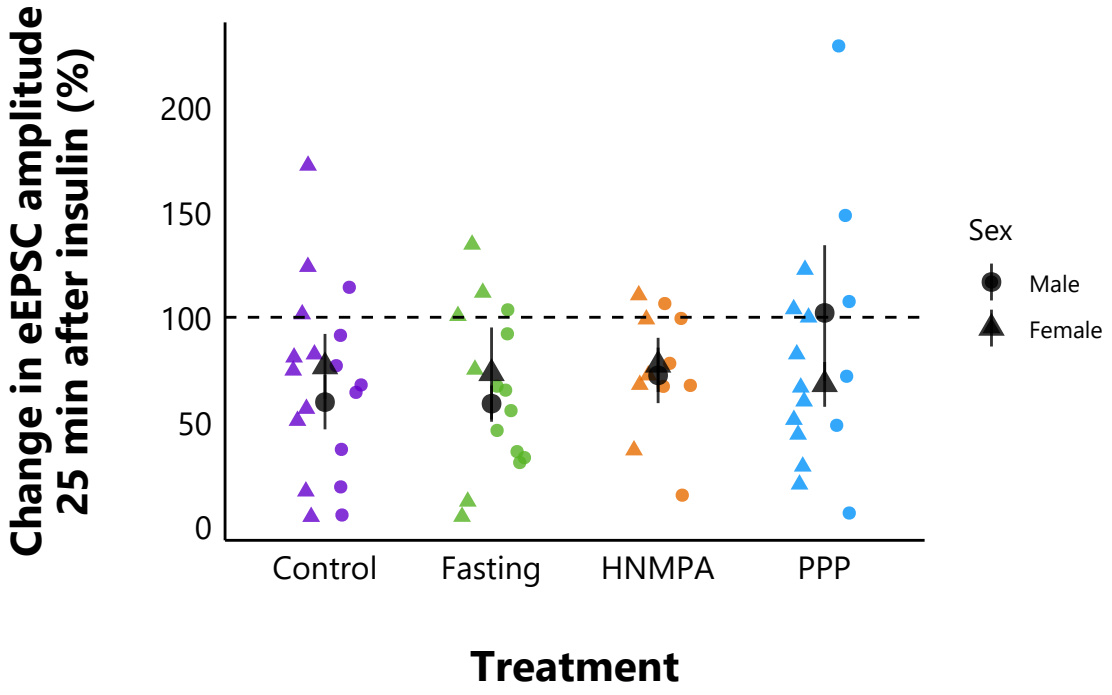

Figure 6: Insulin significantly decreased eEPSC amplitudes in all treatments, with no significant differences between treatments. In this summary figure, each dot represents the mean eEPSC amplitude of a cell relative to the baseline (%) during the last 20-25 minutes of the evoked currents protocol. Black solid shapes indicate the mean ± SE for each sex.

