Sex-specific protection from inhibitory circuit disruption by retinoic acid signaling in a novel mouse model of Alzheimer's Disease

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1 Abstract

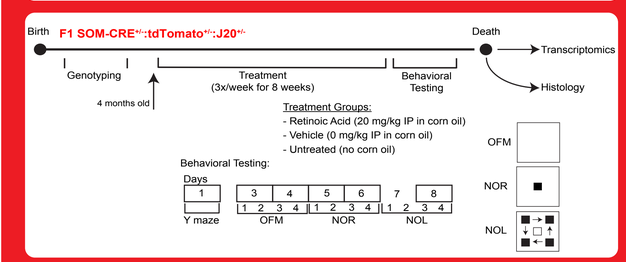
Vitamin A (VA) signaling disruption is observed in Alzheimer's disease (AD). Deficiency of retinoic acid (RA), a VA metabolite, may contribute to hippocampal dentate gyrus (DG) hyperactivity seen in the amnestic mild cognitive impairment stage of AD and alter excitation/inhibition (E/I) balance. Intact inhibitory somatostatin (SOM) and parvalbumin (PV) circuits normally maintain E/I balance, but the impact of RA on DG SOM and PV circuitry has not been investigated. The goal of this study was to evaluate the therapeutic effect of RA on DG SOM circuitry during AD pathogenesis. Triple transgenic J20+/- (AD) mouse models were generated, enabling examination of SOM circuitry via tdTomato (tdT) expression. Mouse models were treated with RA (RT) or vehicle (corn oil,VT) intraperitoneally and compared to age-matched J20-/- (WT) controls. Behavioral testing was peformed in the Y-maze and Open Field Maze. Brains were then hemisected for histological and transcriptomic analyses. Behavioral testing revealed VT AD mice traveled a greater distance than WT AD mice (U-5.000, p=0.009). RT AD and WT mice did not vary in overall distance travelled (U=16.000, p=0.727), suggesting phenotype normalization. Histological analysis revealed SOM:tdT expression in the DG inner molecular layer (IML) of AD, but not WT mice. SOM:tdT expression was absent in the DG IML of 5/6 male RT AD mice, consistent with a rescue of phenotype. However, SOM:tdT expression in the DG IML of 3/3 female RT AD mice persisted, indicating sex differences in RA signaling. Transcriptomic pairwise comparison of VT WT and AD to RT WT and AD showed partial normalization of differentially expressed genes, particularly within the Synaptogenesis Signaling pathway. RA appears to have protective effects against AD pathogenesis among males. The sex differences observed warrant further investigation involving a larger sample size of mice per group matched by age and sex.

2 Introduction

- Alzheimer's Disease (AD) is 6th leading cause of death in US.
- It is estimated that 5 million Americans live with AD and total estimated cost of care per person is $250,174.
- There is a need to investigate risk factors, such as diet that can allow for low-cost prophylactic modifications and could save billions of dollars.
- The Mediterranean diet, known for pairing vegetables and oils, mitigates AD risk, potentially by enhancing bioavailability of vitamin A (VA).
- Low or high serum VA levels correlate with accelerated or delayed AD onset, respectively.
- Hyperactivity in the human hippocampal dentate gyrus (DG) is observed in amnestic mild cognitive impairment stage of AD.
- During hyperactivity, synthesis of VA metabolite, retinoic acid (RA), may be impaired, suggesting RA deficiency may alter excitation/inhibition (E/I) balance, contribute to DG network hyperactivity, and lead to behavioral abnormalities.
- Intact inhibitory somatostatin (SOM) and parvalbumin (PV) circuits normally maintain E/I balance.
- **Objective**: **Investigate impact of RA signaling on DG SOM circuitry during AD pathogenesis**.

