

ORIGINAL RESEARCH COMMUNICATION

Illumination with 630 nm Red Light Reduces Oxidative Stress and Restores Memory by Photo-Activating Catalase and Formaldehyde Dehydrogenase in SAMP8 Mice

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Abstract

Aims: Pharmacological treatments for Alzheimer's disease (AD) have not resulted in desirable clinical efficacy over 100 years. Hydrogen peroxide (H₂O₂), a reactive and the most stable compound of reactive oxygen species, contributes to oxidative stress in AD patients. In this study, we designed a medical device to emit red light at 630 ± 15 nm from a light-emitting diode (LED-RL) and investigated whether the LED-RL reduces brain H₂O₂ levels and improves memory in senescence-accelerated prone 8 mouse (SAMP8) model of age-related dementia.

Results: We found that age-associated H₂O₂ directly inhibited formaldehyde dehydrogenase (FDH). FDH inactivity and semicarbazide-sensitive amine oxidase (SSAO) disorder resulted in endogenous formaldehyde (FA) accumulation. Unexpectedly, excess FA, in turn, caused acetylcholine (ACh) deficiency by inhibiting choline acetyltransferase (ChAT) activity *in vitro* and *in vivo*. Interestingly, the 630 nm red light can penetrate the skull and the abdomen with light penetration rates of ~49% and ~43%, respectively. Illumination with LED-RL markedly activated both catalase and FDH in the brains, cultured cells, and purified protein solutions, all reduced brain H₂O₂ and FA levels and restored brain ACh contents. Consequently, LED-RL not only prevented early-stage memory decline but also rescued late-stage memory deficits in SAMP8 mice.

Innovation: We developed a phototherapeutic device with 630 nm red light, and this LED-RL reduced brain H₂O₂ levels and reversed age-related memory disorders.

Conclusions: The phototherapy of LED-RL has low photo toxicity and high rate of tissue penetration and noninvasively reverses aging-associated cognitive decline. This finding opens a promising opportunity to translate LED-RL into clinical treatment for patients with dementia. *Antioxid. Redox Signal.* 00, 000–000.

Keywords: oxidative stress, hydrogen peroxide, acetylcholine, formaldehyde, memory, light-emitting diode with red light

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Innovation

The major finding in this study is that we developed a medical device to emit 630 nm red light from a light-emitting diode (LED-RL), and this LED-RL not only prevented early-stage memory decline but also rescued late-stage memory deficits in senescence-accelerated prone 8 mouse (SAMP8) mice.

The major results from the study are outlined as follows:

- (i) Age-associated hydrogen peroxide (H_2O_2) inactivates formaldehyde dehydrogenase (FDH).
- (ii) FDH inactivation and semicarbazide-sensitive amine oxidase disorder leads to formaldehyde (FA) accumulation.
- (iii) Excess FA reduces brain acetylcholine (ACh) levels by inhibiting choline acetyltransferase activity.
- (iv) LED-RL reduces brain H_2O_2 and FA levels by activating catalase and FDH.
- (v) LED-RL restores brain ACh levels and rescues memory function.

Introduction

ALZHEIMER'S DISEASE (AD) is the most frequent cause of cognitive dysfunction in elderly individuals (40). However, over the past decades, pharmacological therapies targeting Tau or amyloid-beta by antibodies or vaccines have not resulted in desirable clinical effects (46). Development of γ -secretase inhibitor has been carrying out and worthy for expectation (16, 30). Only medicines for AD targeting the enzymes for regulating acetylcholine (ACh) metabolism have been found to benefit symptoms of dementia by far (46). Oxidative stress has been recognized as a contributing factor in aging and in the pathological processes underlying AD (48, 54). A classic animal model, the senescence-accelerated prone 8 mouse (SAMP8), is widely used to study oxidative stress and age-related dementia (63a). This rodent model exhibits a marked elevation in the levels of reactive oxygen species (ROS) and a decline in levels of brain ACh (43, 77). Consistently, brain ACh deficiency and ROS overload are closely correlated with cognitive decline in patients with AD (34, 55). However, the relationship between brain ROS overload and ACh deficiency is not clear.

Strikingly, hydrogen peroxide (H_2O_2 , a reactive and the most stable compound of ROS) (29), which can be degraded by catalase, directly inhibits alcohol dehydrogenase 3 (ADH3) (39, 51). ADH3 is also named formaldehyde dehydrogenase (FDH, a formaldehyde-degrading enzyme) (65). Inhibition of FDH causes formaldehyde (FA) accumulation (68). Surprisingly, compared with the senescence-accelerated resistant mouse-1 (SAMR1) mice, SAMP8 mice exhibited an abnormally high level of brain FA (50). Healthy, adult wild-type mice exposed to gaseous FA or injected with aqueous FA can mimic age-related memory decline associated with brain ACh deficiency and lower activity of choline acetyltransferase (ChAT) (36, 67). Furthermore, excess FA had been found in the brains of amyloid precursor protein/presenilin 1 (APP/PS1) transgenic AD mice (these double transgenic mice expressed a chimeric mouse/human amyloid precursor protein [Mo/HuAPP695swe] and a mutant human presenilin 1 [PS1-dE9]), the morning urine of patients with dementia, and the autopsy samples of hippo-

campus from AD patients (69, 70). These data strongly suggest that age-associated H_2O_2 may inhibit the FDH activity and induce FA accumulation; consequently, excess FA may suppress the ChAT activity and lead to ACh deficiency-related memory decline.

Substantial studies indicate that visible light affects the structures and/or activities of FDH and catalase. It is known that red light at 630 nm is widely used in traffic lights because human eye is substantially sensitive to 630 nm red light (75). FDH (ADH3) is disturbed in the retina (27) and transfers retinol to retina, which is required for visual sensation (25). FDH is regarded as the ancestral enzyme for the entire ADHs family (28, 61), and ADHs are sensitive to ultraviolet (UV) and visible light in yeast, mouse, and rat (19, 23a, 27). The proteins of horse ADH have an absorbance peak at ~ 650 nm (76). Red light at 630 nm can reduce FA-induced lung inflammation (53). FDH can degrade FA (65). These data suggest that red light at 630 ± 15 nm may activate FDH to scavenge FA. Coincidentally, catalase may be sensitive to 630 nm red light. It has two absorbance peaks at ~ 405 and ~ 630 nm (2, 9), which can be photo activated by 632–640 nm red laser (73). The activity of catalase in plants and animals can be regulated by UV (78, 79) and visible light (21, 41, 57). Therefore, in this study, we designed a medical device to emit red light at 630 ± 15 nm from a light-emitting diode (LED-RL) and investigated whether the LED-RL activates catalase and FDH, reduces H_2O_2 and FA levels, and improves memory in the SAMP8 model of oxidative stress and age-related dementia.

Results

LED-RL at 630 nm penetrates into the skull and the abdomen of SAMP8 mice

To carry out phototherapy on SAMP8 mice (Fig. 1A), we fixed the mice in the mouse sleeve (Lucite pipe) and directly illuminated the skull using LED-RL through a head light source. Similarly, we treated the abdomen close to the liver using an abdominal light source (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/ars) because FDH is widely distributed in the liver and functionally degrades FA rapidly (65). The head light source was placed on the skull, and the spectrophotometric detector was placed under the mandibula of the brain (Fig. 1B, C); the penetration rate in the brain was 49%. Moreover, the penetration rate of the red light penetrating into the abdomen was 43% when the abdomen light source was placed under the abdomen (close to the liver), and the detector was placed on the back (Fig. 1D and Supplementary Figs. S2 and S3). These data indicate that LED-RL at a wavelength of 630 ± 15 nm and light power density of 0.5 mW/cm^2 can penetrate the skull and the abdomen of mice (Supplementary Fig. S4), suggesting that red light might affect the functions of the brain and the liver.

LED-RL prevents early-stage memory decline in SAMP8 mice at 5-month-old age

To examine whether LED-RL prevents age-related memory decline in SAMP8 mice when spatial memory is initially declined at 3-month-old age, we used LED-RL treatment for two consecutive months (Supplementary Fig. S2A) and then tested memory behaviors of mice by using the Morris water

