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Targeting Neuronal Activity and Neuroinflammation in Alzheimer's Disease

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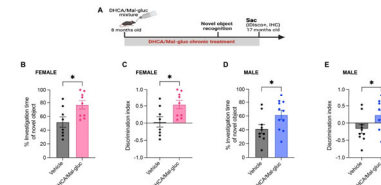
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Abstract

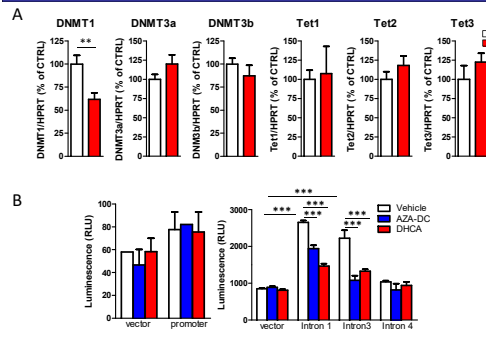
Alzheimer's disease (AD) is characterized by progressive cognitive decline driven by complex pathological processes, including tau hyperphosphorylation (p-Tau), amyloid-beta (A β) accumulation, and neuroinflammation. In this study, we investigated the effects of two bioactive compounds, dihydrocaffeic acid (DHCA) and malvidin-glucoside (Mal-gluc), targeting inflammation and neuronal activity, respectively, on cognitive function and AD pathology in a mouse model of AD. Our results demonstrate that chronic DHCA/Mal-gluc treatment significantly improves recognition memory in 3xTg-AD mice without reducing p-Tau or A β burden. Employing a newly developed whole-brain cFOS and IBA-1 mapping technique, we found that this combination treatment enhances neuronal activity and promotes microglial homeostasis across multiple brain regions in 3xTg-AD mice. These findings underscore the potential of restoring neuronal function and immune homeostasis as a therapeutic approach for AD. Future study will explore the underlying mechanisms and evaluate whether DHCA/Mal-gluc, combined with currently approved A β monoclonal therapy, can synergistically prevent or delay AD onset and progression.

Chronic Treatment Improves Recognition Memory in 3xRg Mice



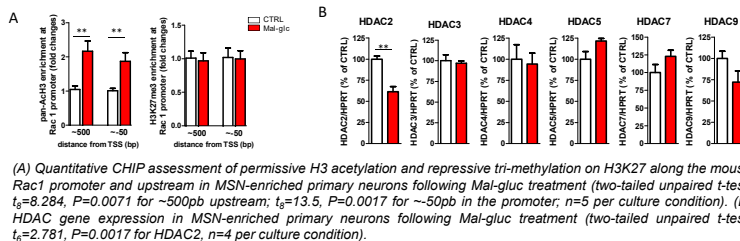
Mice were treated with oral administration of DHCA/Mal-gluc delivered through their drinking water starting at 8 months of age when the mice have already developed synaptic impairment as well as increased neuroinflammation. Following treatment, mice were subjected to novel object recognition (NOR) test that leverages rodents' natural tendency to explore novel stimuli over familiar ones, for recognition memory assessment (A) Experimental design. (B-C) NOR in female 3xTg mice. (B) Percentage of investigation time of novel object in female 3xTg mice; (C) Discrimination index in female 3xTg mice; n=8-9 per group, unpaired t test, p=0.0203. (D-E) NOR in male 3xTg mice. (D) Percentage of investigation time of novel object in male 3xTg mice; (E) Discrimination index in male 3xTg mice; n=10-11 per group, unpaired t-test, p=0.0377. Data are presented as mean $\pm$ SEM. *p < 0.05.

DHCA Attenuates IL-6 Expression by Modulating CpG Methylation of The IL-6 Gene at Select Intronic Regions



(A) The expression of de novo methylation and demethylation genes in PBMCs following DHCA treatment (two-tailed unpaired t-test, $t_{12}=3.220$, $P=0.0074$ for DNMT1, n=7 per culture condition). (B) Assessment of methylation-mediated promoter and enhancer-like activities of CpG-rich sequences in the regions of IL-6 promoter (left panel) and introns (right panel) in transfected NZA cells in the presence or absence of methylation inhibitor AZA-DC or DHCA (Left panel for promoter activity, one-way ANOVA $F_{5,17}=1.278$, $P=0.335$, n=3 per culture condition; Right panel for enhancer activity, one-way ANOVA $F_{1,35}=38.75$, $P<0.0001$, n=3 per culture condition).

