Supplemental Figures For:

Loss of cholinergic input to the entorhinal cortex is an early indicator of cognitive impairment in natural aging of humans and mice

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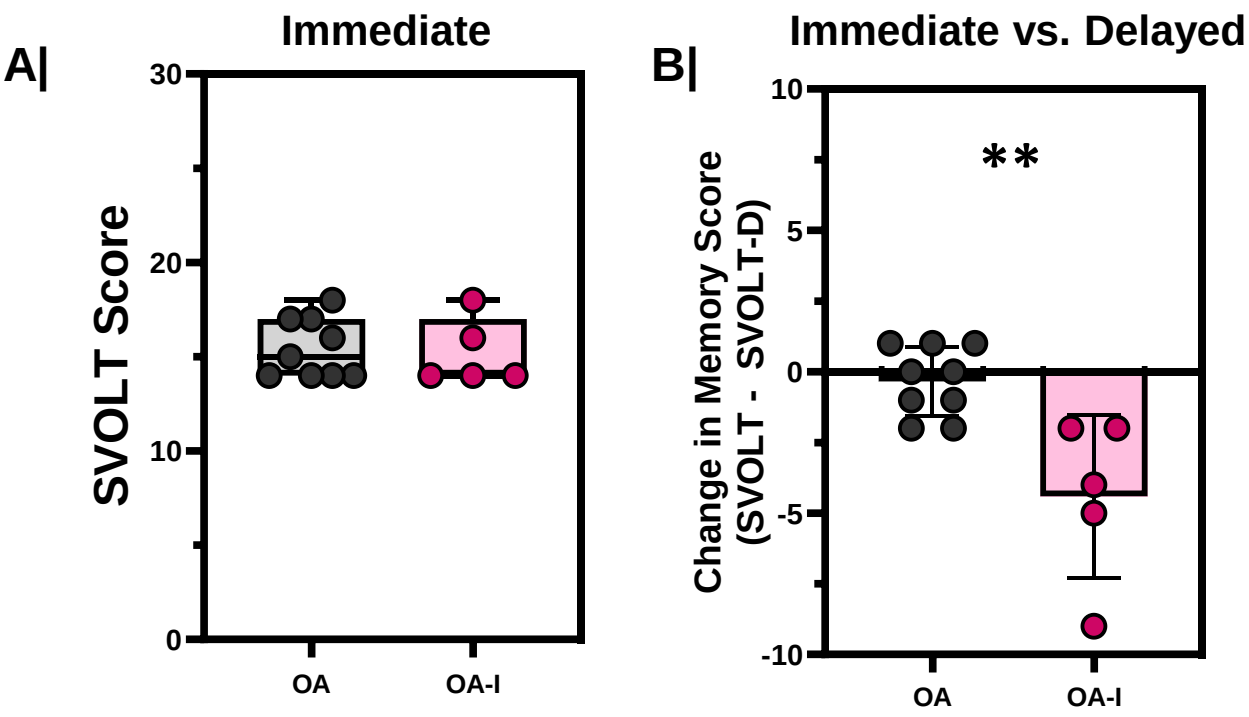
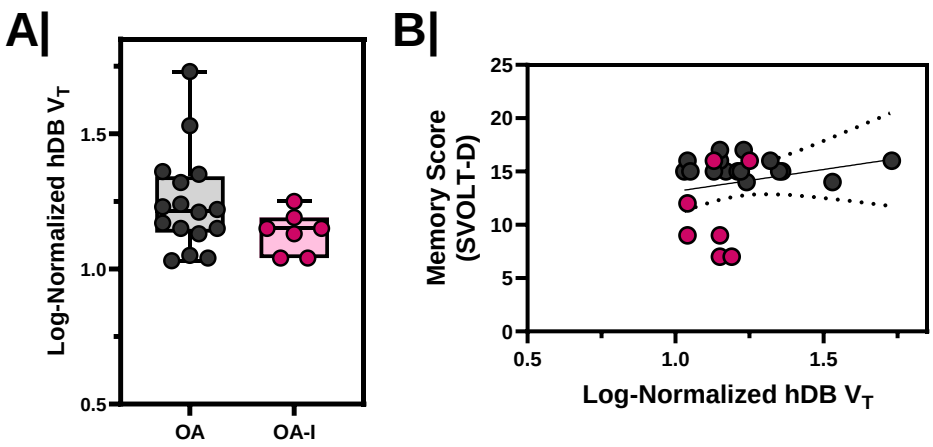