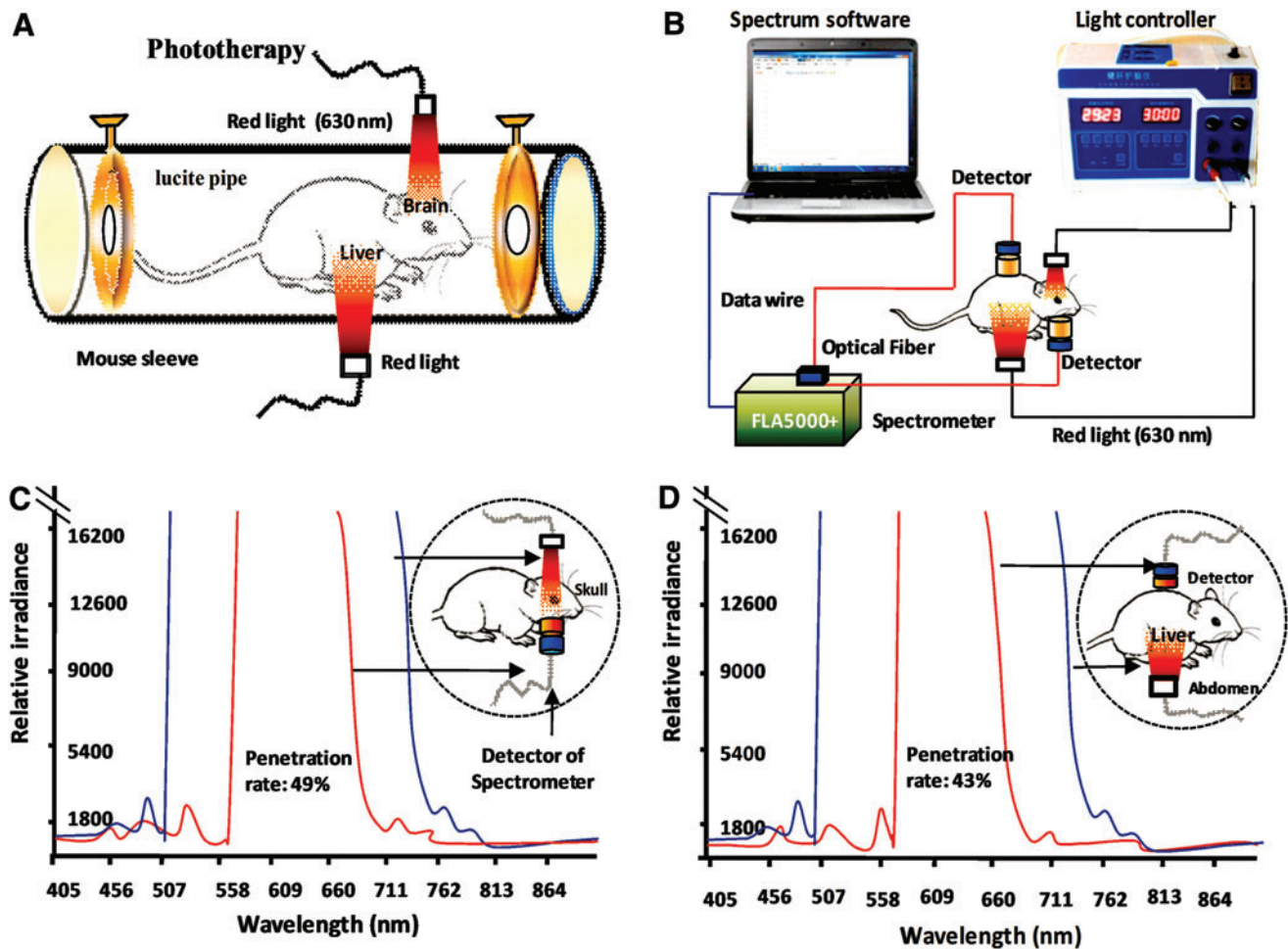


FIG. 1. LED-RL at 630 nm penetrated the skull and the abdomen of SAMP8 mice. (A) Illumination pattern of the phototherapy with LED-RL. (B) Method used to detect the penetration ratio of RL at the skull and the abdomen of the mice. (C, D) The penetration rate of RL to the skull (49%) and the abdomen (43%) of the mice. LED-RL, light-emitting diode with red light; RL, red light; SAMP8, senescence-accelerated prone 8 mouse. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

maze (Fig. 2A). The results showed that SAMP8 mice exhibited a marked decline in their spatial learning ability, in which their escape latency was longer when compared with that of SAMR1 mice (Fig. 2B). Moreover, the time spent in the target quadrant of SAMP8 mice was lower than that of SAMR1 mice (Fig. 2C). However, SAMP8 mice treated with LED-RL showed a marked rescue in the abilities of spatial learning and memory recall compared with SAMP8 mice without LED-RL treatment (Fig. 2B, C and Supplementary Fig. S5). Thus, LED-RL treatment prevents age-associated memory decline.

To test whether excess FA contributes to age-associated memory decline, we measured brain FA levels and the activities of FA metabolism-related enzymes. The results showed that semicarbazide-sensitive amine oxidase (SSAO) activity [a blood FA-generating enzyme (14)] was elevated, and the FDH activity [a FA-degrading enzyme (65)] was decreased in the brains of SAMP8 mice compared with SAMR1 mice (Fig. 2D–F). However, after LED-RL treatment, brain FA levels were decreased, the protein expression levels of FDH and catalase were increased, and the activities of SSAO and FDH were recovered to normal levels in

SAMP8 mice (Fig. 2D–F and Supplementary Fig. S6). These results indicate that LED-RL enhances brain FA metabolism.

To determine whether ROS is a critical factor for inducing brain Ach deficiency, we measured brain H_2O_2 levels, a reactive component of ROS. The results showed that the catalase activity (which degrades H_2O_2) was lower, and brain H_2O_2 levels were higher in SAMP8 mice than SAMR1 mice (Fig. 2G, H). Next, we detected the activity of brain ChAT (which synthesizes Ach) and the levels of brain Ach. We found that both ChAT activity and Ach levels were lower in SAMP8 mice than SAMR1 mice (Fig. 2I, J). However, LED-RL treatment for 2 months reduced brain H_2O_2 levels, improved brain Ach concentrations, and restored the activities of catalase and ChAT in the brains of SAMP8 mice (Fig. 2G–J).

We also found that there were negative correlations between brain H_2O_2 level and FDH activity ($R = -0.729$), between brain FA level and ChAT activity ($R = -0.895$), and between brain FA level and the time spent in the target quadrant ($R = -0.501$) (Fig. 2K–M). However, there were positive correlations between the brain ChAT activity and the time spent in the target quadrant ($R = 0.599$) and between the brain Ach level and the

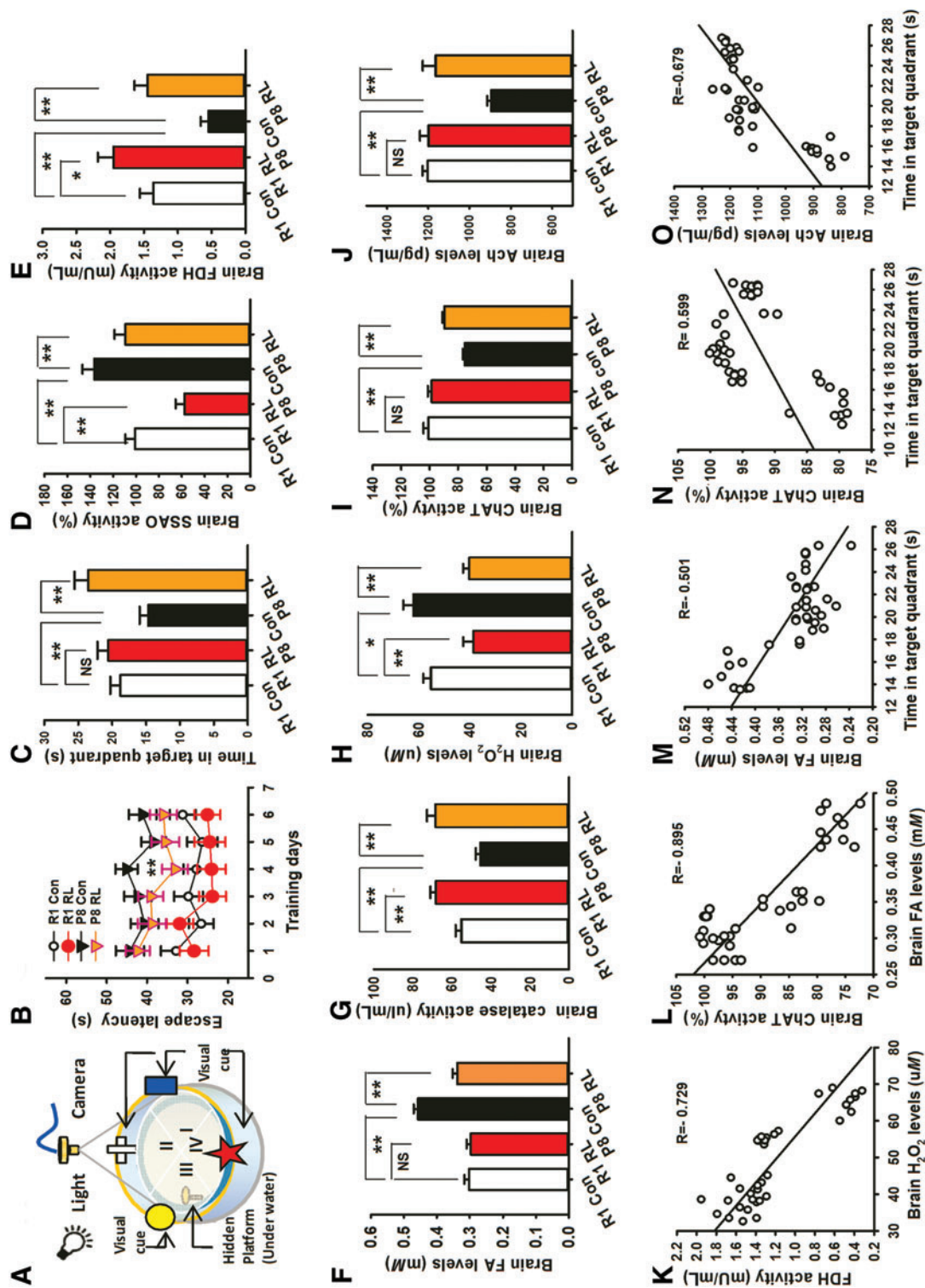


FIG. 2. LED-RL illumination prevents early-stage memory decline in SAMP8 mice at 5-month-old age. (A) Experimental setup of the Morris water maze. (B, C) LED-RL illumination improved the abilities of spatial learning and memory recall in SAMP8 mice. (D) LED-RL reduced the SSAO activity in SAMP8 mice. (E, F) LED-RL enhanced the FDH activity and reduced brain formaldehyde levels in SAMP8 mice. (G, H) LED-RL enhanced the brain catalase activity and reduced brain H₂O₂ levels in SAMP8 mice. (I, J) LED-RL enhanced the brain ChAT activity and improved brain ACh activity and the time spent in the target quadrant in SAMP8 mice. (K–M) Negative relationship between brain H₂O₂ level and FDH activity, between brain formaldehyde level and ChAT activity, and between brain ACh level and the time spent in the target quadrant in SAMP8 mice. (N, O) Positive relationship between brain ChAT activity and the time spent in the target quadrant and the time spent in the target quadrant of SAMP8 mice. $n = 10$; * $p < 0.05$; ** $p < 0.01$. Ach, acetylcholine; ChAT, choline acetyltransferase; FDH, formaldehyde dehydrogenase; H₂O₂, hydrogen peroxide; NS, no statistical significance; SSAO, semicarbazide-sensitive amine oxidase. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

time spent in the target quadrant ($R=0.679$) (Fig. 2N, O). In addition, liver FDH and catalase are important enzymes for degrading FA and H_2O_2 (65). We found that LED-RL restored liver FA and H_2O_2 levels (Supplementary Fig. S7). These data indicate that LED-RL reduces age-related FA accumulation and increases brain Ach levels.

LED-RL reverses late-stage memory deficits in SAMP8 mice at 7-month-old age

To investigate whether LED-RL rescues age-related memory deficits in SAMP8 mice at 7 months of age, we evaluated the changes in memory behaviors of SAMP8 mice before and after LED-RL treatment. The results showed that the two groups of SAMP8 mice before LED-RL treatment exhibited similar spatial learning and memory recall abilities as they showed similar escape latencies and similar amounts of time spent in the target quadrant (Fig. 3A, B and Supplementary Fig. S8A–D). However, after LED-RL illumination for 2 months, SAMP8 mice exhibited better spatial learning and memory recall abilities, as their post-treatment escape latency was shorter and the time spent in the target quadrant was longer than SAMP8 mice without LED-RL treatment (Fig. 3C, D and Supplementary Fig. S8E–H). These results indicate that LED-RL treatment reverses age-related memory deficits.