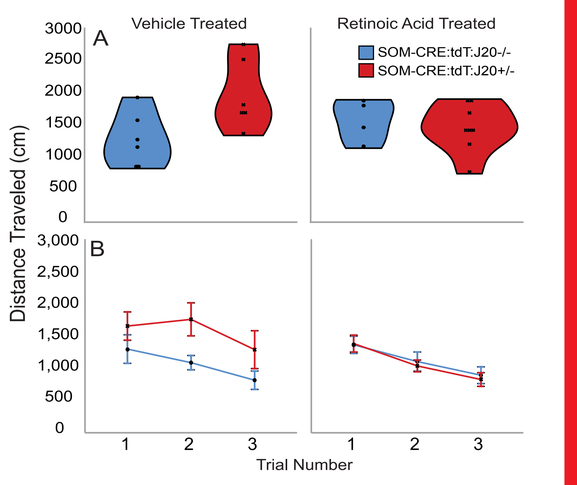
3 Experimental design

SOM-CRE mice (Jax strain #013044) were crossed with floxed tdTomato mice (Jax strain #007914) until homozygous SOM-CRE:tdTomato mice were generated. These mice were subsequently crossed with heterozygous hAPPJ20 AD mice (Jax #006293). F1 age-matched, gender-matched sibling littermates were weaned, genotyped, uniquely identified, and reserved for experiments. Test mice were chosen based on age and genotype, with an emphasis on cage paired J20-positive and wild type (J20-negative; WT) siblings. All test mice were aged within a fifteen-day range from the median age of 4 months for all mice used in injections. RA treated mice were dosed based on individual weight at 20mg RA/kg of mouse. RA was dissolved in DMSO/corn oil for generation of stock solution and storage. IP injections were administered three times a week between 1900 and 2100.



Behavioral tests were conducted over course of 8 days. On Day 1, 31 test mice were subjected to the Y-maze (15 minutes duration). On Days 3-4, 5-6, and 7-8, mice were subjected to 4 x 5 minute trials of Open Field Maze (OFM), Novel Object Recognition (NOR), and Novel Object Location (NOL). For NOL, the object was moved to a random location for each trial. Behavioral tests were conducted at 6.5 months of age. Mouse brains and livers were harvested for transcriptomic and metabolomic analyses and histology at 7 months. One hemisphere and liver was flash frozen with liquid nitrogen; one hemisphere was drop-fixed in 4% PFA in 0.1 M PBS overnight followed by 30% sucrose in 0.1 M PBS. For histological analysis, brains were embedded in OCT, cryosectioned to 40 µm, and stained with Neurotrace 435/455.

4 Retinoic-acid induced normalization of behavioral hyperactivity

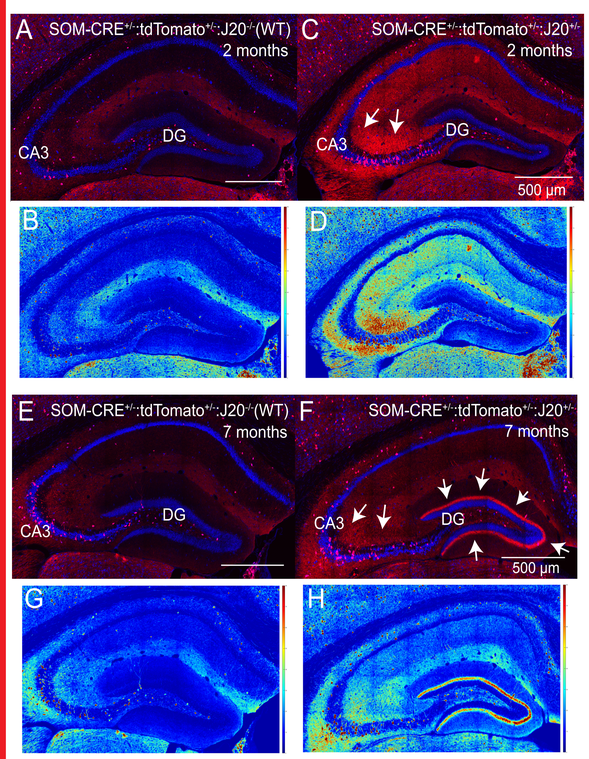


Hyperactivity was observed in both the Y-maze and Open Field Maze (OFM) in vehicle-treated AD mice (red) relative to WT mice (blue). An 8-week treatment with RA normalized behavior in both tasks.

