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Supplemental Information

Early Life Stress Drives Sex-Selective Impairment in Reversal Learning by Affecting Parvalbumin Interneurons in Orbitofrontal Cortex of Mice

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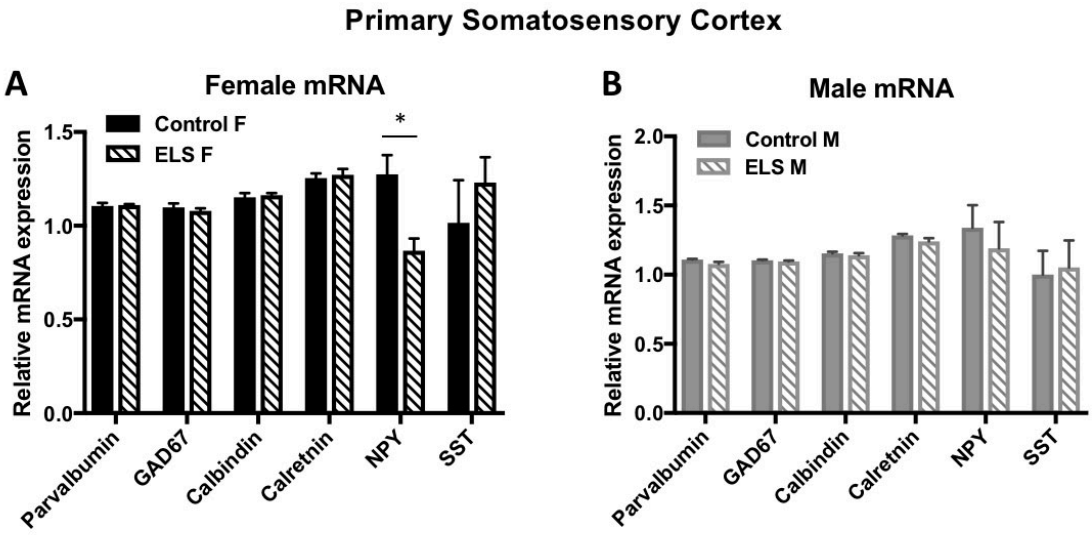
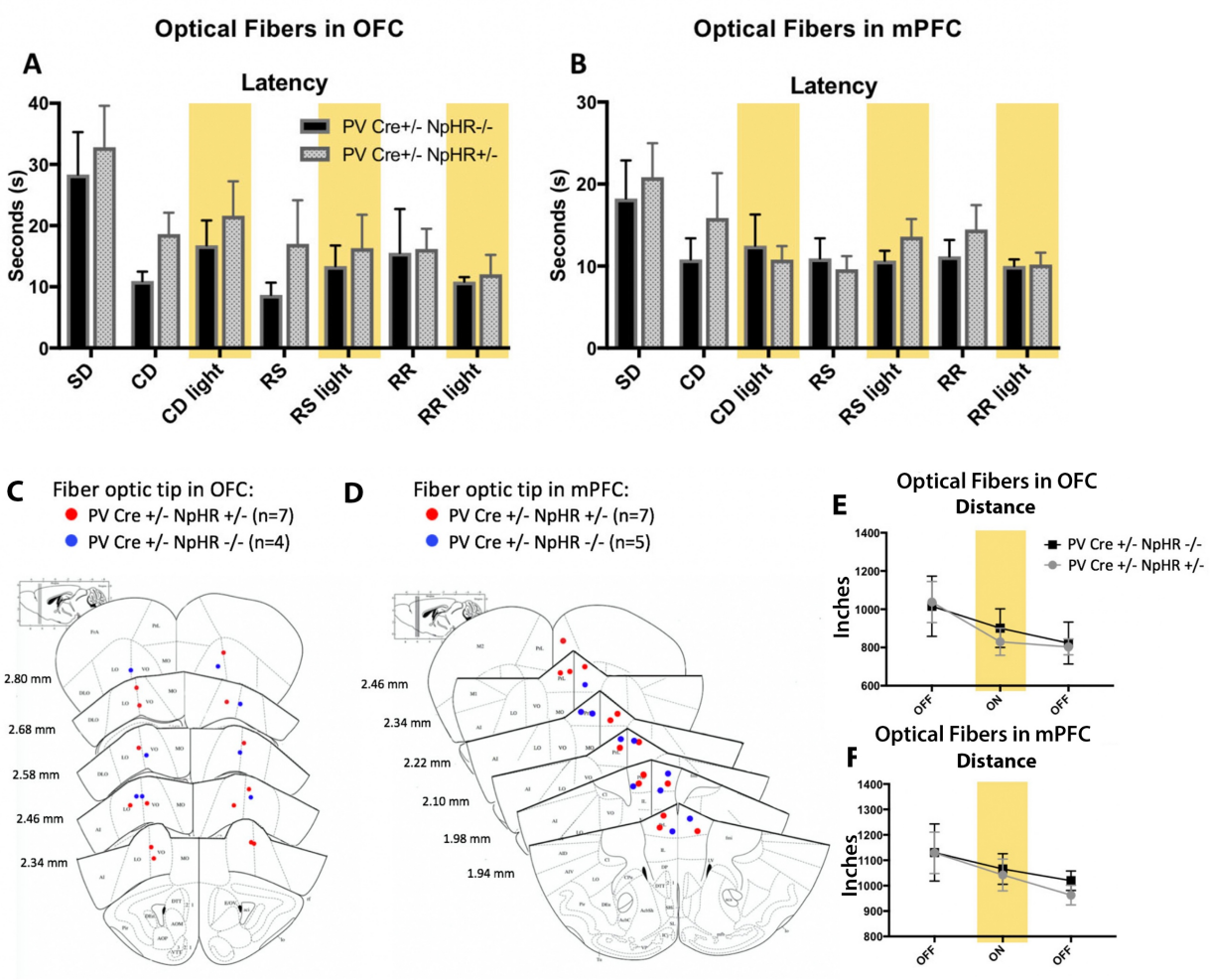
SUPPLEMENTAL INFORMATION