Next, we measured brain FA levels and the activities of enzymes involved in FA metabolism *in vivo*. The results showed that the SSAO activity was higher and the FDH activity was lower in the brains of SAMP8 mice than SAMR1 mice; these trends were associated with higher FA levels in SAMP8 mice than SAMR1 mice (Fig. 3E–G). However, LED-RL treatment for 2 months reduced brain FA levels, increased FDH and catalase expression, and reversed the FDH activity (Fig. 3E–G and Supplementary Fig. S9), indicating that LED-RL restores brain FA metabolism.

We also found that the catalase activity was lower and brain H_2O_2 level was higher in SAMP8 mice than SAMR1 mice (Fig. 3H, I), while both the ChAT activity and Ach level were lower in SAMP8 mice than SAMR1 mice (Fig. 3J, K). However, LED-RL treatment decreased brain H_2O_2 levels, improved brain Ach levels, and restored the activities of catalase and ChAT in the brains of SAMP8 mice (Fig. 3H–K). Moreover, there were negative correlations between the brain H_2O_2 level and FDH activity ($R=-0.926$), between brain FA level and ChAT activity ($R=-0.873$), and between brain FA level and the time spent in the target quadrant ($R=-0.489$) (Fig. 3L–N), but there was also a positive correlation between brain ChAT activity and the time spent in the target quadrant ($R=0.584$) (Fig. 3O). In addition, LED-RL treatment also restored liver FA and H_2O_2 levels (Supplementary Fig. S10). Thus, LED-RL can reverse age-related memory deficits by reducing brain FA accumulation and increasing brain Ach levels.

LED-RL reverses FA-induced memory deficits in healthy adult mice

To investigate whether excess FA is a direct factor for age-associated memory deficits, we injected healthy adult mice with a pathologically high concentration of FA (0.5 mM, intraperitoneally, once daily) for 1 month and assessed their memory behaviors. The results showed that FA injection induced a marked impairment in the abilities of spatial learning and memory recall, as evidenced by the longer es-

cape latencies and the shorter time periods spent in the target quadrant in FA-injected mice than healthy control mice (Fig. 4A, B). In fact, LED-RL treatment reversed FA-induced memory deficits (Fig. 4A, B and Supplementary Fig. S11).

Next, we observed the effects of LED-RL treatment on the activities of FDH, catalase, and ChAT and on the concentrations of brain FA, H_2O_2 , and Ach. The results showed that FA injection induced a marked increase in the levels of brain FA and H_2O_2 and a decline in the activities of catalase, ChAT, and brain Ach levels, in particular, an elevated FDH activity (Fig. 4C–H). This transiently elevated FDH activity induced by acute FA is most likely due to the fact that the organism such as mice needs this rapidly compensatory increase in the FDH activity to degrade FA for preventing FA stress (71). However, LED-RL treatment restored the levels of brain FA, H_2O_2 , and Ach, the activities of FDH, catalase, and ChAT, and increased FDH and catalase expression (Fig. 4C–H and Supplementary Fig. S12). Moreover, LED-RL also restored liver FA and H_2O_2 levels (Supplementary Fig. S13).

To further examine whether H_2O_2 inhibits the FDH activity and FA suppresses the ChAT activity *in vitro*, we incubated the brain sections obtained from healthy adult mice with different concentrations of H_2O_2 and FA and measured the activities of brain FDH and ChAT. The results showed that after 3 h of incubation period, H_2O_2 inhibited the brain FDH activity in a dose-dependent manner (Fig. 4I) and FA dose dependently inhibited the brain ChAT activity (Fig. 4J). These data demonstrate that H_2O_2 leads to FA accumulation by inhibiting FDH and FA reduces brain Ach levels by suppressing the ChAT activity.

LED-RL enhances FDH and catalase activities in cultured N2a cells

Above data indicate that LED-RL treatment enhances the activities of FDH and catalase *in vivo*. We further investigated whether LED-RL increases their activities in cultured N2a cells. First, the results of confocal immunofluorescence microscopy revealed that these two enzymes were colocalized in the cytoplasm of the brains, livers, and N2a cells (Fig. 5A–C and Supplementary Fig. S14). Next, we observed the effects of LED-RL treatment on cell viability after illumination for different time intervals under an air exposure condition (LED-RL device could not be placed inside the cell culture incubator). These results showed that air exposure induced a time-dependent decline in cell viability; however, LED-RL treatment rescued this decline (Fig. 5D). Furthermore, LED-RL treatment for 20 and 40 min markedly enhanced the activities of FDH and catalase (Fig. 5E, F).

We also found that both FA and H_2O_2 induced cellular toxicity in a dose-dependent manner (Fig. 5G–J). However, LED-RL treatment attenuated their toxicity effects and reversed FA-inhibited catalase activity and H_2O_2 -inhibited FDH activity in cultured N2a cells (Fig. 5G–J). It is known that the acidification of culture medium is a common phenomenon in cultured cell death (26). Notably, LED-RL illumination did not induce an elevation in the temperature of the purified protein solution at room temperature of $24 \pm 0.5^\circ\text{C}$ (Supplementary Fig. S15A–C); however, red light obviously resisted the acidification of the culture medium of human SY5Y cells for 24 h (Supplementary Fig. S15D–E). Taken together, LED-RL treatment improves the activities

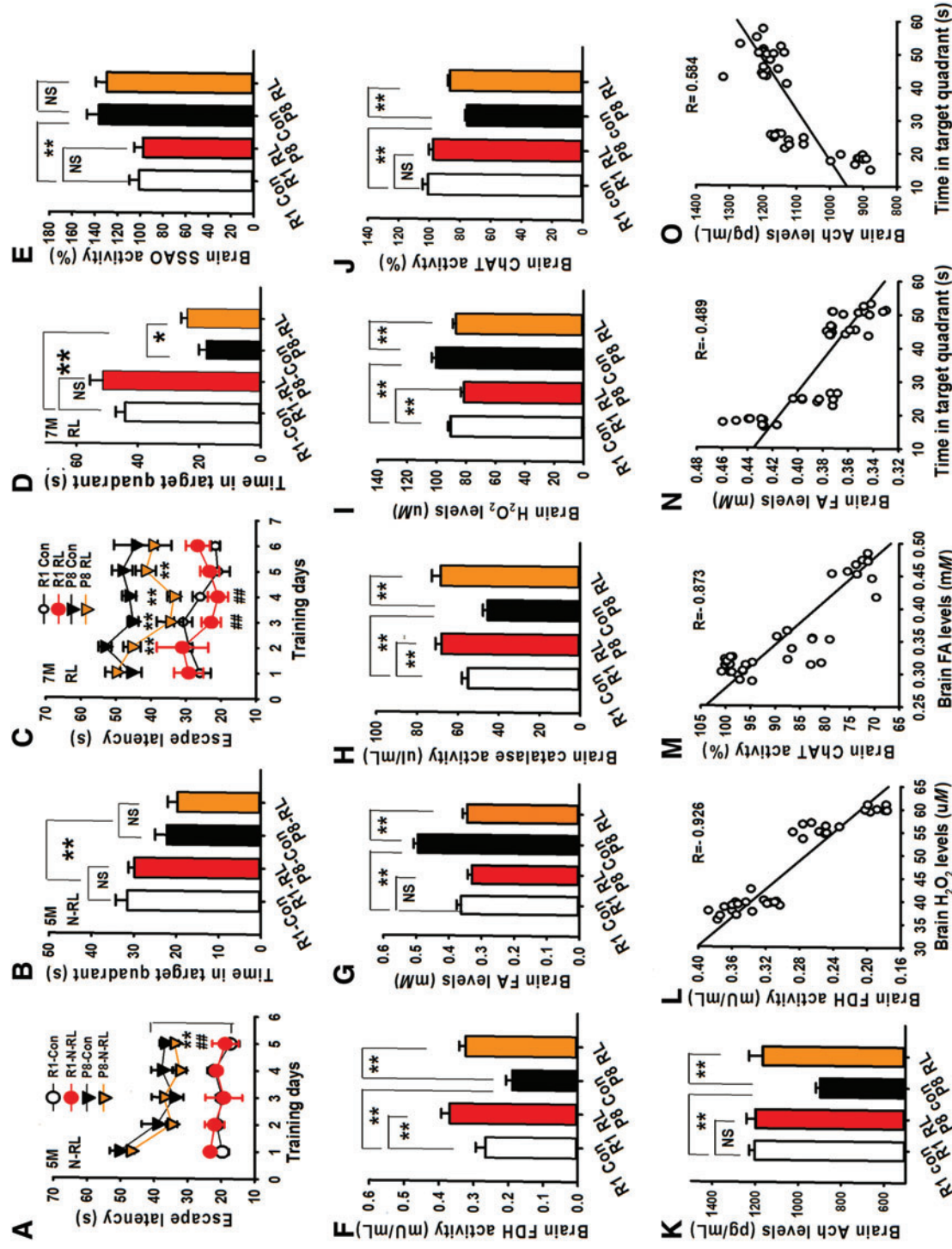


FIG. 3. LED-RL treatment reverses late-stage memory deficits in SAMP8 mice at 7-month-old age. (A, B) The SAMP8 mice showed no differences in the abilities of spatial learning and memory recall before LED-RL illumination. (C, D) LED-RL treatment improved the abilities of spatial learning and memory recall in SAMP8 mice. (E) LED-RL did not affect the SSAO activity in SAMP8 mice. (F, G) LED-RL enhanced the FDH activity and reduced brain formaldehyde levels in SAMP8 mice. (H, I) LED-RL enhanced the brain catalase activity and reduced brain H₂O₂ levels in SAMP8 mice. (J, K) LED-RL enhanced the brain ChAT activity and improved brain Ach concentrations in SAMP8 mice. (L-N) Negative relationship between brain H₂O₂ level and FDH activity, between brain formaldehyde level and ChAT activity, and between brain formaldehyde level and the time spent in the target quadrant in SAMP8 mice. (O) Positive relationship between brain Ach level and the time spent in the target quadrant in SAMP8 mice. $n = 10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, no statistical significance. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

