Title: Neurodevelopmental disorder-associated *Nr2f1* haploinsufficiency disrupts dentate gyrus inhibitory circuits and impairs short-term spatial memory

Authors

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SUPPLEMENTARY INFORMATION

This file contains Supplementary Figures 1-3 with captions supporting the main text. File format: PDF

Supplementary Figure 1

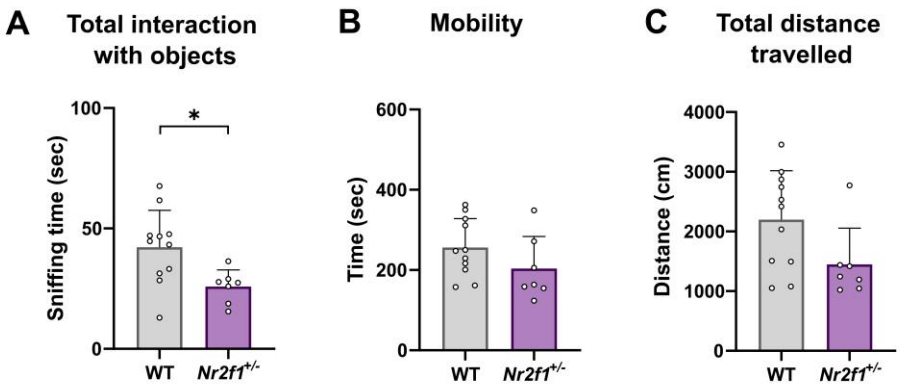


Figure S1. General exploratory behavior and locomotor activity during the Novel Object Location (NOL) test. (A) Bar dot plot showing the total object interaction time (i.e. sniffing time in seconds) during the test phase between WT (grey) and *Nr2f1*$^{+/-}$ (purple) mice. Two-tailed unpaired Student's t-test,**p*=0.0178. **(B,C)** Bar dot plot showing mice locomotor activity during the test phase expressed as mobility and total distance travelled within the arena respectively in WT (grey) and *Nr2f1*$^{+/-}$ (purple) mice. Each dot represents an animal (n = 11 WT / 7 *Nr2f1*$^{+/-}$ mice). Error bars indicate mean± SD.

Supplementary Figure 2

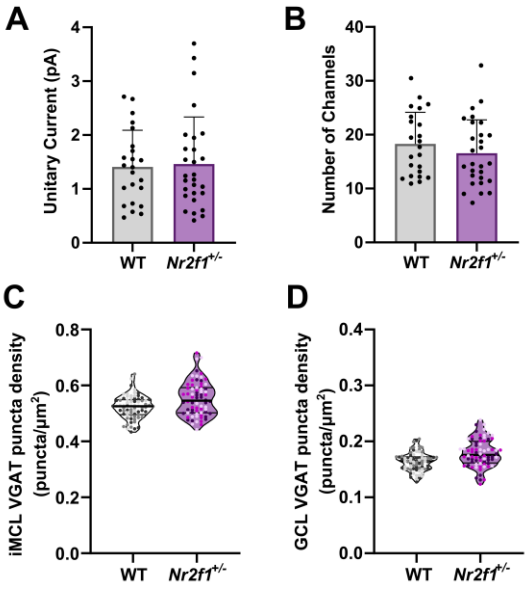


Figure S2. Inhibitory synaptic current properties of granule cells and presynaptic puncta density in the DG layers of WT and *Nr2f1*$^{+/-}$ mice. (A) Unitary current amplitude (pA) measured in WT and *Nr2f1*$^{+/-}$ neurons. **(B)** Estimated number of channels per cell in WT and *Nr2f1*$^{+/-}$ neurons. In both panels, each dot represents an individual neuron, and bars indicate mean ± SD (WT, n = 23 cells; *Nr2f1*$^{+/-}$, n = 28 cells). Statistical analysis using an unpaired t-test revealed no significant differences between groups for either unitary current amplitude (P = 0.8062) or number of channels (P = 0.3123). **(C-D)** Violin plot representing the density (puncta/µm^2) of vGAT-positive puncta in the MCL and GCL respectively of WT (white) and *Nr2f1*$^{+/-}$ (purple) mice (Two-tailed nested t-test, n.s.). Three animals per genotype were analyzed (n = 3 WT/ *Nr2f1*$^{+/-}$mice), with ROIs from each animal coded by a distinct color.

Supplementary Figure 3

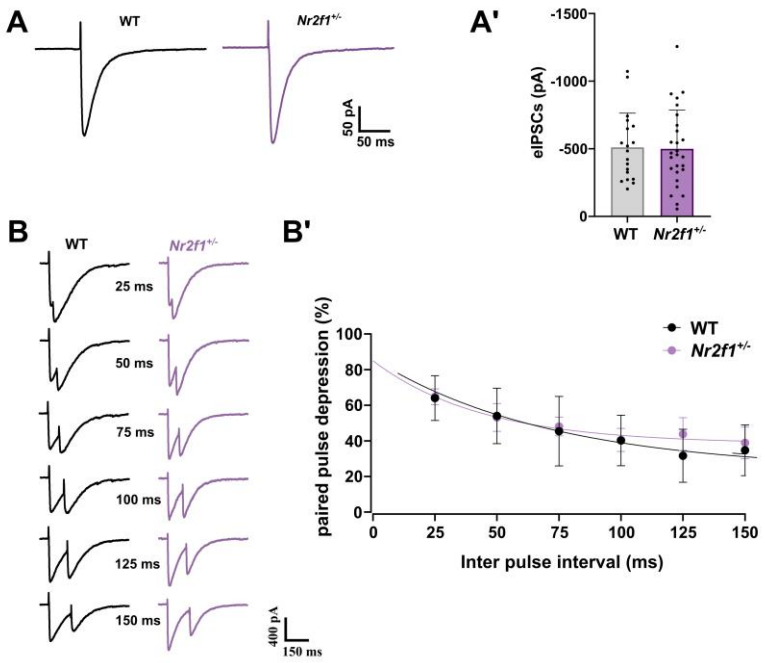


Figure S3. Analysis of paired-pulse depression (PPD) and evoked inhibitory currents (eIPSCs) showed preserved inhibitory synaptic properties in DG granule cells of *Nr2f1*$^{+/-}$ mice. (A, A') Representative traces and mean amplitude values of evoked inhibitory postsynaptic currents (eIPSCs) recorded at DG granule neurons in *ex-vivo* hippocampal slices of WT (black and grey) and *Nr2f1*$^{+/-}$ (purple) mice. **(B)** Average paired pulse depression (PPD) of three consecutive traces evoked by paired stimuli separated by inter-stimulus intervals (ISIs) increased duration (from 25 ms to 150 ms) in DG granule neurons of WT (black traces) and *Nr2f1*$^{+/-}$ (purple traces) mice. **(B')** Mean percentage of PPD as a function of ISIs (from 25 ms to 150 ms) recorded in granule neurons of WT (full black circles) and *Nr2f1*$^{+/-}$ (full purple circles) mice (e.g. at 25 ms of interpulse interval the percentage pf PPD was 63.99 ± 12.56% in WT vs. 64.79 ± 4.44% in *Nr2f1*$^{+/-}$; at 150 ms the percentage of PPD was 34.77 ± 14.323% in WT vs. 38.90 ± 8.84% in *Nr2f1*$^{+/-}$; multiple t-tests, $p > 0.05$ for all intervals).