Y-maze. Consistent with the hyperactivity phenotype of AD mice, VT AD mice traveled a greater distance compared to WT mice (U=5.000, p = 0.009). In contrast, RT AD and RT WT mice did not vary in overall distance traveled, indicating normalization of phenotype (U-16.000, p=0.727).

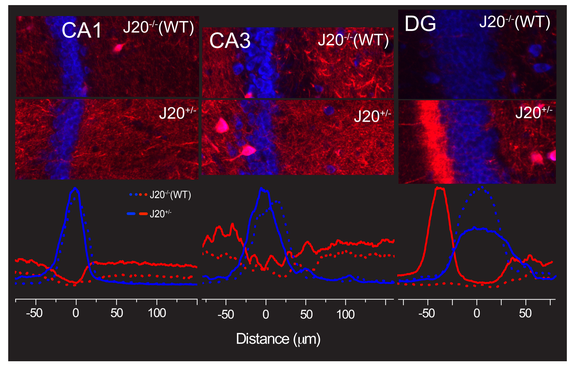
Open Field Maze (OFM). VT AD mice traveled a greater distance compared to WT (mean differece: 677 ± 277 cm) while, in contrast, RT AD and RT WT mice did not vary in overall distance traveled (mean difference = 127 ± 262 cm, p = 0.031).

5 Increased tdTomato expression in SOM-CRE+/-:tdTomato+/-:J20+/- mice



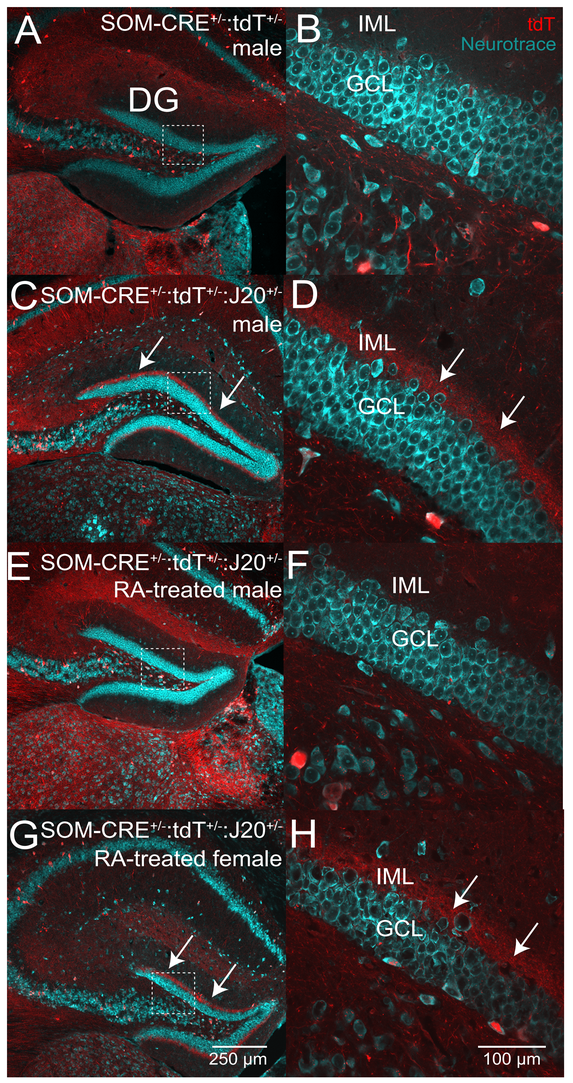
tdTomato expression in the dorsal hippocampus of sibling- and age-matched 2 month-old SOM-CRE+/-:tdTomato+/- littermates in which the J20 gene is (A) absent or (B) present. (C, D) tdTomato intensity heatmaps for (A,B) images, respectively. (E-H) Similar format for 7 month-old SOM-CRE+/-:tdTomato+/-:J20-/- and PV-CRE+/-:tdTomato+/-:J20+/- mice, respectively. Red channels have been evenly intensified for display.