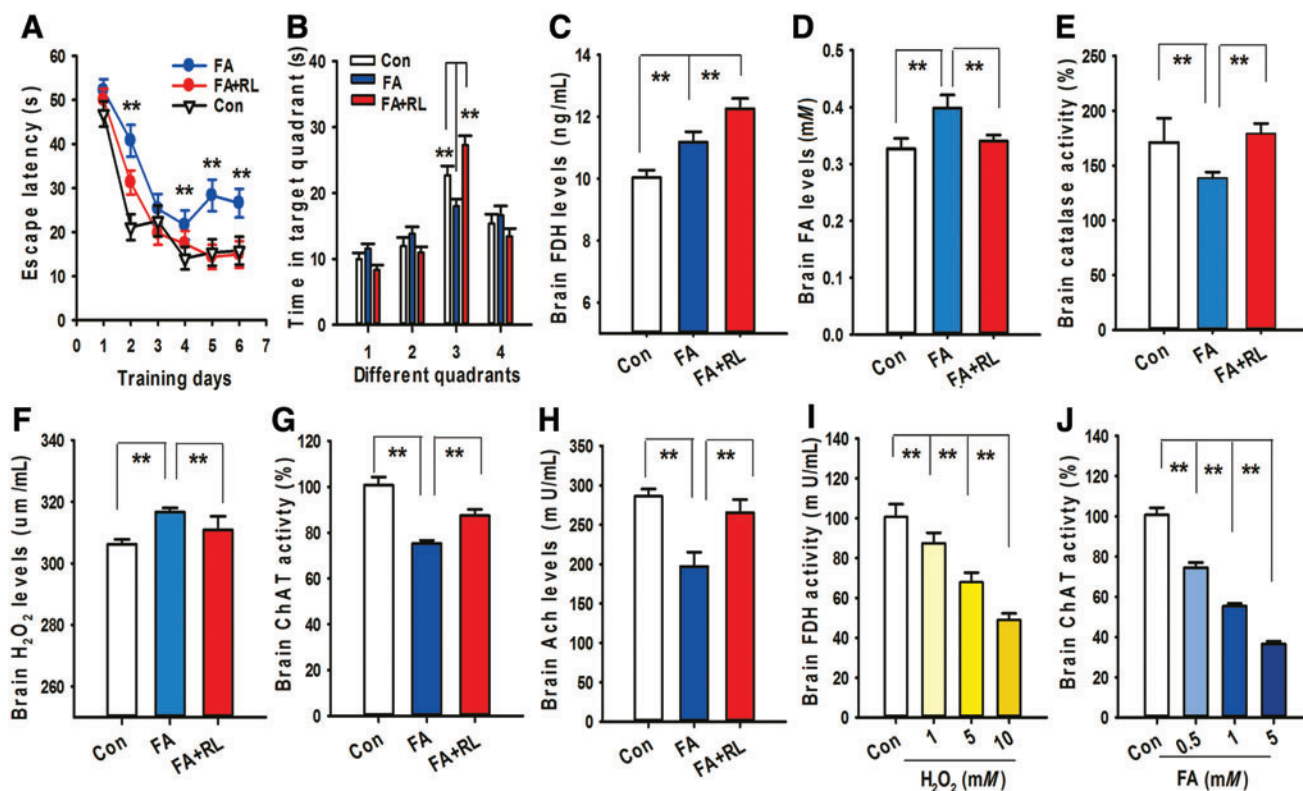


FIG. 4. LED-RL illumination reverses formaldehyde-induced memory deficits in healthy adult C57BL/6 mice. (A, B) LED-RL illumination improved the abilities of spatial learning and memory recall in formaldehyde-injected mice ($n=10$). (C, D) LED-RL enhanced the FDH activity and reduced brain formaldehyde levels in formaldehyde-injected mice ($n=10$). (E, F) LED-RL enhanced the brain catalase activity and reduced brain H₂O₂ levels in formaldehyde-injected mice ($n=10$). (G, H) LED-RL enhanced the brain ChAT activity and improved brain Ach concentrations in formaldehyde-injected mice ($n=10$). (I) H₂O₂ inhibited the FDH activity in brain slices ($n=6$). (J) Formaldehyde inhibited the ChAT activity in brain slices ($n=6$). ** $p < 0.01$. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

of FDH and catalase and rescues cell viability, and this effect is probably not due to the thermal effects of light.

Excess FA inhibits ChAT activity in the purified protein solutions

To investigate how FA directly affects the ChAT activity, we first theoretically simulated the binding of FA with rat ChAT. First, using the three-dimensional (3D) crystal structures of rat ChAT (PDB ID: 1Q6X) (Fig. 6A) and the molecular simulation software-AutoDockTools-1.5.6, we found that there were hydrogen bonds formation between FA and TRP-90 and TYR-446 of rat ChAT (Fig. 6B). The site of FA binding to ChAT is precisely the active center of ligand binding to ChAT, suggesting that FA directly disturbs the ChAT activity (Supplementary Fig. S16). Previous study has shown that TRP-90 is required for the activity of ChAT (8). Unsurprisingly, we found that excess FA led to a dose-dependent inhibition in the activity of ChAT in the purified protein solutions (Fig. 6C). Taken together, H₂O₂-inactivated FDH activity results in FA accumulation, and excess FA directly suppresses the ChAT activity.

LED-RL illumination activates human catalase in the purified protein solutions

To provide molecular evidence that red light at 630 nm directly enhances the activity of catalase, we measured the

catalase activity in purified protein solution after LED-RL treatment for 40 min. Previous studies have shown that the heme binding with tyrosine residue (Tyr-357) of rat catalase or Tyr-358 of human catalase (PDB ID: 1QQW) is the active center of this enzyme (3, 37) (Fig. 6D, left). Therefore, we proposed that the group of Tyr-358-binding heme is the sensitive site to UV and red light (Fig. 6D, right). This is due to the fact that the Tyr binding to the fifth HEME iron ligand can assist in the oxidation of the Fe(III) to Fe(IV) (23). We first measured the K_m (Michaelis constant) of catalase *in vitro*. The results showed that the K_m of catalase exposed to UV light was higher than that exposed to LED-RL (Fig. 6E), indicating that UV is easier to inactivate catalase. UV as a negative control, LED-RL positively enhanced the catalase activity (Fig. 6F). These data indicate that LED-RL can directly activate catalase.

LED-RL illumination activates FDH in the purified protein solutions

To provide molecular evidence that red light at 630 nm directly promotes the activity of FDH, we measured the FDH activity in purified protein solution after LED-RL treatment for 40 min. First, we purified the recombinant human FDH (hFDH) and identified the hFDH proteins (molecular weight = 40 kDa) by nondenaturing gel (Fig. 7A, B). We found that H₂O₂

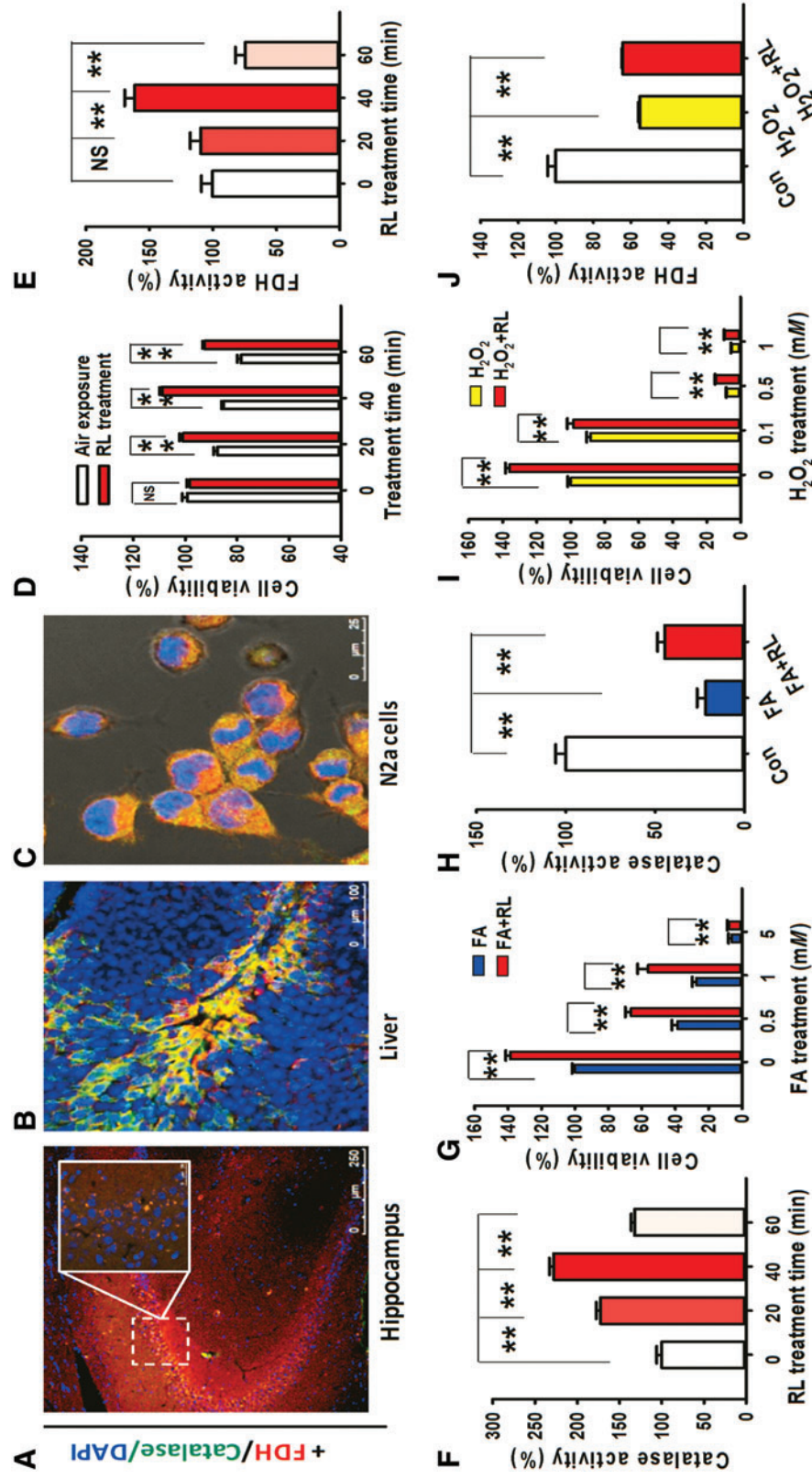


FIG. 5. LED-RL illumination enhances FDH and catalase activities in cultured N2a cells. (A–C) Colocalization of FDH and catalase in the brain, liver, and cultured neuronal N2a cell line. (D) LED-RL reversed air exposure-induced cell viability decline. (E) RL irradiation for 40 min increased the FDH activity. (F) RL illumination for 40 min enhanced the catalase activity. (G) LED-RL reversed formaldehyde-induced cellular toxicity. (H) LED-RL reversed formaldehyde-inhibited catalase activity. (I) LED-RL reversed H_2O_2 -induced cellular toxicity. (J) LED-RL reversed H_2O_2 -inhibited FDH activity. $n = 6$; NS, no statistical significance; ** $p < 0.01$. DAPI, 4',6-diaminophenylindole. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