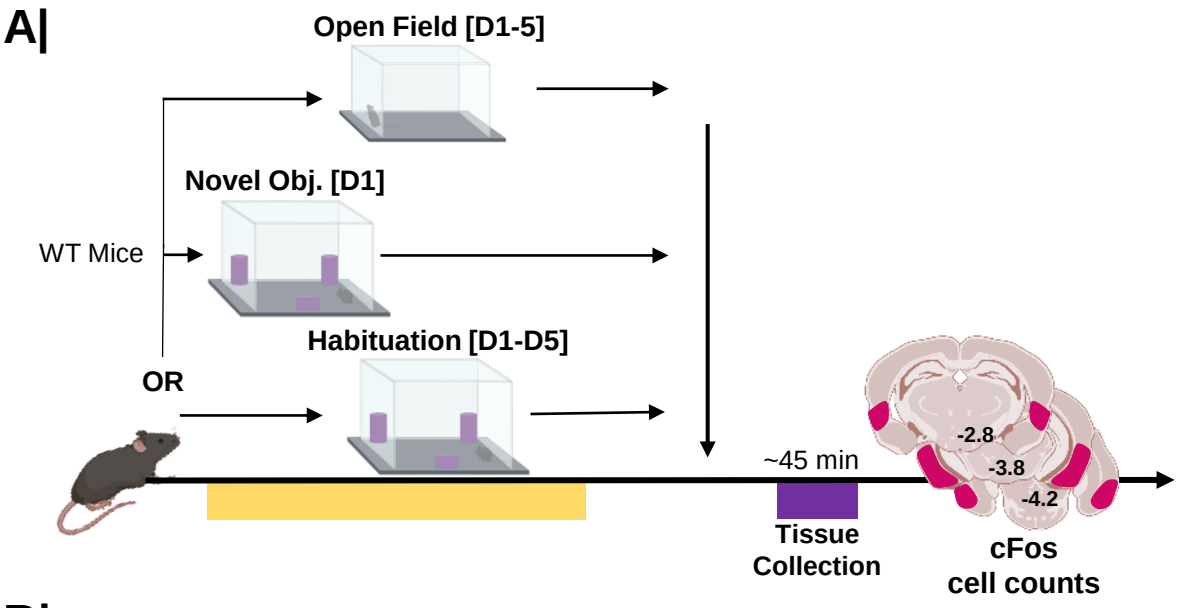
Supplemental Figure 1| (related to Figure 1) Delayed, but not immediate, object location memory is altered in older adults with cognitive impairment

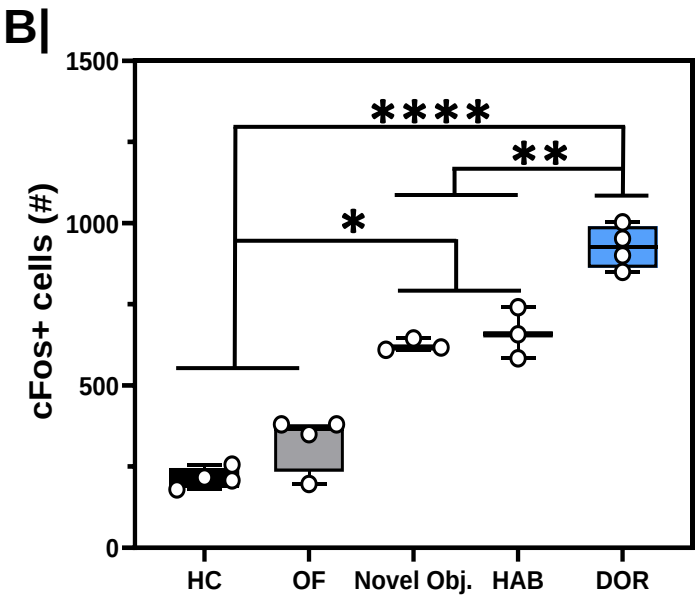
A. SVOLT immediate recall test scores (immediately after observation period) of the object location learning task between older adults (OA) and impaired, older adults (OA-I) (p=0.80). Older adults = **Grey**; Impaired, older adults = **Magenta**. **B.** Change in object location memory performance from immediate to delayed recall tests between older adults (OA) and impaired, older adults (OA-I) (**, p=0.005). Older adults = **Grey**; Impaired, older adults = **Magenta**.