6 Laminar distribution of tdTomato expression in CA1, CA3, and DG



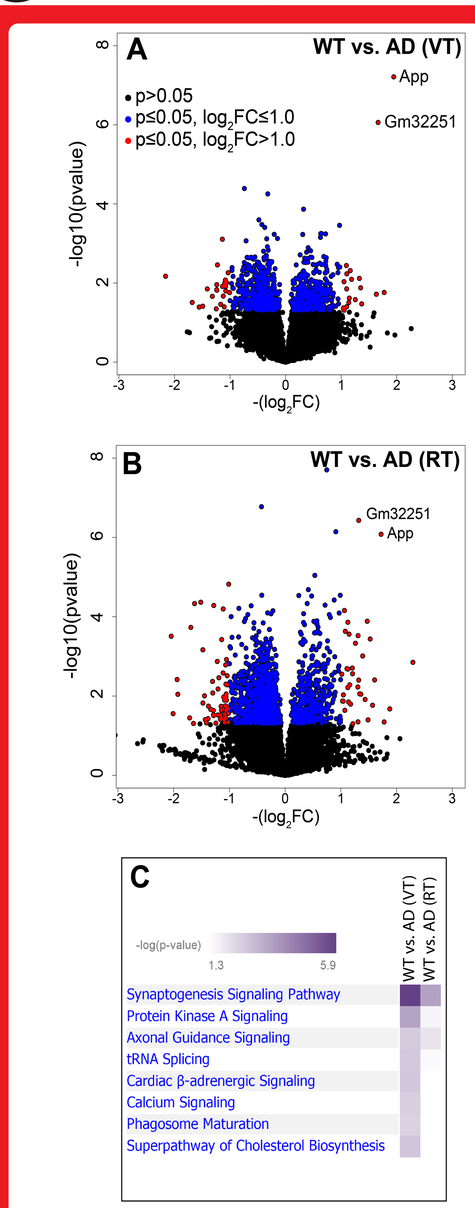
Laminar distribution of tdTomato expression in CA1, CA3, and DG. Dotted lines are WT; solid lines are from J20 mice. tdTomato expression is enhanced outside of the principal cell layer. Zero distance is estimated to be the peak of Neurotrace intensity in the prinicpal cell layers. Note the peak in tdTomato expression in the inner molecular layer of the DG.

7 Sex-specific prevention of DG SOM:tdT circuitry by RA treatment



Representative DG confocal images from male WT (A, B), male AD (C, D), RT AD male (E,F), and RT AD female (G, H). SOM: tdT expresion was found in the DG inner molecular layer (IML) of AD mice (C, D), but not WT mice (A, B). SOM: tdT expression was absent in the DG IML layer of RA treated AD mice, consistent with rescue of phenotype (E, F). SOM:tdT expression persisted in DG IML layer of female mice (G, H). RA tratment prevented SOM:tdT circuitry in DG IML in male, but not female mice.

8 Molecular pathways are normalized by RA treatment



Volcano plot illustrates distribution oF differentially expressed genes (DEGs) for WT vd AD VT populations (A) and WT vs AD RT populations (B). RPKM threshold was set to be greater than 2. Gene labels are for top two genes that fall within less than or equal to 0.05 and have a log2. Fold Change (FC) greater than -1 or 1. Pairwise comparison of WT VT vs AD VT groups revealed molecular pathways dysregulated (B). The top 8 dysregulated molecular pathways are shown based on -log(p-value). Pairwise comparison of WT vs AD RT data revealed that RT normalized 6/8 molecular pathways to WT expression levels and reduced 2/8 pathways towards WT expression levels.

9 Conclusions

1. Retinoic acid rescues the hyperactivity phenotype of AD mice.

2. Increased tdTomato expression in SOM-CRE: tdTomato J20 mice suggests SOM circuits are affected differently than PV circuits during AD pathogenesis.

3. RA appears to have a protective effect in the AD pathogenesis of males.

4. The Synaptogenesis Signaling pathway may be involved in the mechanism by which retinoic acid rescues the behavioral hyperactivity phenotype of AD mice.

10 Acknowledgements

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