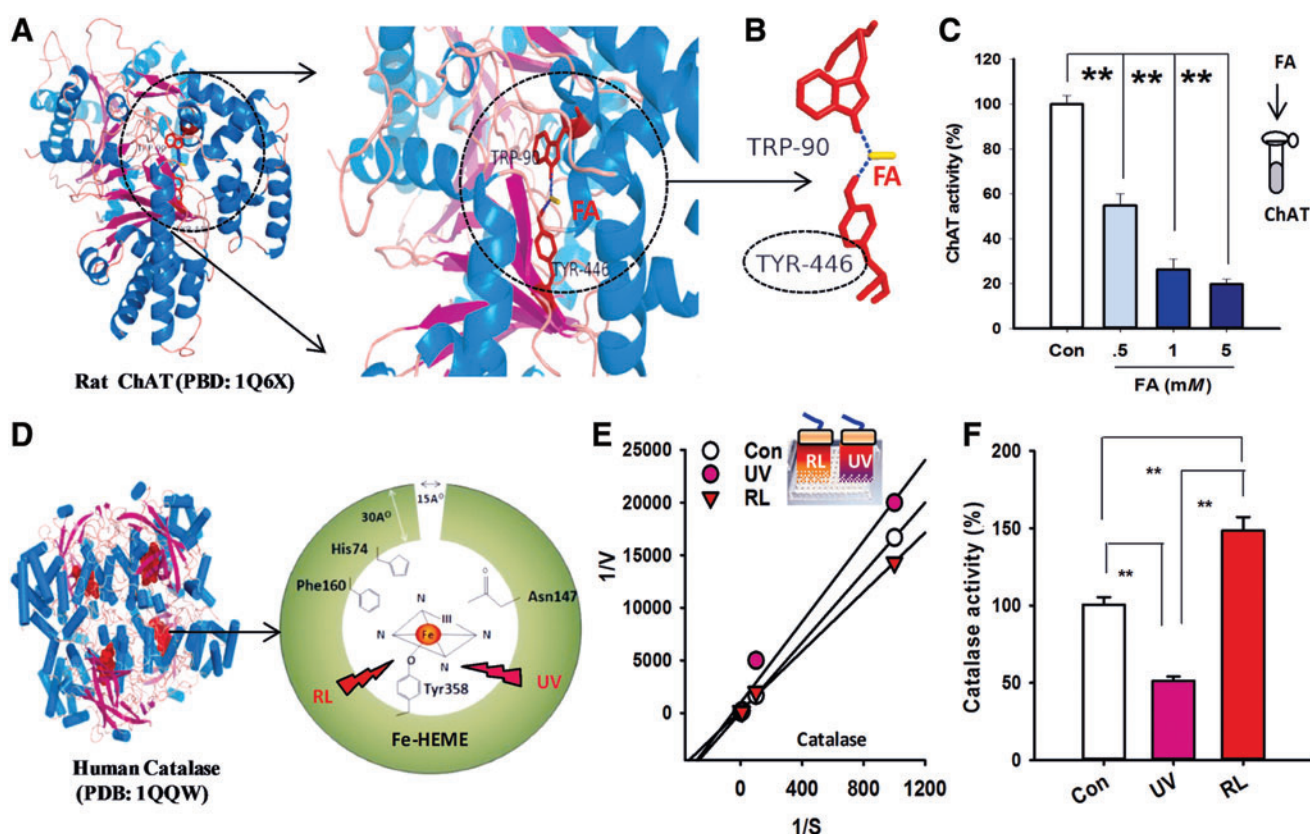


FIG. 6. Excess formaldehyde reduces the ChAT activity and RL activates human catalase in the purified protein solutions. (A, B) Molecular simulation of the structure of formaldehyde binding with rat ChAT (the 3D crystal structures of ChAT, PDB ID: 1Q6X). (C) H_2O_2 inhibited the ChAT activity in a dose-dependent manner ($n=6$). (D) The 3D crystal structures of human catalase (PDB ID: 1QYW). (E) K_m of human catalase exposed to UV and RL ($n=6$). (F) UV inhibited the catalase activity, while RL enhanced the catalase activity ($n=6$). $^{**}p < 0.01$. 3D, three-dimensional; UV, ultraviolet. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

induced a dose-dependent inhibition of the hFDH activity *in vitro* (Fig. 7C).

According to previous studies, ADH is sensitive to UV (18) and H_2O_2 (51), and loss of catalytic Zn^{2+} from the active center of ADH leads to enzyme inactivation (5). Therefore, we observed the effects of UV and LED-RL on the hFDH activity in the purified protein solutions. The 3D crystal structure of hFDH (PDB: 15MC) contains the catalytic Zn^{2+} binding to Cys45 residue and the structural Zn^{2+} binding to Cys97, Cys100, Cys103, and Cys111 residues of FDH (Fig. 7D). The results showed that the K_m of hFDH exposed to UV light was higher than that exposed to LED-RL (Fig. 7E). UV as a negative control, LED-RL positively enhanced the hFDH activity (Fig. 7F). As a strong oxidizing agent, H_2O_2 markedly inhibited the hFDH activity, as it induced Zn^{2+} release from FDH; however, LED-RL treatment reversed H_2O_2 -inhibited FDH activity by reducing Zn^{2+} release from this Zn^{2+} -enzyme (Fig. 7G, H). These data indicate that LED-RL not only activates FDH but also reverses H_2O_2 -inhibited FDH activity by decreasing Zn^{2+} release.

LED-RL reversed H_2O_2 -oxidized cysteine residues of hFDH analyzed by liquid chromatography with tandem mass spectrometry

To further provide molecular evidence that LED-RL reverses H_2O_2 -inhibited FDH activity by protecting free -SH

of cysteine binding to Zn^{2+} , we examined the status of the multi-oxidative modifications of multiple cysteine residues of human recombinant FDH by using the technology of liquid chromatography with tandem mass spectrometry (LC-MS/MS). The results showed that after incubation with hFDH, H_2O_2 induced a marked oxidation of Cys45 (a decrease of 25% in -SH), Cys97 and Cys100 (a decrease of 7.6% in -SH), and a slight change in Cys111 (a decrease of 0.5% in -SH) residues of hFDH than control hFDH group *in vitro* (Fig. 8A-C). However, 40-min LED-RL illumination obviously increased -SH by 15.6% in Cys45, 7.2% in Cys97 and Cys100, and 25.4% in Cys111 residues in the group of H_2O_2 incubated with hFDH when compared with the group without LED-RL (Fig. 8A-C). H_2O_2 -treated hFDH induced a total of $\sim 20\%$ decrease in free -SH; however, LED-RL obviously reversed H_2O_2 -oxidized cysteines of hFDH (Fig. 8D).

Furthermore, we observed the relationship between the oxidized cysteines and catalytic Zn^{2+} of hFDH and between the oxidized cysteines and structural Zn^{2+} of hFDH. The catalytic Zn^{2+} of hFDH is covalently bonded to Cys45 in the 3D structure of FDH (PDB: 15MC) (Fig. 8E, up). Loss of catalytic Zn^{2+} from yeast ADH or mutation Cys47 binding to catalytic Zn^{2+} of plant FDH (also named GSNOR) leads to enzyme inactivation (5, 39). In this study, H_2O_2 obviously oxidized free -SH and decreased the reversed MTHIO-labeling modifications of Cys45 (Fig. 8A), indicating that