Supplemental Figure 2| (related to Figure 3) hDB VAT density was not found to be significantly altered in older adults with cognitive impairment

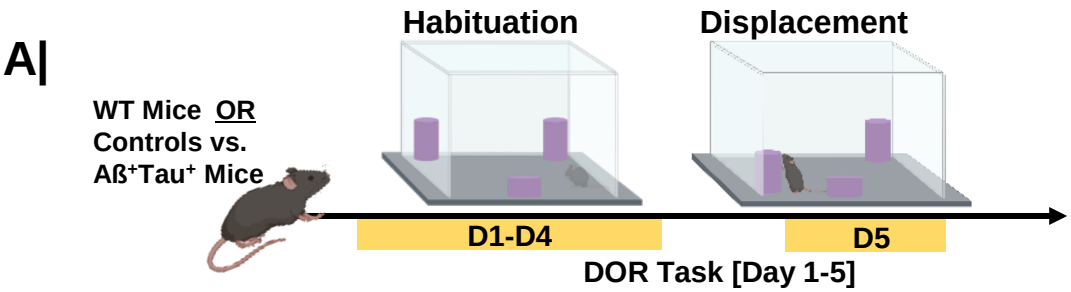
A. Log-normalized right and left hemisphere horizontal zone of the diagonal band (hDB, CH3) distribution volumes (V_T) between older adults (OA) and impaired, older adults (OA-I) (p=0.16); **B.** Correlation plots with linear regression line comparing the relationship between hDB distribution volume and delayed memory score (SVOLT-D) (r=0.16, p=0.47; linear regression not significant; confidence interval marked by dashed black lines). Older adults = **Grey**; Impaired, older adults = **Magenta**. Hemispheres plotted individually.