Paired Pulse Ratio

Shapiro

PPR Plots

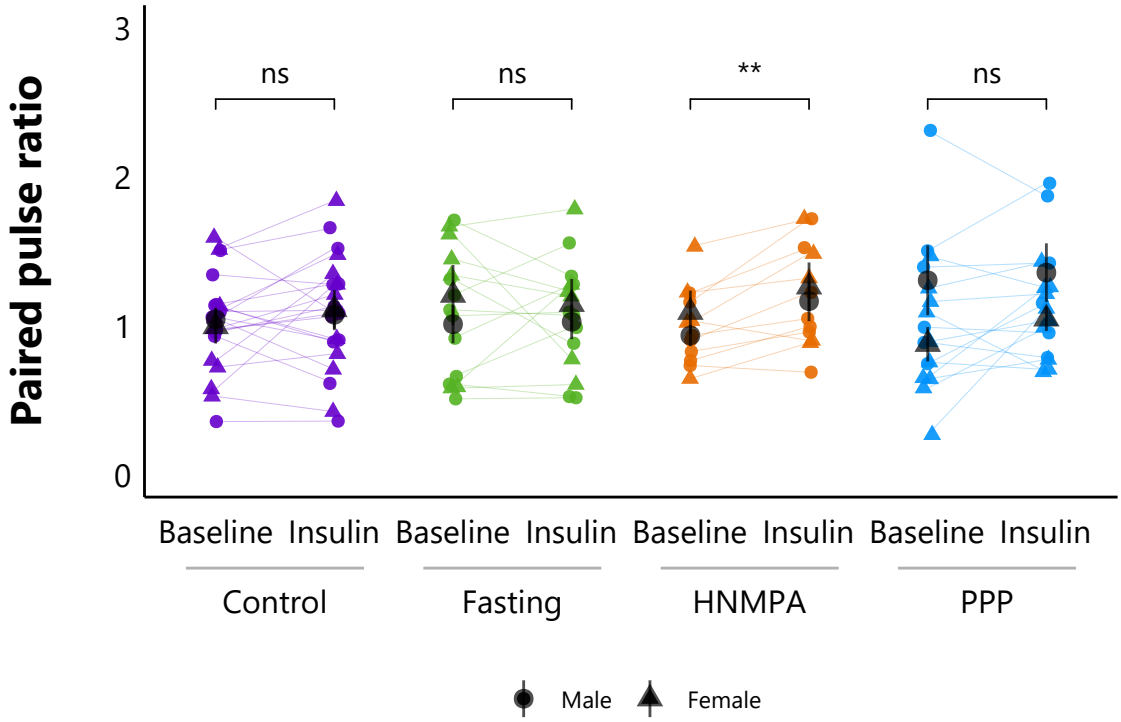

Figure 7: The paired pulse ratio (PPR) did not change significantly after insulin exposure for most of the treatment groups. The change in the PPR relative to the baseline was non-significant for the control, fasting, and PPP treatment groups, but it was statistically significant for the HNMPA-treated cells. Each dot represents the mean PPR of a single cell averaged over the baseline period (0 to 5 minutes) or the last 20 to 25 minutes of the evoked currents protocol. The asterisks indicate a statistically significant difference in the PPR between the two intervals (t-test; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). The solid black circle and triangle overlay represents the mean PPR (± SE) per sex.

Table 2: A paired t-test comparing the mean paired pulse ratio (PPR) per cell during the baseline period (0-5 min) to the mean PPR after insulin exposure (20-25 min) shows that insulin does not significantly affect the PPR during experiments with no additional treatments, fasting, or PPP. Insulin significantly increases the PPR when the insulin receptor blocker HNMPA is applied.

Parameter	Statistic	DF	*p*-value
Control	-1.05	21	0.30
Fasting	0.15	14	0.88
HNMPA	-3.64	11	0.004
PPP	-1.43	15	0.17

Action Potentials

AP Frequency t-test

Shapiro test

Wilcoxon Test

Summary plot

Individual AP plots

Table 3: A Wilcoxon signed-rank test shows that insulin significantly affects action potential amplitudes, thresholds, and half-widths. Insulin does not significantly affect the latency to fire, after-hyperpolarization amplitude, or the time of afterhyperpolarization.

Parameter	df	Statistic	*p*-value
Peak amplitude	15, 15	120	< 0.001
Threshold	15, 11	63	0.005
Latency to fire	15, 11	31	0.90
Half-width	15, 11	2	0.003
After-hyperpolarization amplitude	15, 11	35	0.90
After-hyperpolarization time	15, 11	17	0.17