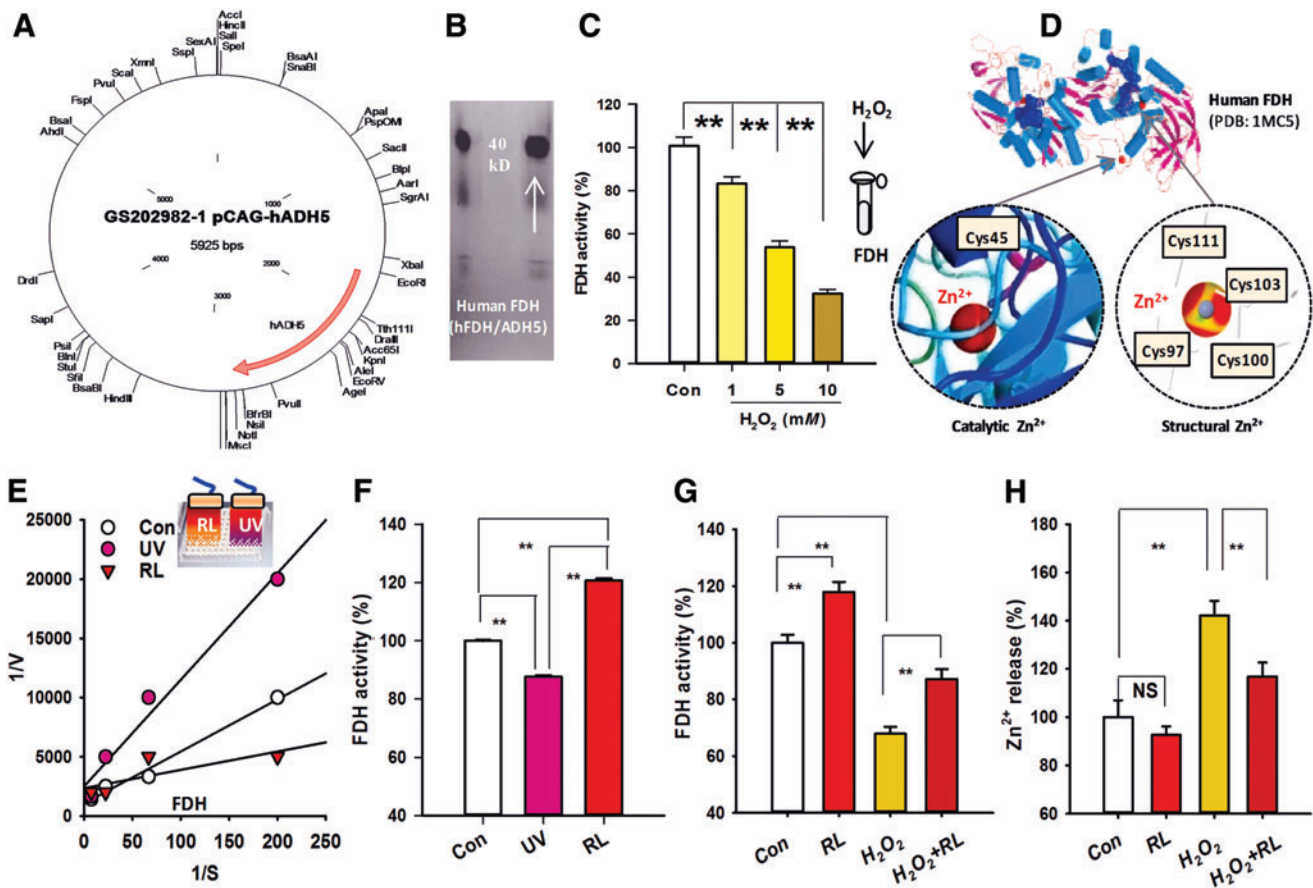


FIG. 7. The effects of UV, RL, H_2O_2 on the activity of recombinant hFDH in the purified protein solutions. (A) Full-length recombinant hFDH (also named ADH5). Synthetic gene was cloned into *EcoRI*-*NotI* digested pCAG-CreERT2. (B) The proteins of hFDH are identified by nondenaturing gel (hFDH: molecular weight = 40 kDa). (C) H_2O_2 dose dependently inhibited the hFDH activity. (D) The 3D crystal structure of hFDH (PDB: 1MC5). (E) K_m of hFDH exposed to UV and RL ($n=6$). (F) UV inhibited the hFDH activity, while RL enhanced the FDH activity ($n=6$). (G) LED-RL reversed H_2O_2 -inhibited FDH activity ($n=6$). (H) LED-RL reversed H_2O_2 -induced Zn^{2+} release from FDH ($n=6$). NS, no statistical significance; $**p<0.01$. hFDH, human FDH. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Cys45 residue is very sensitive to oxidation. Notably, LED-RL reduced the oxidation of Cys47 residues of hFDH (Fig. 8A). Therefore, the inactivation of hFDH induced by H_2O_2 is mainly due to the oxidized Cys45 and the release in the catalytic Zn^{2+} of hFDH. These four cysteine residues (Cys97, Cys100, Cys103, and Cys111) are located in the structural Zn^{2+} site highlighted in the 3D structure of hFDH (Fig. 8E, down). However, H_2O_2 treatment did neither induce a change in -SH of Cys103 residues (-SH content was 100% in hFDH, H_2O_2 -incubated FDH, and red light plus H_2O_2 -incubated FDH; data not shown) nor a statistically significant increase in oxidative modifications of -SH (only ~8.1%) of the Cys97, Cys100, and Cys111 residues around the structural Zn^{2+} when compared with a marked oxidation (~25%) of Cys45-connected catalytic Zn^{2+} of hFDH (Fig. 8A–C and Supplementary Tables S1–S4). These data suggest that Cys45 residue plays a critical role in the oxidized inactivation of hFDH, and LED-RL can protect the -SH of hFDH.

Discussion

In this study, we found that the age-associated accumulation of H_2O_2 directly induces the FDH inactivity and FA

accumulation. FA, in turn, reduces brain Ach levels by inhibiting the ChAT activity and results in age-related memory deficits in SAMP8 mice. However, the phototherapy of LED-RL (wavelength: 630 ± 15 nm) can restore brain Ach levels and ameliorate age-related memory decline by directly activating catalase and FDH (Supplementary Fig. S17).

Is the selection of 630 ± 15 nm the best choice for therapy of age-related cognitive impairments? The most important concern is the penetration rate and phototoxicity of light. UV (200–280 nm) can induce cross-linking of proteins and/or DNA (12). Blue light (470–495 nm) directly damages mitochondrial DNA (24). Blue light and green light (495–570 nm) can induce apoptosis of skin cells (4) and penetrate the skin only 0.5–1 mm deep (20). However, red light can deeply penetrate the skin to about 6 mm (56). In this study, the 630 nm red light penetrated the skull of mice with ~49% penetration rate. In fact, the light with longer wavelength has the stronger penetration (38). Red light and infrared light have been found to penetrate the skull of human (22). However, near-infrared light (NIR) with a longer wavelength in the ranges of 780–1080 nm has the stronger thermal effects on the brains (32). Recent clinical investigations have shown that NIR sometimes induces the side effects, such

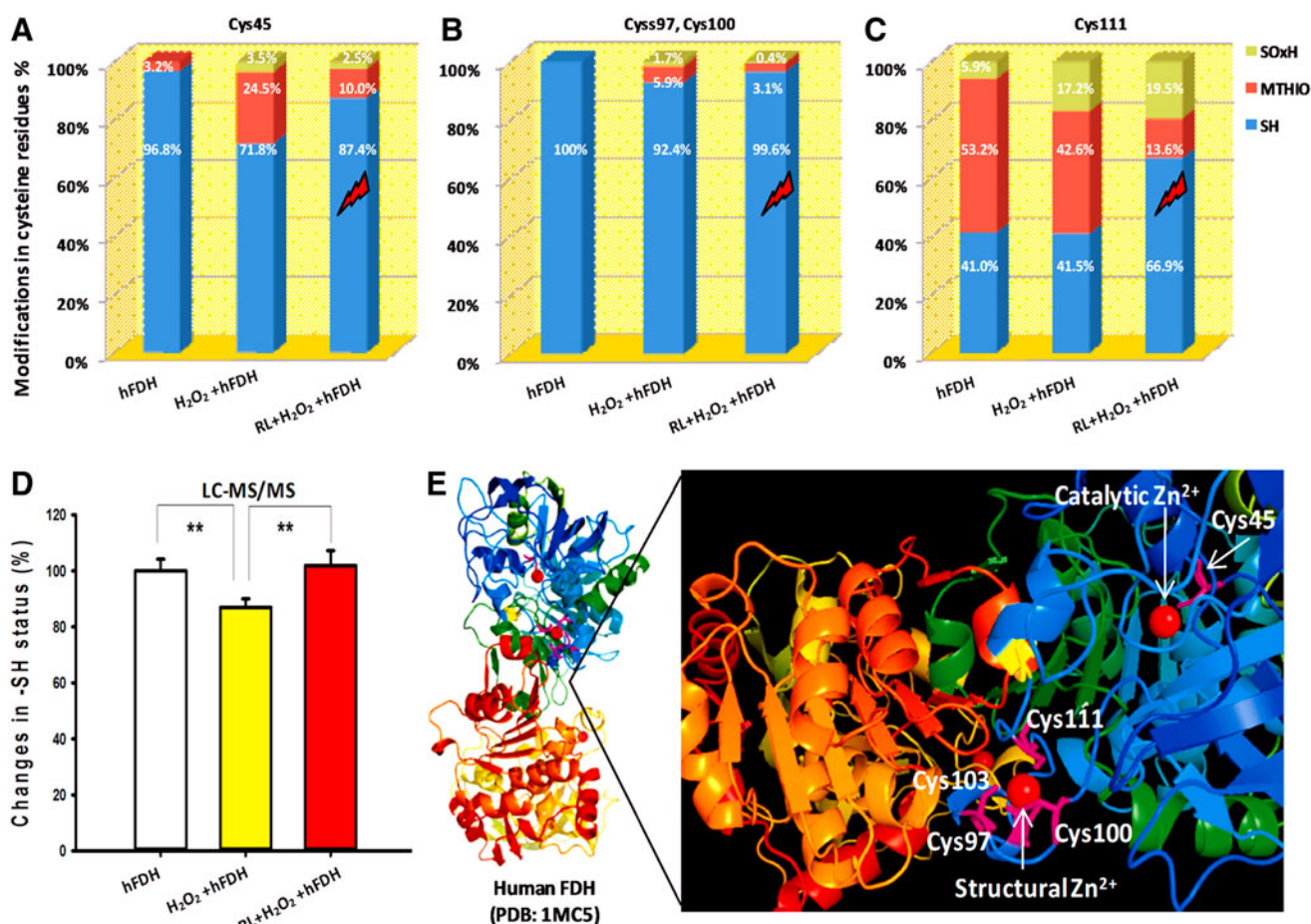


FIG. 8. The protective effects of RL on H₂O₂-oxidized cysteine residues of recombinant hFDH analyzed by LC-MS/MS. (A–C) Percentage distribution of different modifications (SH, MTHIO, and SOxH) of Cys45, Cys97, Cys100, and Cys111 residues of hFDH, hFDH oxidized by H₂O₂ (0.1 mM), and RL-treated oxidized hFDH. These samples were analyzed by LC-MS/MS. SH represents free cysteine; MTHIO labeling shows reversibly modifications; and SOxH represents irreversibly oxidative modifications. **(D)** RL at 630 nm reversed H₂O₂-induced decrease in free cysteines (–SH) of hFDH. **(E)** The 3D crystal structure of hFDH (also named GSNOR) (PDB code: 1MC5) as a ternary complex. Cys45, Cys97, Cys100, Cys103, and Cys111 residues of hFDH are highlighted in purple. The catalytic and structural Zn²⁺ is labeled in red. LC-MS/MS, liquid chromatography with tandem mass spectrometry. ***p* < 0.01. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

as irritability, headache, eye strain, sleep disturbance, and insomnia (66). NIR (for instance 980 nm) has more severe thermal effects than red light (650 nm) (32). Thus, UV/blue light and the NIR are not suitable for long-term therapeutic application based on their impairments and thermal effects. Hence, in this study, we designed a medical device to emit 630 ± 15 nm red light with a temperature overload protection device ($T \leq 40^\circ\text{C}$ to avoid the thermal effects on the brains). Actually, illumination of red light (<650 nm) does not induce a thermal effect in the brains (32). In this study, the LED-RL is 2 cm away from the brains of SAMP8 mice, the cultured N2a cell lines, the purified protein solutions. Red light irradiation at 630 nm did not induce an elevation in the test temperature under air exposure condition (room temperature: $24 \pm 0.5^\circ\text{C}$) (Supplementary Fig. S15B, C). Thus, the red light-enhanced activities of catalase and FDH are due to the light energy and not due to the temperature of solutions or environment.

Oxidative stress is a contributing factor for aging and the pathological processes underlying AD (48, 54). Brain ROS overload exacerbates cognitive impairments in AD (1).