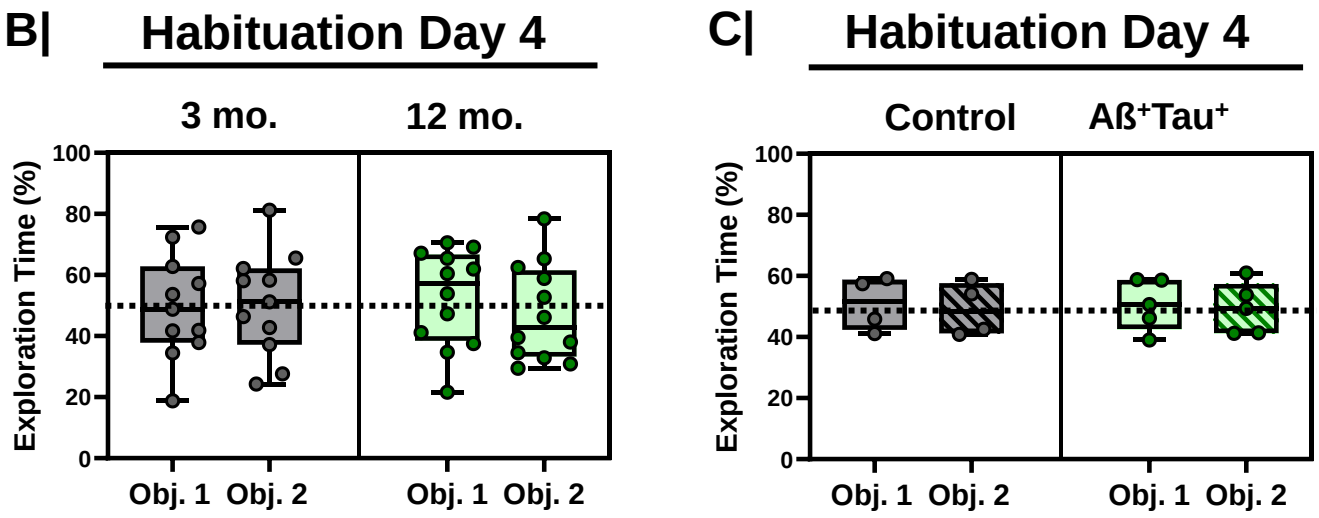




Supplemental Figure 3| (related to Figure 5) The lateral entorhinal cortex is engaged during object exploration

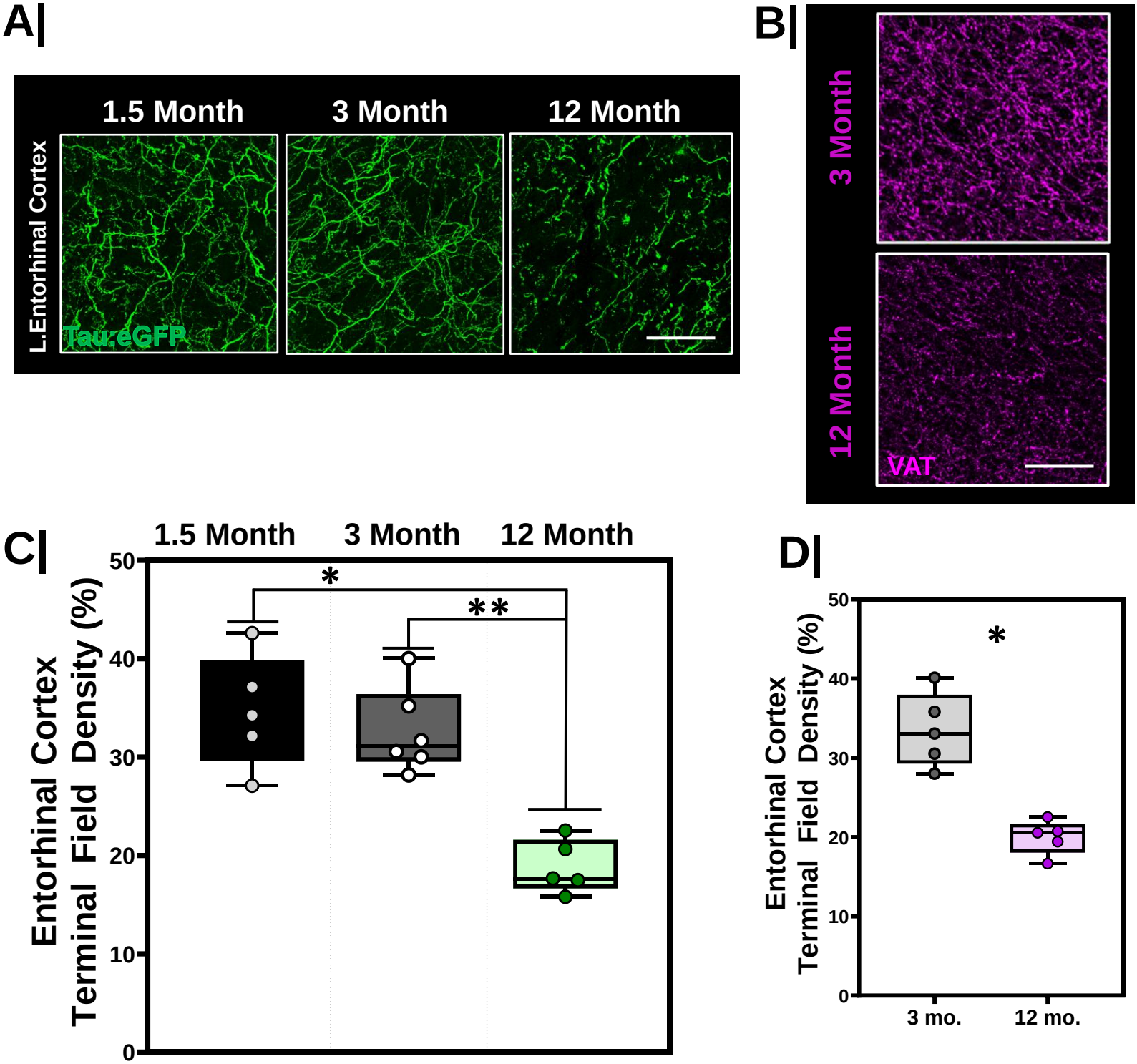
A. Experimental workflow for behavioral control conditions. WT mice underwent one of three conditions: open field exploration without objects (days 1-5), first-time novel object exploration (day 1), or five days of habituation (days 1-5). Brain tissue was harvested 45 mins after the behavior session and processed for cFos immunoreactivity and compared to home cage control and DOR conditions displayed in Figure 5. **B.** Quantification of total cFos activation in the LEC during homecage only (HC, n=2), open field (OF, no object, n=4), first time object exposure (Novel Obj., n=3) controls compared to the habituation (HAB, n=3) vs. displacement (DOR, n=4) sessions (*, HC/OF vs. Novel Obj./HAB, $p<0.01$) (**, Novel Obj./HAB vs. DOR, $p<0.005$) (****, HC/OF vs. DOR, $p<0.0001$). Data from HC and DOR conditions are identical to those in Figure 5C, and re-presented here for comparison.