This Supplemental Information includes

-**Supplemental Data**



Supplemental Figure 1 (Related to Figure 2). *ELS does not affect relative gene expression of PV or GAD67 in primary somatosensory cortex of female or male mice.* Quantitative real-time PCR analysis of mRNA expression of several interneuron markers and GAD67 in the primary somatosensory cortex (n = a minimum of 5 per group, with 3 technical replicates for each sample). (A) ELS females show a significant decrease in NPY mRNA expression in comparison with controls (P=0.009). No other interneurons were affected by ELS in this region. (B) No significant differences between ELS and control males are observed in S1. Data show mean ± SEM and are analyzed using two-tailed Student's t tests; * $p < 0.05$.



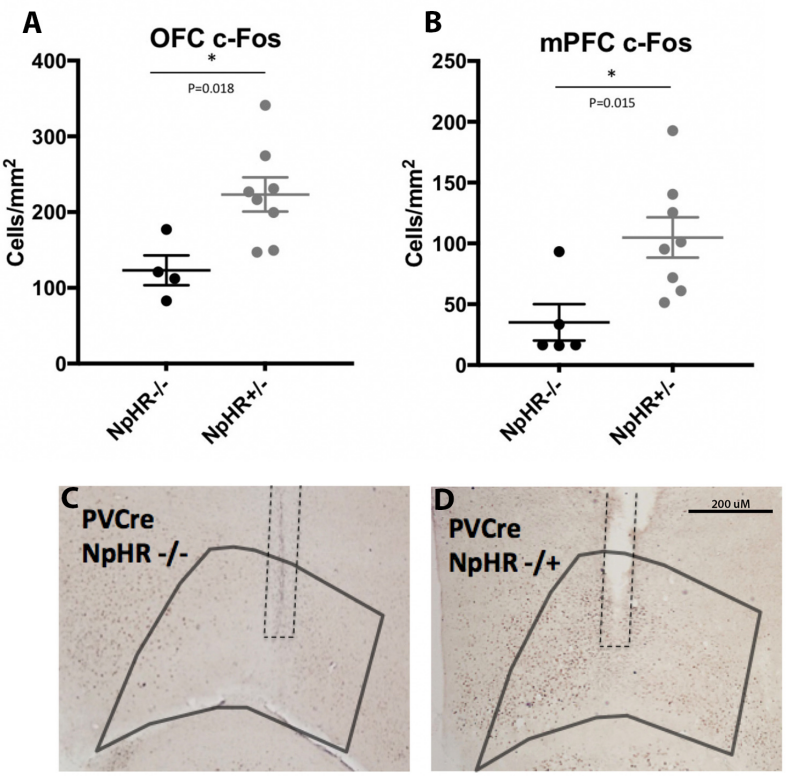
Supplemental Figure 2 (Related to Figure 3).

Optical stimulation does not affect latency to make a decision in NpHR+/- mice or control littermates. Latency to dig was recorded during the attentional set-shifting task, and no main effects or interactions between light stimulation, trial phase or genotype were observed between groups when the optical fibers were (A) in the OFC or (B) in the mPFC. Data show mean ± SEM and analyzed using repeated-measures ANOVAs.