SAMP8 model mice also exhibit a marked elevation in brain ROS levels (77). Photobiomodulation of ROS generation or reduction has been investigated for several decades. For example, red light at ~630 nm has been found to reduce ROS generation (42). However, blue light and NIR cause a marked increase in ROS levels (10, 45). It is known that catalase can reduce ROS levels by degrading H₂O₂ (74). Catalase has been found to reduce ROS and rescue memory function in the APP/PS1 transgenic mice model of familial AD (13, 47) and in the streptozotocin-induced rat model of sporadic AD (63). Catalase activity can be inactivated by UV exposure (78, 79) and affected by visible lights (21, 41, 57). For the possible molecular mechanism of light-regulating catalase activity, blue light (>400 nm) is effective in inactivation of rat mitochondrial catalase with the maximal absorbance at the heme site (405 nm) (11). Surprisingly, the heme groups of catalase can be photoreactivated using 632–633 nm red laser (73). Rat tyrosine residue (Tyr-357) or human Tyr-358 of catalase is a functional site for binding with the fifth iron of heme (37, 59), which is considered to assist in the oxidation of the Fe(III) to

Fe(IV) (17, 23). These data suggest that light with different energy (wavelengths) causes the different oxidative effects of heme in catalase. In this study, both UV and excess FA can inactivate human catalase. Tyr-358 is the catalytic center of catalase. Consistently, UV has been found to cross-link tyrosine in proteins (15), and FA directly has spontaneous chemical reaction with tyrosine (6, 52). Thus, excess FA most likely binds to tyrosine residue and inactive catalase, which needs to be further investigated. Notably, ~630 nm laser light can activate cytochrome *c* oxidase (CCO), which also contains an active center, Tyr244 binding to heme; and the redox status of heme in CCO is responded to red laser light (35); Tyr244 participates in the oxidation–reduction reaction of heme (64). Herein, we speculated that the active center of Tyr-358-binding heme in human catalase may be similar to the model of CCO.

Another interesting finding is that LED-RL not only directly activated FDH but also reversed H₂O₂-inhibited FDH activity. FDH is considered as the ancestral enzyme for the entire ADHs family (28, 61) and is sensitive to UV and visible light in yeast, mice, and rats (19, 23a, 27). This is because ADHs including FDH contain the common structure Zn²⁺-thiolate catalytic center (Fig. 7D). The thiol oxidants of these cysteine residues are proposed to be the common mechanisms that visible lights reversibly activate ADH or other photosensitive enzymes (7, 62). Reasonably, H₂O₂ has been found to inhibit the FDH activity by oxidizing free –SH (39, 51), and inactivation of FDH leads to FA accumulation (68). Furthermore, loss of catalytic Zn²⁺ or mutation of Cys45 binding to catalytic Zn²⁺ of plant FDH (also named GSNOR) leads to the FDH inactivation (5, 39). In this study, red light directly activates hFDH in the purified protein solutions. Our results of LC-MS/MS analysis showed that Cys45 residues binding with catalytic Zn²⁺ were oxidized by H₂O₂ and associated with a release in Zn²⁺ and a loss activity of hFDH. However, LED-RL illumination reversed H₂O₂-oxidized cysteine residues, including Cys45, Cys99, Cys100, and Cys111, and increased the free –SH of hFDH, suggesting that red light improves the FDH activity by activating Cys45 residue and protecting –SH against oxidation. This can explain why red light at 630 nm reverses FA-induced lung inflammation (53).

Endogenous or exogenous FA overload, including gaseous FA exposure, aqueous FA injection in animals, and age-related FA accumulation in elderly people and AD patients, all induced memory disorders (36, 67) or dementia (69, 70). Inhibition of FDH obviously impaired spatial memory in Sprague Dawley rats (68). Knockout of FDH indeed damaged the visual pattern memory in *Drosophila* (33). Excess FA markedly reduced brain Ach and directly inhibited the ChAT activity in this study. Brain Ach or ChAT deficiency has been found to contribute to memory deficits in SAMP8 mice and AD patients (31, 34, 72). Our results and these data support the notion that age-associated H₂O₂ inhibits the FDH activity and leads to FA accumulation; consequently, excess FA inhibits the ChAT activity and causes Ach deficiency-related memory decline.

There are some limitations in this study. First, SAMP8 model is not an AD transgenic mouse model. However, it is a typical age-related dementia rodent model (63a). The effects of LED-RL on the APP/PS1 mouse model will be investigated in the future. Second, although LED-RL did not affect

the visual sense and swimming speed of SAMP8 mice, the auditory and taste senses were not examined in this study. Third, the phototoxicity of LED-RL with a longer time treatment was not addressed.

In conclusion, these findings indicate that the phototherapy of LED-RL has low phototoxicity and strong tissue penetration, and this medical device of LED-RL is easy to operate and effective to treat animal model of dementia noninvasively. Therefore, this study provides evidence for translating LED-RL into clinical application.

Materials and Methods

Animals

All protocols involving the use of animals were conducted in accordance with the guidelines of Biological Research Ethics Committee, Capital Medical University, China. C57BL/6 mice (body weight 25 ± 5 g) were obtained from the Experimental Animal Center of the Peking University, China. SAMR1/SAMP8 male adult mice at 3 or 5 months of age were provided by the Weitong Lihua Animal Center, China. All the animals were maintained in cages at room temperature (25°C) under an alternating 12-h light/dark cycle (lights on at 7 a.m.) with food and water provided *ad libitum*.

Illumination at the skull and abdominal cavity of mice with LED-RL

SAMP8 prevention group. Two groups of 3-month-old mice, namely SAMP8 and SAMR1 mice (*n* = 10 per group), were fixed in the mouse sleeve (Lucite pipe) and were illuminated for 30 min daily at the skull using a head-mounted light source and at the abdomen using an abdominal light source for 5 days per week within two consecutive months (Supplementary Fig. S1A–D). The mean optical power density was 0.5 ± 0.1 mW/cm². Another two groups of SAMP8 and SAMR1 mice (*n* = 10 per group) were fixed in the mouse sleeve and used as control mice illuminated by using a sunlight lamp. The head light source (China utility model patent: CN204890973U, 201520438526.8), abdominal light source (China utility model patent: CN204864568U, 201520438951.7), and light controller were developed by the Beijing Institute for Brain Disorders, China (Supplementary Fig. S1A–H).

SAMP8 treatment group. Two groups of 5-month-old SAMP8 and SAMR1 mice (*n* = 10 per group) were fixed in the mouse sleeve and illuminated for 30 min daily at the skull using a head light source and at the abdomen using an abdominal light source for 5 days per week within two consecutive months (0.5 mW/cm²) (Supplementary Fig. S1A–D). Another two groups of SAMP8 and SAMR1 mice (*n* = 10 per group) were fixed in the mouse sleeve and used as control mice illuminated by using a sunlight lamp.

FA-injected group. One group of 3-month-old C57BL/6 (*n* = 10 per group) mice was intraperitoneally injected with FA (0.5 mM, 0.4 mL) and then received LED-RL illumination for 30 min per day at the skull using a head light source and at the abdomen using an abdominal light source for 5 days per week within 1 month (0.5 mW/cm²) (Supplementary Fig. S2A). Another group of FA-injected mice was used as a sunlight-irradiating control. One group of healthy

C57BL/6 mice was served as negative control. After treatment, these three groups of mice were used for the memory behavior assessments and biochemical detection assays.

Examination of LED-RL penetration in the skull and abdominal cavity

To detect light penetration in the skull of SAMP8 mice, a spectrophotometric detector (FLA5000+; Hangzhou Crystal Fly Technology Co., Ltd., China) was placed under the mandibula and the head light source was placed on the skull (Supplementary Fig. S2B, C). For detecting light penetration in the abdomen of SAMP8 mice, a spectrophotometric detector was placed on the back of the mice and the abdominal light source was placed under the abdomen of the mice (Supplementary Fig. S2D).

Examination of LED-RL light power density

The mean optical power density of 0.5 ± 0.1 mW/cm² was detected by using a laser radiation detector (model: GB8898.6.2, NOVA II; OPHIR, Israel) with an optical fiber detector (model: part of S/N 719261, 1.44 cm², NOVA II; OPHIR, Israel) (Supplementary Fig. S4).

Memory behavior assessment using the Morris water maze

Spatial memory behaviors were assessed using the Morris water maze test, as described previously (69, 70). They were tested in a pool filled with water after receiving different interventions. Mice were trained to mount a hidden/submerged (1.5 cm below water surface) escape platform in a restricted region of the pool. Spatial memory was assessed by recording the latency time to escape from the water onto a submerged escape platform, which was regarded as a function of the number of learning trials during the learning phase. Six days after the learning phase, mice were subjected to a 60-s probe trial wherein the escape platform was removed. The water maze activity was monitored with the Instrument of Morris water maze (Shanghai Biowill Co. Ltd., Shanghai, China).

Detection of tissue FA, H₂O₂, and Ach levels

FA concentration analysis. All samples were collected and immediately placed on ice before storing at -70°C until further processing. After centrifugation (8000 g, 4°C , 10 min), FA concentrations in the brain homogenate and the liver homogenate were measured using high-performance liquid chromatography with fluorescence detection (44).

H₂O₂ concentration analysis. Brain and liver H₂O₂ levels were measured using a commercially available kit according to the manufacturer's instructions (S0038; Jiancheng Co., Nanjing, China).

Ach concentration analysis. Brain Ach levels were detected using an enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (CSB-E17521m; Cusabio Biotech Co., Newark, NJ).

Measurement of tissue FDH, catalase, SSAO, and ChAT activities

FDH activity. FDH (ADH3) activity was measured using a commercially available kit according to the manufacturer's instructions (K787-100; Biovision, Milpitas, CA).

SSAO activity. SSAO activity was measured using a commercially available kit according to the manufacturer's instructions (GMS50538.2; Genmed Scientifics, Inc., Arlington, MA).

Catalase activity. Catalase activity was measured using a commercially available kit according to the manufacturer's instructions (S0051; Beyotime Biotechnology, Jiangsu, China).

ChAT activity. ChAT activity was measured using a commercially available kit according to the manufacturer's instructions (KGT037-1; KeyGen Biotech, Nanjing, China).