Supplemental Figure 4| (related to Figures 6/11) Habituation behavior is intact in Aß+Tau+ Animals and 12-month aged mice.

A. Experimental workflow. Aß+Tau+ or control mice underwent displaced object recognition task. **B.** Quantification of percent time spent exploring objects during the habituation session (day 4) in 3-month (n=11; p=0.85) and 12-month animals (n=12; p=0.29). 3 month = **Grey box**; 12-month = **Green box**. **C.** Quantification of percent time spent exploring objects during the habituation session (day 4) between control (n=4; p=0.69) and Aß+Tau+ animals (n=5; p>0.99). Controls = **Grey box**; Aß+Tau+ = **Green box**.

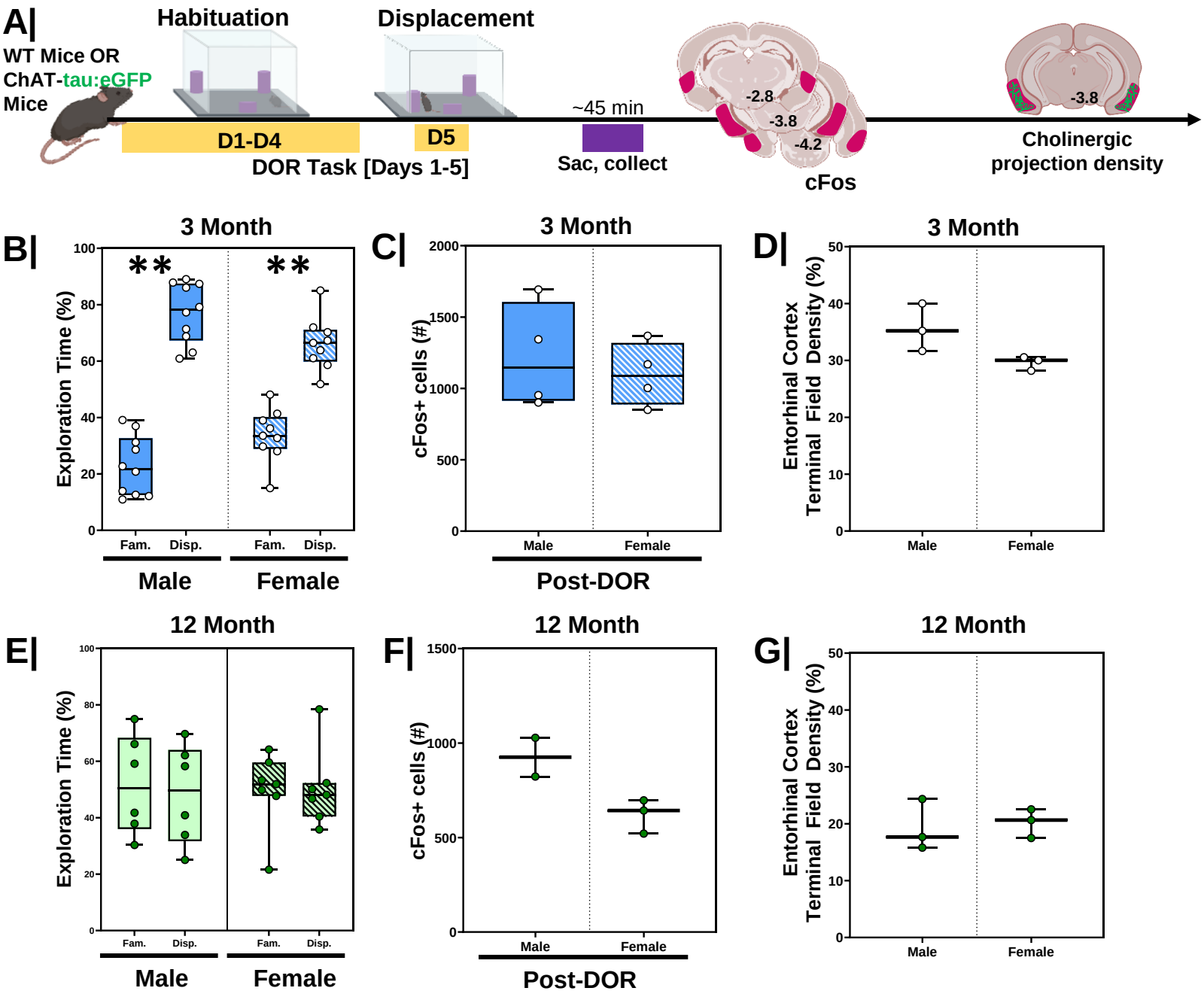


Supplemental Figure 5| (related to Figure 8) Lateral entorhinal cortex cholinergic terminal density differs between 3-month and 12-month mice

A| Representative high magnification confocal images of lateral entorhinal cortex cholinergic terminal field density from Chat-tau:eGFP mice across 1.5-month (left), 3-months (middle), and 12-months (right) of age. Scale bar = 50um **B|** Representative high magnification confocal images of lateral entorhinal cortex cholinergic terminal field density as visualized by vesicular acetylcholine transporter (VAT) immunoreactivity at 3-months of age between 3-month (top row) vs. 12-month (bottom row) mice. Scale bar = 50um **C.** Quantification of cholinergic terminal field density as measured by tau:eGFP distribution in the entorhinal cortex across 1.5-month (n=5), 3-month (replicated from Figure 8C-top, n=6), and 12-month (replicated from Figure 8C-top, n=5) mice (1.5 vs. 3mo, p=0.54; 1.5 vs. 12 mo., *, p=0.008; 3 mo. vs. 12 mo., **, p=0.004)**.** **(continued on next page)**

Supplemental Figure 5| (related to Figure 8) Lateral entorhinal cortex cholinergic terminal density differs between 3 month and 12 month (naturally aged) mice (continued)

D. Quantification of cholinergic terminal density as measured by vesicular acetylcholine transporter (VAT) immunoreactivity at 3-months vs. 12-months of age (n=5/group, *, p=0.008).