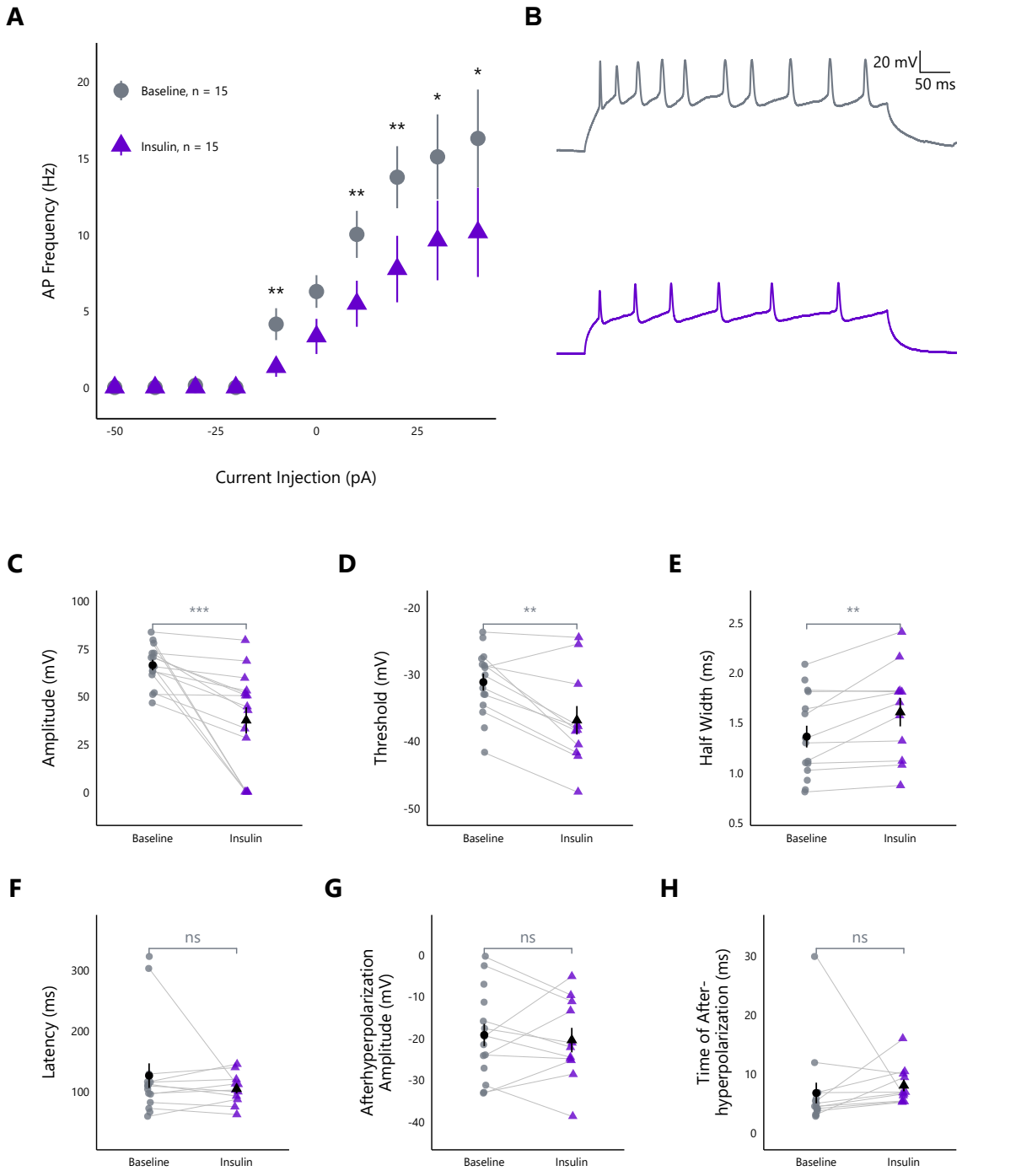

Figure 8: Insulin decreases the excitability of DMH neurons. Data were recorded before and then after 25 minutes of insulin exposure. *A)* Insulin significantly decreases action potential frequency (mean ± SE; *n* is the number of cells). *B)* Representative traces from a current injection of 40 pA (top: baseline, bottom: insulin). *C)* Insulin significantly decreases action potential amplitudes *C)* and thresholds *D)*, while significantly increasing half-widths *E)*. Insulin does not significantly affect latency to fire *F)*, after-hyperpolarization amplitude *G)* or after-hyperpolarization time *H)*. Overlay on Figures C-H: mean ± SE. $n = 15$ for baseline and $n = 11$ for insulin because 4 cells did not fire any action potentials after insulin exposure. Wilcoxon signed-rank test, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

References