Double immunofluorescence labeling of FDH and catalase in vitro and in vivo

Tissue fluorescence histochemistry. Brain and liver sections (thickness 30 μm) were prepared from the SAMP8 and control mice. The sections were permeabilized by immersing in phosphate-buffered saline (PBS) containing Triton-100 (PBS with Tween-20 [PBST]; 0.3% Triton-100 in PBS) for 1 h at room temperature (25°C), washed thrice with PBS for 10 min each, followed by antigen retrieval at 95°C for 10 min and three more washes with PBS for 10 min each. Next, the sections were blocked with 5% goat serum (ZLI-9021; ZSGB-BIO, Beijing, China) and 1% bovine serum albumin in PBST (0.3% Triton-100 in PBS) at room temperature for 1 h. For the first labeling, the sections were incubated overnight with the rabbit anti-ADH5 polyclonal antibody (1:100, TA327239; Origene, Rockville, MD) at 4°C . The sections were then washed thrice with PBS for 10 min each. For the second labeling, the sections were incubated overnight with the anti-catalase antibody (1:50, ab16771; Abcam, Cambridge, United Kingdom) at 4°C . These sections were washed thrice with PBS for 10 min and then incubated with the goat anti-rabbit IgG H&L (Alexa Fluor[®] 594) at a 1:300 dilution (a11012; Invitrogen) for 1 h at room temperature, after being washed three times with PBS each for 10 min and then incubated with the goat anti-mouse IgG H&L (Alexa Fluor 488) at a 1:300 dilution (ab150117; Abcam) for 1 h at room temperature. They were marked with 4',6-diaminophenylindole (DAPI, C1002; Beyotime Biotechnology) at a 1:5000 dilution for 5 min and washed three times with PBS for 10 min each. Finally, the sections were washed thrice with PBS for 10 min each before they were viewed under the Leica TCS SP5 confocal laser microscope (Leica, Wetzlar, Germany).

Cellular fluorescence histochemistry. Cultured N2a cells were fixed with 4% paraformaldehyde in PBS at room temperature for 25 min and then washed thrice and permeabilized by immersing in 0.1% Triton-100 in PBS for 10 min. After these steps, the cells were blocked with 5% goat serum in PBST (0.1% Triton-100 in PBS) at room temperature for 1 h. As mentioned in the above section, the cells were dual marked with ADH5 and catalase. Following this, the cells were

incubated with DAPI (1:500, C1002; Beyotime Biotechnology) for 5 min, washed thrice with PBS for 10 min each, and viewed and photographed using confocal microscopy.

Quantification of FDH and catalase by Western blotting. The proteins of FDH and catalase in the hippocampus of different groups of mice were collected and used for Western blotting. Antibodies included were anti-FDH (1:1000, #ab108203; Abcam), anti-catalase (1:1000, #ab16771; Abcam), mouse anti-glyceraldehyde 3-phosphate dehydrogenase (1:1000, #8884; CST), and goat anti-mouse IgG (1:5000, #7074, #7076; CST).

Cell viability detection

The N2a cells were cultured as described previously (49). The cell viability of N2a cells was measured using a commercially available CCK-8 kit according to the manufacturer's instructions (E1CK-000208-10; EnoGene, Nanjing, China).

Detecting red light-irradiated thermal temperature

The cultured N2a cell lines were taken out of the cell culture box and illuminated with 630 nm red light at 0, 20, 40, 60 min, respectively. Room temperature was measured by a room temperature meter (Anymetre, model: Th108; Yunalue Instrument Co., Ltd. Shanghai, China), with an accuracy of $\pm 0.5^\circ\text{C}$. The temperature of the red light-irradiating 96-well plate was detected by a portable digital thermometer (Zhihui, model: TC-01; Sansixing Technology Co. Ltd., Beijing, China), with an accuracy of $\pm 0.1^\circ\text{C}$ (Supplementary Fig. S15B).

Molecular simulations of FA binding with rat ChAT

The 3D crystal structures of rat ChAT (PDB ID: 1Q6X) was downloaded (Protein Data Bank). FA-ChAT binding was simulated using AutoDockTools-1.5.6 and the MGLTools software. The hydrogen bonds between FA and ChAT were simulated using the PyMOL 1.7 software.

In vitro human catalase and recombinant hFDH activity measurement

Human catalase activity measurement. The catalase samples (1–30 μL , human catalase, C3556; Sigma-Aldrich, St. Louis, MI) were placed in cuvettes containing 0.1% H_2O_2 in 1 mL of 50 mM KPO_4 , pH 7.0. Human catalase activity was detected as described previously (2).

Recombinant hFDH purification. The full-length, recombinant human glutathione-dependent FDH was provided by the Institute of Zoology, Chinese Academy of Medical Sciences, China. The synthetic gene was cloned into the *EcoRI*-*NotI*-digested pCAG-CreERT2 vector. After ion exchange chromatography, the pooled fractions were dialyzed and loaded on a 5-mL Blue Sepharose 6 Fast Flow column (GE Healthcare) and eluted with 200 mM NaCl and 5 mM NAD^+ . A final gel filtration step (HiLoad 16/60, Superdex 200; GE Healthcare) was performed in 10 mM Tris-HCl (pH 8.0), 200 mM NaCl, and 1 mM dithiothreitol. The purity

of the protein was verified by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Recombinant hFDH activity analysis. The hFDH samples (1–30 μL) were placed in cuvettes, and the FDH activity was tested at 25°C by monitoring the production of NADH at 340 nm for FA degradation. Briefly, the FDH activity was measured at pH 8.0 in 0.1 M sodium pyrophosphate, with S-hydroxymethyl glutathione (HMGS; formed by mixing FA and glutathione) and NAD^+ . The kinetic constants for NAD^+ and NADH were determined with 1 mM of glutathione and 1 mM of FA. The kinetic constant for HMGS was determined using 2.4 mM NAD^+ . The kinetic constants were calculated using a nonlinear regression program GraFit (version 3.0; Erithacus Software, Staines, United Kingdom).

Detection of Zn^{2+} release in recombinant hFDH solution

Free Zn^{2+} ions were detected using a metallochromic indicator, 4-(2-pyridylazo)-resorcinol (PAR), which binds to Zn^{2+} in a 2:1 complex and turns its color from yellow to orange with a strong absorbance at 490 nm. Recombinant hFDH (50–100 μg) was oxidized by increasing molar excess (100–3000 to protein) of H_2O_2 for 1 h in 50 mM Tris-HCl (pH 7.2) and mixed with 100 μM PAR. The absorbance of the $\text{PAR}_2\text{-Zn}$ complex was measured at 490 nm, and the Zn content was calculated using the ZnCl_2 standard curve.

LC-MS/MS for analyzing the oxidized cysteine residues of hFDH

hFDH (0.29 $\mu\text{g}/\mu\text{L}$) in 10 mM sodium phosphate buffer (pH 7.5, buffer A) was treated with 0.1 mM H_2O_2 at 25°C for 10 min, and the reactions were terminated by adding 5 mM L-methionine. The oxidized hFDH was subsequently alkylated with 55 mM iodoacetamide in 8 M urea in the dark for 30 min, and the buffer of the resulting hFDH solution was exchanged with 50 mM NH_4HCO_3 (pH 8.0, buffer B) by using a Microcon YM-10 microcentrifuge filter (Millipore, Billerica, MA). The protein samples were then digested with chymotrypsin or trypsin (Roche Diagnostics, Indianapolis, IN) at an enzyme-to-substrate ratio of 1:40 (w/w) in a 100 μL NH_4HCO_3 buffer at 37°C overnight.

The samples were then subjected to LC-MS/MS analyses, and then, samples were performed on an LCQ Deca XP Ion Trap Mass Spectrometer (Thermo Finnigan, San Jose, CA). MS/MS experiments were carried out in a data-dependent scan mode by selecting the most abundant protonated ions observed in MS mode for collisional activation with relative collision energy of 35%. A 0.30×150 mm capillary C_{18} column (Micro-Tech Scientific, Vista, CA) was used for the separation; and the flow rate was $\sim 5 \mu\text{L}/\text{min}$, which was obtained from a 120 $\mu\text{L}/\text{min}$ pump flow after precolumn splitting. The mobile phases were 0.6% acetic acid in water (mobile phase A) and 0.6% of acetic acid in acetonitrile (mobile phase B). A linear gradient of 2–42% mobile phase B in A in 63 min was employed for the separation. The resulting MS/MS data were searched against the protein database by using SEQUEST (Thermo Finnigan) or against the sequence of hFDH by using MassAnalyzer 1.03. To carry out quantitative measurement of specific peptides, LC-MS/MS experiments were also performed in selective ion monitoring mode.

Statistical analyses

In the Morris water maze experiment, measurements of performance during acquisition trials (*i.e.*, escape latency) were averaged for each day for each animal. The animal performances across days were compared using repeated measures analysis of variance (ANOVA) with day as a within-subjects factor and treatment as a between-subjects factor. Differences between treatment groups for each day were analyzed using one-way ANOVA; Fisher's least significant differences test was used for *post hoc* comparisons. SPSS 16.0 (SPSS, Inc., Chicago, IL) was used for all the statistical analyses. The statistical significance of the results was determined by the Student's *t*-test (for independent or dependent samples, as appropriate), and results with $p < 0.05$ (two-tailed) was considered statistically significant. Data are reported as mean $\pm$ standard error.

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Authors' Contributions

Z.T. conceived and designed the experiments. J.Z., X.Yu., H.L., W.J., Y.M., L.A., G.G., Y.W., H.Y., J.A., S.D., X.Ya., and B.S. performed the experiments. W.L., R.H., J.J., J.L., and Z.T. analyzed the data. J.L., R.H., and Z.T. edited the manuscript.

Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

3D = three dimensional
Ach = acetylcholine
AD = Alzheimer's disease
ADH3 = alcohol dehydrogenase 3
ANOVA = analysis of variance
APP = amyloid precursor protein
CCO = cytochrome *c* oxidase
ChAT = choline acetyltransferase
DAPI = 4',6-diaminophenylindole
FA = formaldehyde
FDH = formaldehyde dehydrogenase
H₂O₂ = hydrogen peroxide
hFDH = human FDH

HMGSH = S-hydroxymethyl glutathione
LC-MS/MS = liquid chromatography with tandem mass spectrometry
LED-RL = light-emitting diode with red light
NIR = near-infrared light
PAR = 4-(2-pyridylazo)-resorcinol
PBS = phosphate-buffered saline
PBST = PBS with Tween-20
PS1 = presenilin 1
ROS = reactive oxygen species
SAMP8 = senescence-accelerated prone 8 mouse
SAMR1 = senescence-accelerated resistant mouse-1
SSAO = semicarbazide-sensitive amine oxidase
UV = ultraviolet