Supplemental Figure 6| (related to Figure 5-8) Comparison of EC-related behavioral performance, EC activation, and EC cholinergic terminal density across sex in healthy aging

A. Experimental workflow. WT or ChAT-tau:eGFP animals were assessed in the displaced object recognition (DOR) task followed by tissue harvesting and cFos immunostaining OR measurement of cholinergic projection density in the EC.

B/E. Quantification of percent time spent exploring familiar vs. displaced object during the displacement session between male and female mice in **B.** 3-month (Male, n=10, Fam vs. Disp: **, p=0.002; Female, n=9, Fam vs. Disp: **, p=0.004; Male vs. Female: familiar object, p=0.3; displaced object, p=0.30) and **E.** 12-Month animals (Male, n=6, p=0.81; Female, n=7, p=0.69; Male vs. Female: familiar object, p=0.99; displaced object, p=0.99).

C/F. Quantification of total cFos activation in the EC during the displacement test between male and female mice **C.** 3-month (Male, n=4 vs Female, n=4, p=0.89) and **F.** 12-month (Male, n=2 vs Female, n=3, p=0.2).

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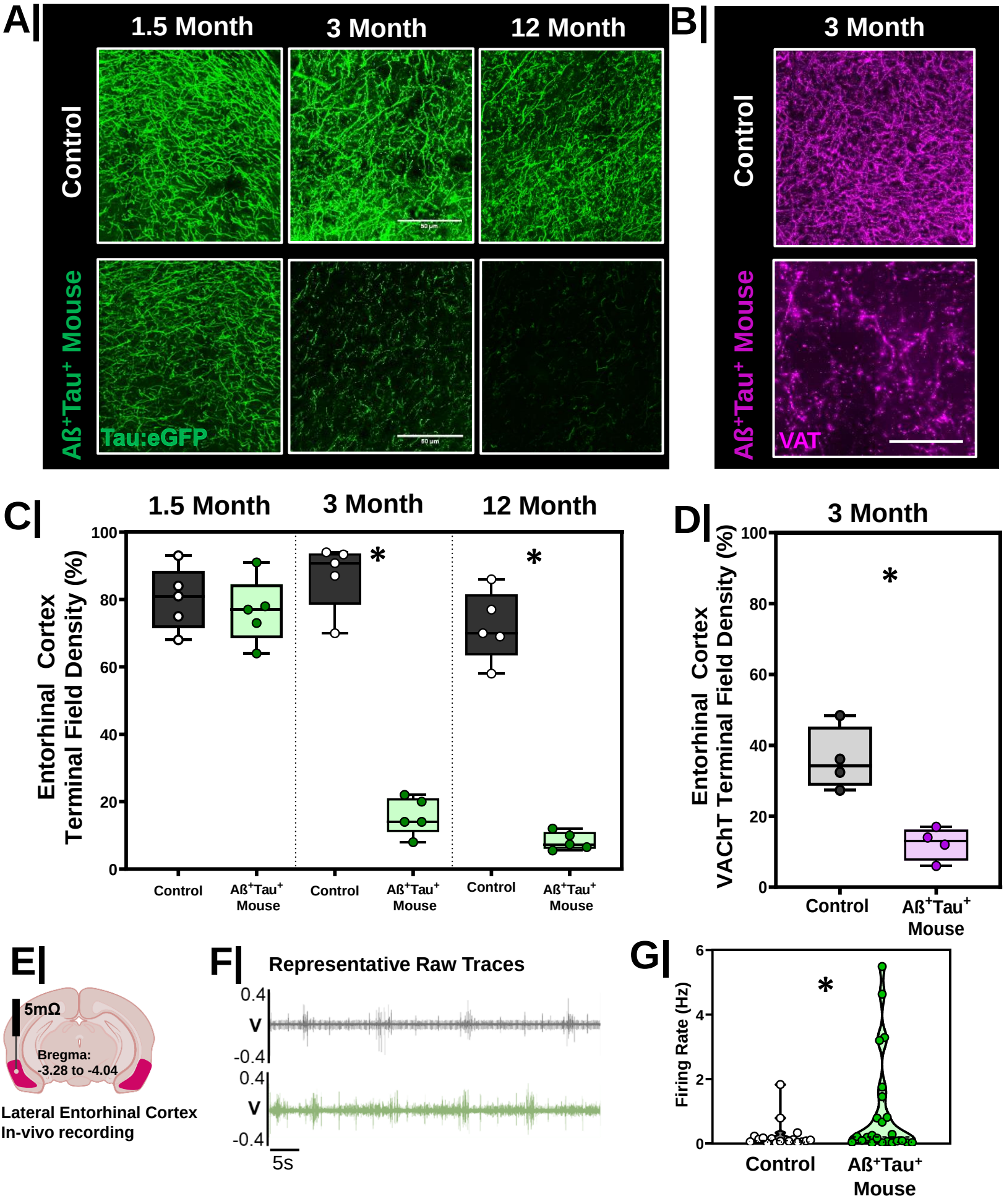
Supplemental Figure 6| (related to Figure 5-8) Comparison of EC-related behavioral performance, EC activation, and EC cholinergic terminal density across sex in healthy aging (continued)