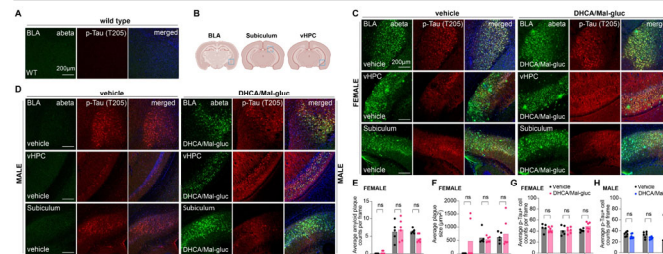
Mal-gluc Increases Rac1 Expression by Modulating the Acetylation Status of The Rac1 Gene Promoter Regions



(A) Quantitative CHIP assessment of permissive H3 acetylation and repressive tri-methylation on H3K27 along the mouse Rac1 promoter and upstream in MSN-enriched primary neurons following Mal-gluc treatment (two-tailed unpaired t-test, $t_8=8.284$, $P=0.0071$ for ~ 500 bp upstream; $t_8=13.5$, $P=0.0017$ for ~ 50 bp in the promoter; n=5 per culture condition). (B) HDAC gene expression in MSN-enriched primary neurons following Mal-gluc treatment (two-tailed unpaired t-test, $t_8=2.781$, $P=0.0017$ for HDAC2, n=4 per culture condition).

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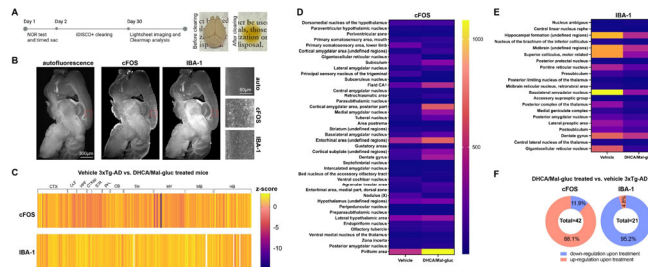
Assessment of AD Pathology in 3xTg mice Following Treatment



(A) Representative images of A β and p-Tau (T205) staining in basolateral amygdala (BLA) of age matched wild type mice. (B) Schematic diagrams of brain regions assessed for A β and p-Tau (T205) expression. (C) Representative images of A β and p-Tau (T205) staining in BLA, ventral hippocampus (vHPC) and subiculum in 17-month-old vehicle vs. DHCA/Mal-gluc treated 3xTg female mice. (D) Representative images of A β and p-Tau (T205) staining in BLA, vHPC and subiculum in 17-month-old vehicle vs. DHCA/Mal-gluc treated 3xTg male mice. (E) Quantification of A β plaque counts in BLA, subiculum and vHPC of vehicle vs. DHCA/Mal-gluc treated 3xTg female mice. 3 frames (1 frame = 105 μ m²) per

mouse in each brain region, n=5 or 6 mice/group. The average count of different frames per mouse in each brain region was presented as independent point in graphs. Two-way ANOVA for analysis. (F) Quantification of area per A β plaque in BLA, subiculum and vHPC of female 3xTg-AD vs. (3xTg-AD+ treatment) mice. 3 frames (1 frame = 105 μ m²) per mouse per mouse in each brain region, n=5 or 6 mice/group. The average plaque size per mouse in each brain region was presented as independent point in graphs. Two-way ANOVA for analysis. (G) Quantification of number of p-Tau (T205) positive cells in BLA, subiculum and vHPC of vehicle vs. DHCA/Mal-gluc 3xTg female mice. 3 frames (1 frame = 105 μ m²) per mouse in each brain region, n=5 or 6 mice/group. The average count of different frames per mouse in each brain region was presented as independent point in graphs. Two-way ANOVA for analysis. (H) Quantification of number of p-Tau (T205) positive cells in BLA, subiculum and vHPC of vehicle vs. DHCA/Mal-gluc 3xTg male mice. 3 frames (1 frame = 105 μ m²) per mouse in each brain region, n= 6 mice/group. The average count of different frames per mouse in each brain region was presented as independent point in graphs. Two-way ANOVA for analysis. Data are presented as mean $\pm$ SEM. n.s. p>0.05, ***p<0.001, Scale bar, 200 μ m.

Whole-brain cFOS and IBA-1 Mapping Following Treatment



number of cFOS-positive cells in different brain subregions. (E) Brain subregions with significant difference in IBA-1 protein expression (p<0.05) between vehicle and treated 3xTg mice. Heatmap shows the numbers of IBA-1-positive cells in different brain subregions. (F) Pie chart illustrating up- or down-regulation of cFOS and IBA-1 protein levels across brain regions following treatment.

Summary:

- Bioactive phytochemicals target neuronal activity and neuroinflammation
- Whole-brain c-Fos and Iba-1 mapping confirms target engagement
- Restore brain function and immune homeostasis without interfering A β or tau