Placement of optical fibers was verified in the OFC and the mPFC. Halo positive mice (PV Cre+/- NpHR+/-) are shown in red, while littermate controls (PV Cre +/- NpHR -/-) are shown in blue in both (C) the OFC (N= 11) and (D) the mPFC (N=12).

Optogenetic inhibition of PV interneurons in the OFC or mPFC does not affect locomotor activity. NpHR+/- and NpHR-/- animals were recorded in the open field arena for 5 minutes with no photostimulation, followed by 5 minutes with continuous illumination (570nm) and another 5 minutes without photostimulation, for a total of 15 minutes.

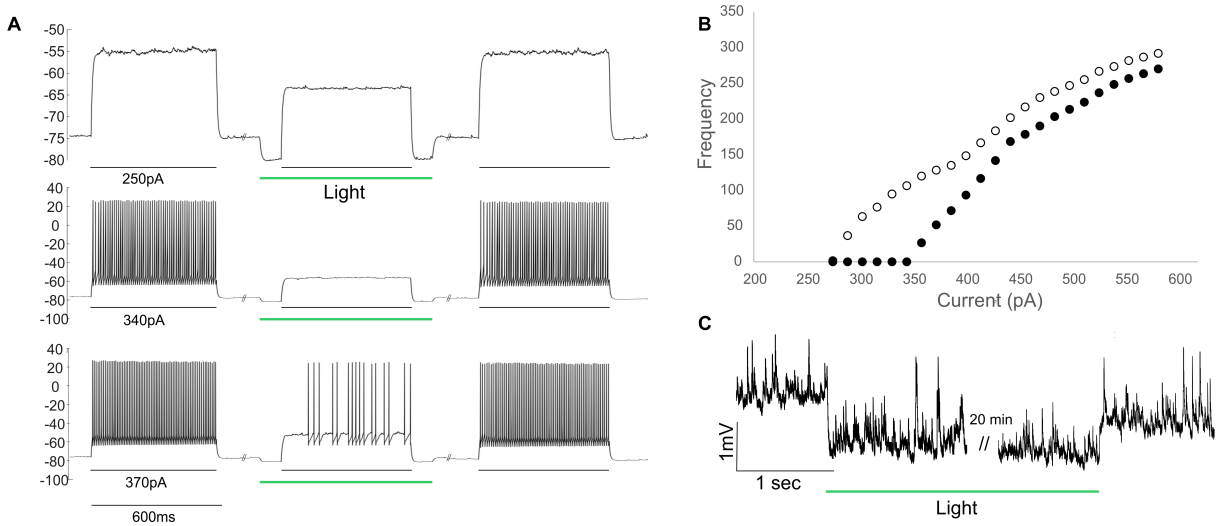
(E) In the OFC, optogenetic inhibition of PV interneurons did not have an overall effect on distance travelled in the open field test in NpHR+/- or NpHR-/- animals. (F) In the mPFC, photostimulation had no effect on distance travelled. Data show mean ± SEM and analyzed using repeated-measures ANOVAs.



Supplemental Figure 3 (Related to Figure 3). *Optogenetic stimulation increases immediate early gene c-Fos, activation*. (A)Post-mortem c-Fos analysis revealed that optically silencing PV interneurons in the OFC lead to an increase in c- Fos expression in the ventral orbitofrontal (VO) region, (N = 12, p = 0.018)

(B) Optically silencing PV interneurons in the mPFC lead to an increase in c-Fos expression in the prelimbic mPFC (PL), (N = 13, p = 0.015).

Representative photomicrographs of c-Fos immunohistochemistry in the OFC of (C) NpHR-/- and (D) NpHR-/+ mice following optogenetic stimulation.



Supplemental Figure 4 (related to Figure 3). in vitro optogenetic inhibition of parvalbumin positive cells using NpHR. (A) Traces depicting three pulses (columns) of three different current intensities (rows) in the same cell from a PV Cre x NpHR mouse. Green lines depict halorhodopsin activation, and black lines indicate current stimulation. (B) Frequency of action potentials as a function of various levels of current stimulation in the same cell without (open circles) and with (filled circles) halorhodopsin activation. (C) Response of membrane potential to halorhodopsin activation immediately after it was turned on, then when the light was turned off twenty minutes later. For slice experiments we used a 545 +/- band-pass excitation filter on white LED light. The light power at the plane of focus was 28.2 μW/mm^2.