D/G. Quantification of cholinergic terminal field density in the entorhinal cortex of male and female mice in **D.** 3-month (Male, n=3 vs Female, n=3, p=0.1) and **G.** 12-month animals. (Male, n=3 vs Female, n=3, p>0.99).



Supplemental Figure 7| (related to Figure 12) Lateral entorhinal cortex cholinergic terminal density and physiology differ in control vs. Aß$^{+}$Tau^{+} mice at 3 months of age (legend on next page)

Supplemental Figure 7| (related to Figure 12) Lateral entorhinal cortex cholinergic terminal density and physiology differ in control vs. Aß$^{+}$Tau^{+} mice at 3 months of age

A| Representative high magnification confocal images of lateral entorhinal cortex cholinergic terminal field density from Aß$^{+}$Tau^{+} X Chat-tau:eGFP mice across 1.5-month (left), 3-months (middle), and 12-months (right) of age between control (top row) vs. Aß+Tau+ (bottom row) mice. Scale bar = 50um **B|** Representative high magnification confocal images of lateral entorhinal cortex cholinergic terminal field density as visualized by vesicular acetylcholine transporter (VAT) immunoreactivity at 3-months of age between control (top row) vs. Aß$^{+}$Tau^{+} (bottom row) **mice.** Scale bar = 50um **C.** Quantification of cholinergic terminal field density as measured by tau:eGFP distribution in the entorhinal cortex across 1.5-month (p=0.55), 3-month (*, p=0.008, replicated from Figure 12B to show comparison), and 12-month (*, p=0.008) between control (n=5) and Aß$^{+}$Tau^{+} (n=5) animals**.** Controls = **Grey box**; Aß$^{+}$Tau^{+} = **Green box**. **D.** Quantification of cholinergic terminal density as measured by vesicular acetylcholine transporter (VAT) immunoreactivity at 3-months of age between control (n=4) and Aß$^{+}$Tau^{+} (n=4) mice (*, p=0.03). Controls = **Grey box**; Aß$^{+}$Tau^{+} = **Magenta box**. **E.** Schematic showing recording site for in vivo electrophysiological recording experiments in layer II-VI of lateral entorhinal cortex. **F.** Representative traces from control (top, **Grey trace**) and Aß$^{+}$Tau^{+} (bottom, **Green trace**) mice. Note differences in pattern and frequency of firing, with more disorganized firing patterns evident in Aß$^{+}$Tau^{+} mice. **G** Quantification of firing rate distribution in control (n=6) vs. Aß$^{+}$Tau^{+} (n=9) animals (*, p=0.012). Controls = **Grey violin distribution**; Aß$^{+}$Tau^{+} = **Green violin distribution.**